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ORIGINAL RESEARCH

# Enhancing Root Development: The Impact of Indole-3-Butyric Acid on Nodal Leaf and Tip Cuttings of *Dendrocalamus longispathus* and *Bambusa tulda*

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**ABSTRACT:** This study examines the effects of two propagation techniques—nodal leaf cuttings and tip cuttings—on root development in two bamboo species, *Dendrocalamus longispathus* and *Bambusa tulda*. The experiment aimed to determine the most effective propagation method for enhancing root growth, addressing the challenge of optimizing bamboo cultivation for both conservation and commercial purposes. The experiment was conducted using a non-mist propagator system, under controlled humidity and temperature, to observe differences in root number and length after four weeks. Our results indicate significant differences between the two cutting methods across both species, with *D. longispathus* generally demonstrating more substantial root growth compared to *B. tulda* in both metrics. Specifically, for *D. longispathus*, nodal leaf cuttings showed a consistently higher mean root length, whereas for *B. tulda*, tip cuttings eventually resulted in slightly longer roots, highlighting a species-specific response to the propagation methods. Statistical analysis confirmed significant differences ($p<0.05$) in root growth dynamics between treatments, underscoring the importance of choosing appropriate propagation techniques based on species-specific responses. This research contributes to the understanding of bamboo propagation strategies and suggests further exploration into species-specific cultivation methods to enhance growth and conservation efforts.

**KEYWORDS:** Nodal leaf cuttings, tip cuttings, Principal Component analysis, IBA, branch cutting

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## 1. Introduction

Bamboo, a tall grass from the Poraceae family, is incredibly versatile and economically significant, especially in regions like Bangladesh (Md Numan, 2015). Known as "poor man's timber," bamboo's strength and rapid growth make it a valuable resource for construction and manufacturing. In Bangladesh (Goyal & Sen, 2016), the demand for bamboo spikes notably due to the needs of the world's largest refugee camp, which requires substantial supplies for building and maintenance (Jamatia, 2012). The country's climate is ideally suited for bamboo cultivation, promoting its rapid growth. However, despite these favorable conditions, there is a critical need for advanced research to optimize production methods and enhance yield (Suwal et al., 2020). Bangladesh harbors 9 genera and 34 species of bamboo (Hossain et al., 2018), each with unique physical and chemical properties that make it suitable for a range of applications—from building materials to crafting utensils (Ojha et al., 2009). This diversity also positions bamboo as a key player in sustainable development, considering its role in carbon sequestration and its potential to replace less sustainable raw materials. To maximize these benefits, significant investment in research and development is essential to explore innovative uses and improve cultivation practices, making bamboo an even more valuable asset for the future.

*Dendrocalamus longispathus*, one of the most abundant bamboo species in Bangladesh, is renowned for its significant height (1-37m) and diameter (1-30.5cm), although specific measurements are not provided (Alamgir et al., 2007; Sohel et al., 2015). This species is widespread across the country, particularly thriving in the districts of Chittagong, Cumilla, Mymensingh, and Dinajpur. *D. longispathus* is notable for producing a large number of primary and secondary branches starting from just above the ground level, around 0.5 meters, up to 10.5 meters high. This characteristic branching pattern supports its use in various applications, from construction to crafts, making it a vital component of the local bamboo industry (Jha et al., 2013).

*Bambusa tulda*, which is prevalent in Chittagong, typically grows to heights ranging from 1 to 2.5 meters and achieves a diameter of 9 to 20 cm. This species is integral to the bamboo industry in the region due to its structural robustness and versatile applications (Mishra et al., 2019). Traditionally, bamboo has been propagated through techniques such as offset cuttings, branch cuttings (Hamalton, 2022), and clump nodal cuttings (Alamgir et al., 2007; Sohel et al., 2015). These methods, however, are not cost-effective for large-scale production because they involve bulky materials that are expensive and difficult to transport(Alamgir et al., 2007). As a viable alternative, tissue culture and nodal leaf and tip cuttings have been identified as efficient strategies for mass propagation. Tissue culture is particularly beneficial as it enables the rapid multiplication of plants, maintaining genetic uniformity and enhancing disease resistance across large batches (Kumar Ahirwar & Bagchi, 2014). Meanwhile, nodal leaf and tip cuttings (Rout, 2020), though less conventional, are being explored for their potential to propagate bamboo quickly and economically. Leaf cutting is currently a focal point of research due to its varied success across different bamboo species (Kalanzi & Mwanja, 2023). The effectiveness of this method depends significantly on the species in question and the specific cutting techniques employed. Notably, species like *B. balcooa* and *B.polymorpha* do not adequately produce branches from leaf cuttings, which poses a challenge for their propagation using this method (Hamalton, 2022; Ilorkar et al., 2021; Suwal et al., 2020). Ongoing research into propagation techniques is essential to improve the scalability and sustainability of bamboo cultivation, ensuring that this valuable resource can continue to support economic development and environmental sustainability in Bangladesh and beyond.

Studies by (Hossain et al., 2018; Islam et al., 2011)have demonstrated that cuttings begin to show active bud development within 6-10 days and can develop extensive root systems in propagation beds within 4-8 weeks, with the timing influenced by seasonal conditions. However, the availability of suitable branches (those larger than 5 mm in diameter with a base or node) from a single clump for root production in propagation beds is limited, and the branch cutting method (Shirin et al., 2021; Singh et al., 2012; Sood et al., 2002) is typically restricted to the rainy months of June and July. Our approach overcomes the limitation of traditional methods by using smaller, leafy branch cuttings for vegetative propagation, which enables the year-round production of a high volume of cuttings from a single bamboo clump at minimal cost. In this study, we investigated the root development of these small leafy branch cuttings, each approximately 20 cm long and 3 mm in diameter (Figure 1), using a non-mist propagator with or without the application of the rooting hormone Indole-3-Butyric Acid (IBA). We also evaluated their ability to establish under nursery conditions, with the aim of producing a large number of propagules from a single clump. The primary objective was to assess the effectiveness of nodal leaf cuttings and tip cuttings in promoting root development in two bamboo species, *Dendrocalamus longispathus* and *Bambusa tulda*, under controlled nursery conditions. The research aimed to identify species-specific responses to these propagation techniques to optimize bamboo cultivation practices for conservation and commercial purposes. We hypothesized that nodal leaf cuttings would result in greater root growth in D. longispathus, while tip cuttings would be more effective for B. tulda. Additionally, we anticipated that higher concentrations of IBA would significantly enhance rooting success in both species, with the effects varying depending on the cutting type and species.

## 2. 2. Materials and methods

### 2.1 Study area and Rooting Trials

The study was conducted from June to November 2023 at the nursery of the Institute of Forestry and Environmental Sciences, located at the University of Chittagong, Bangladesh. Positioned at a latitude of 22°27′27″N and a longitude of 91°48′30″E, the location is marked by a tropical monsoon climate that features hot, humid summers and cool, dry winters. Temperatures at this site typically fluctuate between 22.9°C and 29.1°C monthly (Hossain et al., 2018), with annual extremes reaching 27°C and dipping to 15°C. Relative humidity varies, with a low of 65% in February and a peak of 95% during the rainy months from June to September. This area accumulates approximately 300 cm of rainfall each year, primarily between June and September, and daylight hours range from 10 hours and 35 minutes in December to 13 hours and 15 minutes in June(Islam et al., 2011). For the purposes of this research, robust five-year-old bamboo clumps were selected based on several characteristics: their maturity, evidenced by sufficient branch production; growth potential, measured by the number of culms per clump, as well as culm height, diameter, and internode length; and a clear absence of disease and pests(Das et al., 2020; Ojha et al., 2009). Cuttings were then obtained from these clumps, involving the removal of small secondary or tertiary branches (E.M. El-Keltawi & S.A. Abdel-Rahman, 2010). The study prepared two types of cuttings: nodal leafy cuttings and tip cuttings. Nodal leafy cuttings were made up of small branches containing one node from a primary or secondary branch, including about 2 cm on both sides of the node as the base, and topped with 3−4 nodes with leafy growth (Jha et al., 2013). (Figure 1)Tip cuttings captured the healthy leading shoot apex, incorporating 2−3 nodes from the secondary or tertiary branches, excluding any swollen bases. The lengths of the nodal leafy and tip cuttings varied between 25-28 cm and 18-20 cm respectively, with diameters of 4-5mm for nodal leafy cuttings and 2-3 mm for tip cuttings.

In total, 480 cuttings were utilized in rooting experiments, divided equally between the two species each for 240 (Figure 1). To mitigate fungal infections, these cuttings were briefly soaked in a fungicide solution of Diathane M-45 (Rohm & Co. Ltd., France; 2 g/L). After rinsing, the cuttings were air-dried in the shade for about 15 minutes.

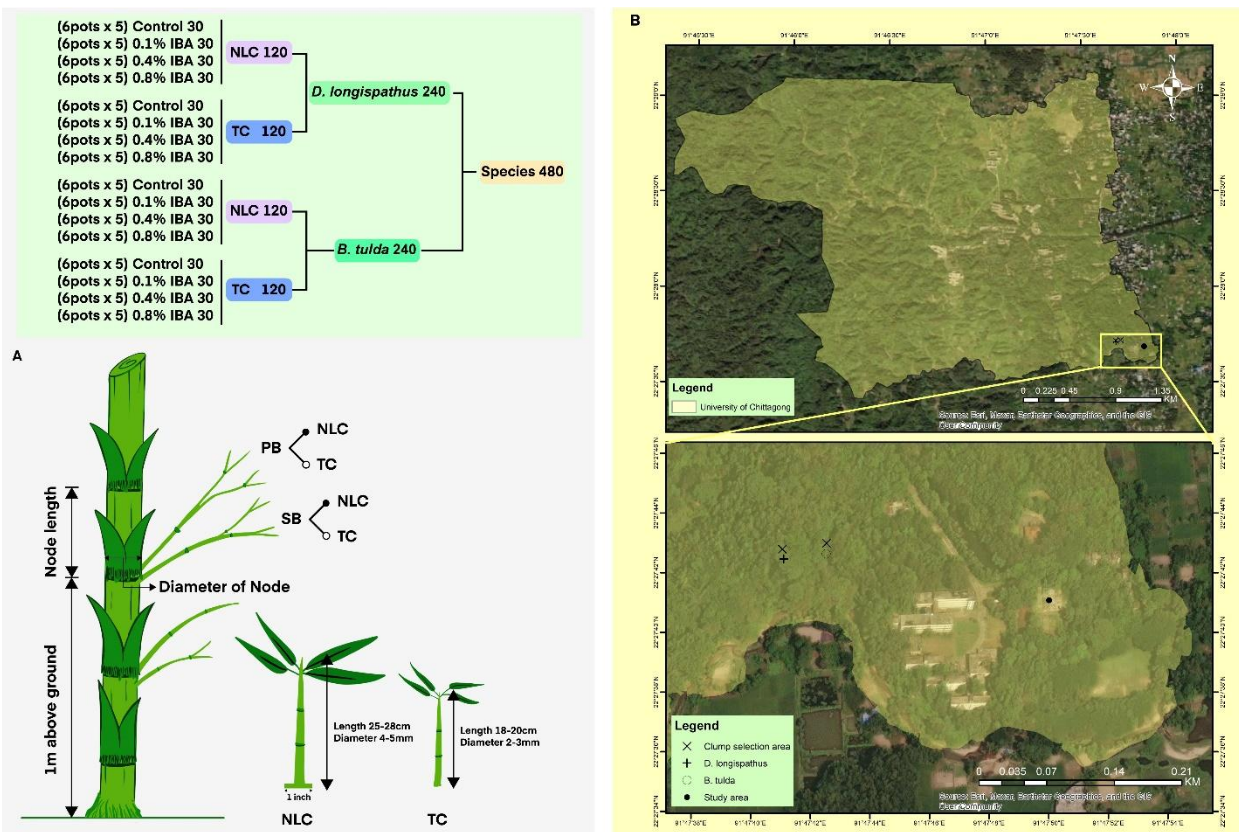


Figure 1. A) Nodal leaf cuttings of *D. longispathus* and *B. tulda* are ready for rooting trials. Note: PB for primary branch cuttings, SB for secondary branch cuttings, NLC for nodal leaf cuttings, and TC for tip cuttings. B) Location of the study area at the University of Chittagong and clump selection areas.

The cuttings underwent treatment for 1 hour with various concentrations of Indole-3-Butyric Acid (IBA) at levels of 0%, 0.1%, 0.4%, and 0.8% (w/v) to determine the hormone's effectiveness in promoting root development (Hossain et al., 2018). The bases of the cuttings were dipped briefly into the IBA solutions as part of their treatment.

### 2.2. Measurements and Analysis

The cuttings were arranged in perforated plastic trays that were filled with a blend of coarse sand and gravel, with each tray accommodating 5 cuttings (Figure 1). This setup resulted in a total of 30 replicated cuttings per treatment (E.M. El-Keltawi & S.A. Abdel-Rahman, 2010; Jha & Das, 2021; Pawar et al., 2002; Ramanayake & Yakandawala, 1997), distributed across six trays in a complete randomized block design (CRBD). To support rooting, these trays were placed within a non-mist propagator, where humidity was kept between 85% and 90% (Hossain et al., 2018; Kalanzi & Mwanja, 2023; SINGH, 2016). To ensure adequate air exchange and to prevent the buildup of excessive heat, the propagator was opened each morning and again in the late afternoon. Additional heat protection was provided by situating the propagator under a bamboo shed, which was further shaded by a jute mat over the roof. This arrangement reduced the light reaching the cuttings to about 12% of full sunlight intensity. Throughout the 4 weeks of the experiment, the average maximum temperature recorded was 32℃, while the average minimum was 25℃.

### 2.3. Data analysis

All data were analyzed using Microsoft Excel and R programming (version 4.2.2). We explored possible treatment variations through analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). The formulas used were: ANOVA formula and Duncan's test formula. Additionally, we assessed the density of different treatments and performed principal component analysis (PCA) to evaluate the data further.

## 3. Results

### 3.1. Number of roots per cuttings

The investigation explored the comparative efficacy of nodal leaf cuttings (NLC) versus tip cuttings (TC) in promoting the growth of *D. longispathus* (DL) and *B. tulda* (BT), (Figure 2) with a focus on root number dynamics. Disparities were evident across treatments and species. For *D. longispathus* treated with nodal leaf cuttings, the mean root number started at 2.64 (±0.10) at T0, and surged to 10.02 (±0.37) by T3. Conversely, *D. longispathus* with tip cuttings had a starting mean root number of 3.02 (±0.56) at T0, peaking at 10.35 (±0.72) by T3, showing a significant difference ($p<0.05$). Similarly, *B. tulda* treated with nodal leaf cuttings showed a root number mean of 3.4 (±0) at T0, escalating to 10.98 (±0.44) by T3. In contrast, *B. tulda* with tip cuttings demonstrated a root number mean of 3.4 (±0) at T0, culminating at 11.25 (±0.76) by T3.Principal component analysis explained the variance with PC1 (94.2%) and PC2 (5.7%) and the distribution of principal component scores across different treatments. The study analyzed the effects of treatments T0, T2, and T3 on the growth of *D. longispathus* and *B. tulda* using nodal leaf and tip cuttings. For *D. longispathus*, both nodal leaf and tip cuttings showed highly significant differences between T0 and T3, and T0 and T2, with p-values extremely close to zero, confirmed by chi-square and Z-values. Similarly, *B. tulda* exhibited significant differences in nodal leaf cuttings between T0 and T2, and T0 and T3, with consistent results for tip cuttings. All p-values remained extremely low after Bonferroni adjustment, indicating robust, statistically significant differences between treatments.

### 3.2. Root length of per cuttings

Figure 3 illustrates the root length (RL) comparison between non-leafy cuttings (NLC) and treated cuttings (TC) under various treatments (T0, T1, T2, and T3). The density distribution plots (panels A and B) reveal that NLC cuttings have broader variations with peaks around 11 cm and 14 cm, whereas TC cuttings show a more consistent distribution, peaking around 12 cm. This suggests variability in root growth patterns between the two types of cuttings. The bar graphs (A1 and B1) indicate a general increase in RL with progressive treatments, with TC cuttings consistently exhibiting higher mean RL values than NLC cuttings across all treatments, achieving the highest mean RL at treatment T3. This highlights the positive impact of treatments on root development, especially for TC cuttings.

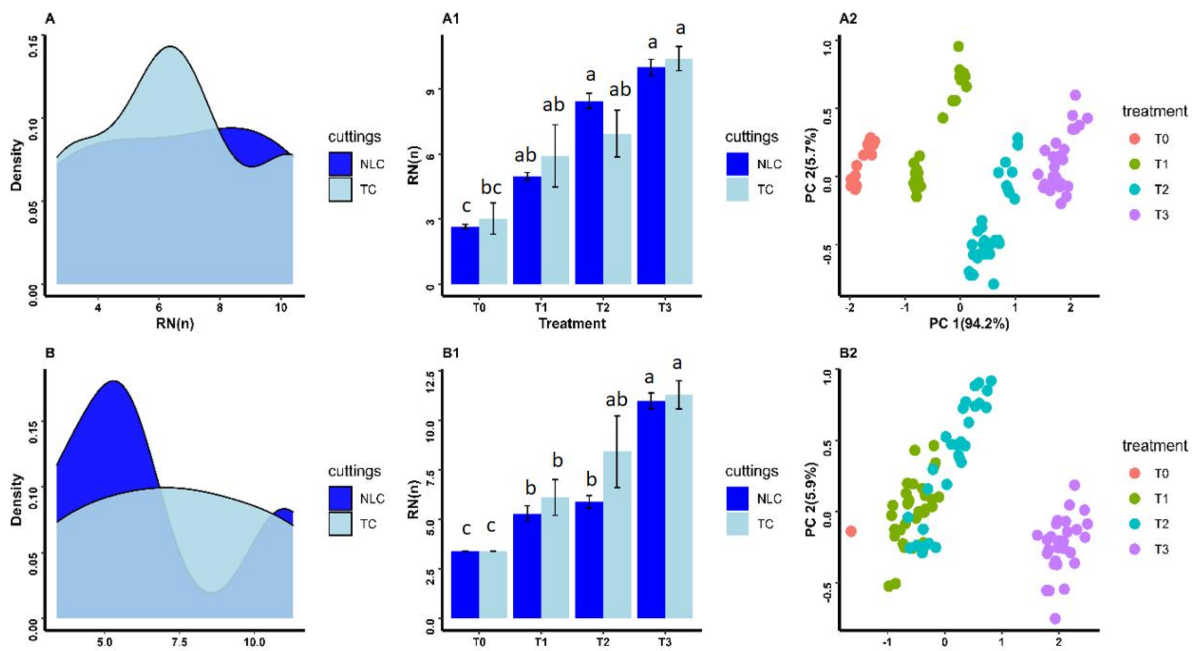


Figure 2. Comparative analysis of root number (n) development from cuttings of *D. longispathus* (DL) and *B. tulda* (BT) developed after four weeks. A) Density plot: displays root development in *D. longispathus* of cuttings.A1) Bar plot: mean ± sd of roots in *D. longispathus* of cuttings. A2) PCA: Principal component analysis shows distribution of root numbers with PC1 (94.2%) and PC2 (5.7%). B) Density plot: compares root development in *B. tulda* of cuttings.B1) Bar plot: mean ±sd of roots in *B. tulda* of cuttings. B2) PCA: Illustrates variability in root numbers with two principal components having PC 1(94.0%) and PC 2(5.9%).

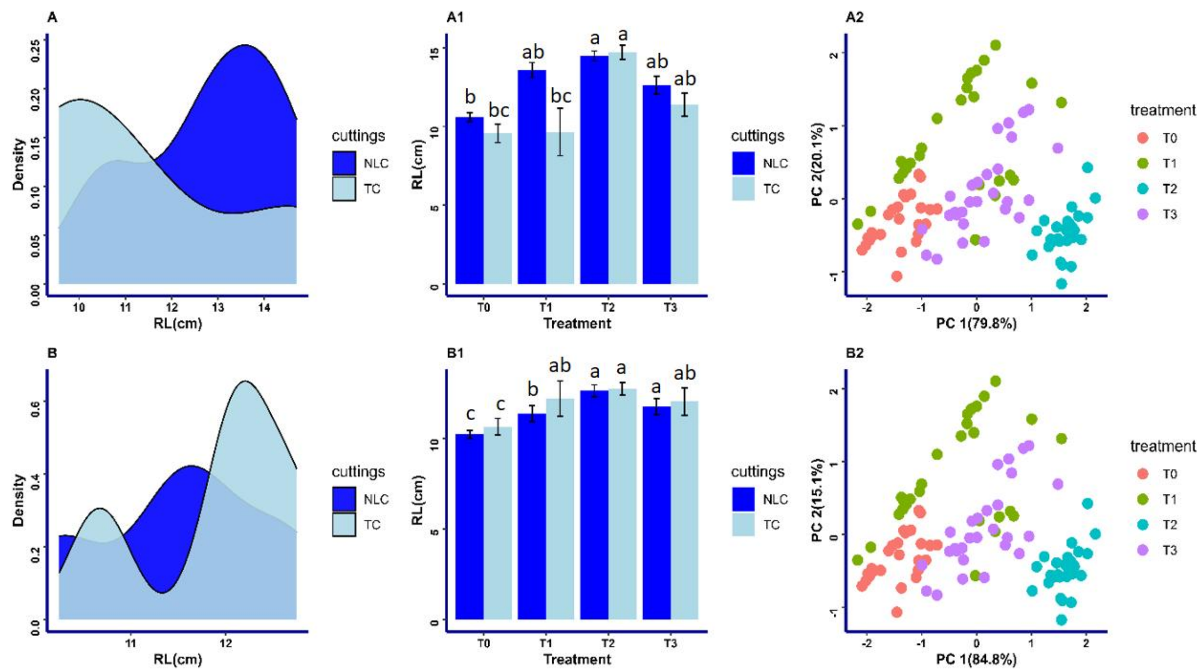

Figure 3. Comparative analysis of root length (cm) development from cuttings of nodal cuttings (NLC) and tip cuttings (TC) of *D. longispathus* (DL) and *B. tulda* (BT) developed after four weeks. A) Density plot: displays root development in *D. longispathus of* cuttings. A1) Bar plot: mean ± sd of roots in *D. longispathus* of cuttings. A2) PCA: Principal component analysis shows distribution of root length with PC1 (79.8%) and PC2 (20.1%). B) Density plot: compares root development i*n B. tulda of* cuttings. B1) Bar plot: mean ±sd of roots length in *B. tulda* of cuttings. B2) PCA: Illustrates variability in root numbers with two principal components having PC 1(84.8%) and PC 2(15.1%).

The principal component analysis (PCA) plots (A2 and B2) demonstrate distinct clustering for each treatment group, with minimal overlap, indicating that the treatments lead to significant differences in root growth characteristics. The first two principal components account for most of the variance, underscoring the effectiveness of the treatments. Overall, the results suggest that treated cuttings (TC) not only exhibit better root growth but also respond more positively to treatments, as reflected in both the density and PCA analyses, thereby confirming the beneficial effects of the treatments on root development.

**3.3. Number of roots per species**

The investigation delved into the propagation dynamics of *D. longispathus* and *B. tulda*, two eminent taxa frequently encountered in botanical discourse (Figure 4). We scrutinized the influence of distinct treatments of IBA, namely nodal leaf cuttings (NLC) and tip cuttings (TC), on root development metrics. The results delineate a discernible pattern: for *D. longispathus*, NLC yielded a mean root number of 2.64 (± 0.10), while TC exhibited a comparable mean root number of 3.4. Conversely, in the case of *B. tulda*, NLC manifested a mean root number of 5.95 (± 0.33), whereas TC showcased a notably higher mean root number of 8.43 (± 1.80). These findings underscore the species-specific responses to different propagation techniques, offering valuable insights for tip cuttings optimizing cultivation strategies and fostering the conservation endeavors of both species.

### 3.4. Root length of per species

Figure 5 illustrates the intriguing ways in which different cutting techniques and treatment levels affect root growth in two types of bamboo, *D. longispathus* and *B. tulda*. In *D. longispathus*, nodal leaf cuttings (NLC) generally show superior results compared to tip cuttings (TC), especially in treatment T2 where NLC reached an impressive root length of 14.48 cm. Interestingly, this species also exhibits significant variability with tip cuttings—treatment T1, for instance, showed a high standard deviation of 1.50 cm, pointing to unpredictable outcomes.

Meanwhile, *B. tulda* paints a different picture, where the distinction between the effectiveness of NLC and TC isn't as clear-cut. Both types of cuttings peaked in treatment T2, with root lengths very close to each other, suggesting a similar level of adaptability to the treatment conditions.

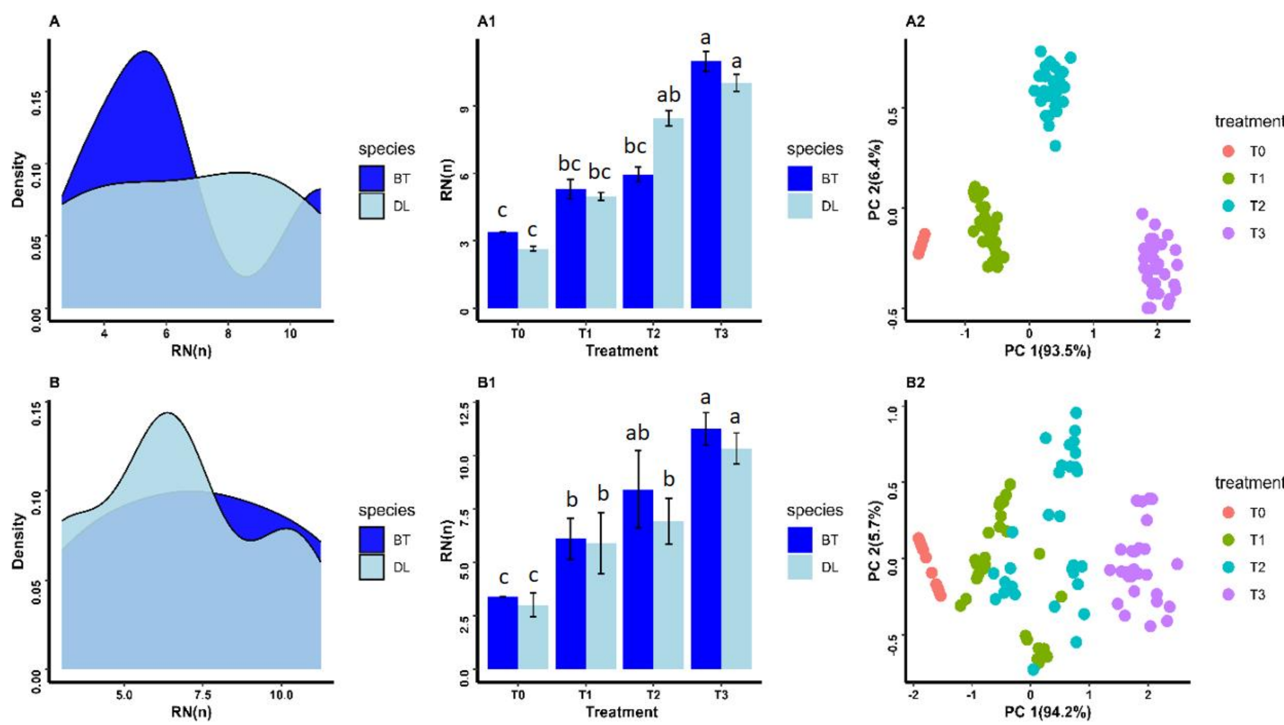


Figure 4. Comparative analysis of root number (n) development from cuttings of *D. longispathus* (DL) and *B. tulda* (BT) developed after four weeks. A) Density plot: displays root development in nodal leaf cuttings (NLC) of *D. longispathus* (DL) and *B. tulda* (BT). A1) Bar plot: mean ± sd of roots in NLC of *D. longispathus* (DL) and *B. tulda* (BT). A2) PCA: principal component analysis shows distribution of root number with PC1 (93.5%) and PC2 (6.4%). B) Density plot: compares root development in tip cuttings (TC) of *D. longispathus* (DL) and *B. tulda* (BT) after four weeks of cuttings. B1) Bar plot: mean ± sd of roots in TC of DL and BT. B2) PCA: shows two species with PC1 (94.2%) and PC2 (5.7%).

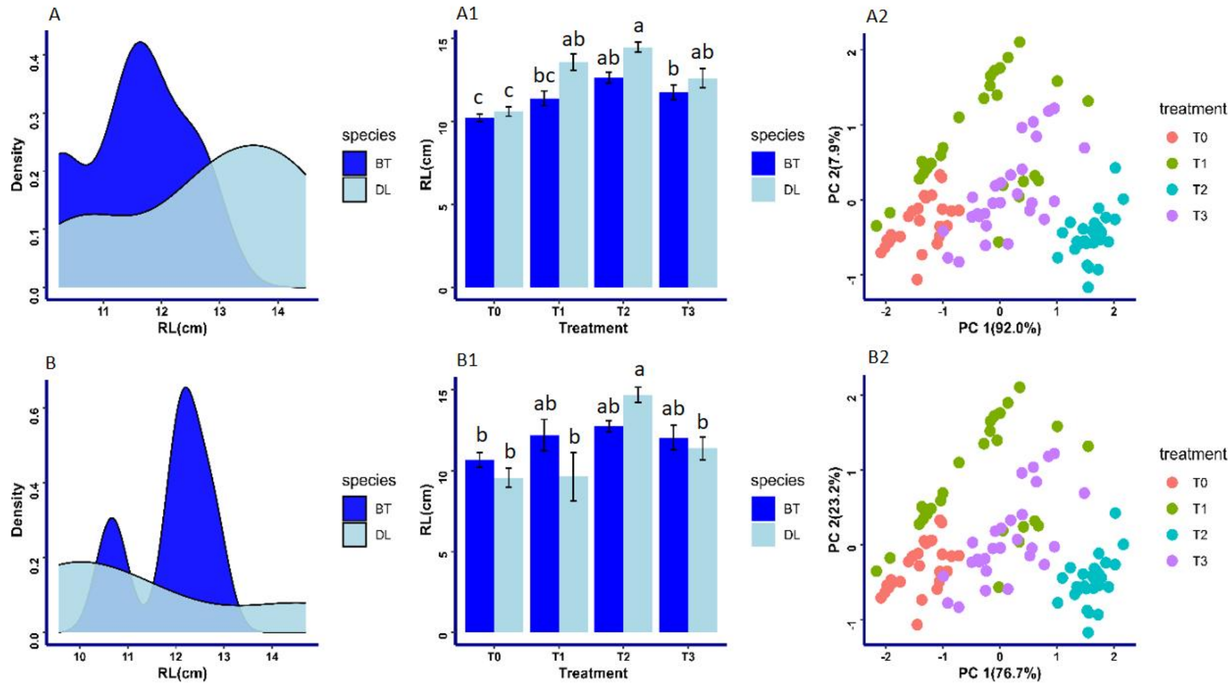


Figure 5. Comparative analysis of root length (cm) development from cuttings of *D. longispathus* (DL) and *B. tulda* (BT) developed after four weeks. A) Density plot: displays root development in nodal leaf cuttings (**NLC**) of *D. longispathus* (DL) and *B. tulda* (BT). A1) Bar plot: mean ± sd of roots in NLC of *D. longispathus* (DL) and *B. tulda* (BT). A2) PCA: principal component analysis shows distribution of root number with PC1 (92.0%) and PC2 (7.9%). B) Density plot: compares root development in tip cuttings (**TC**) of DL and BT after four weeks of cuttings. B1) Bar plot: mean ± sd of roots in TC of DL and BT. B2) PCA: Shows two species with PC1 (76.7%) and PC2 (23.2%).

However, the fluctuating standard deviations across various treatments indicate that there's still a degree of unpredictability, which could influence how these findings are applied in real-world agricultural or botanical settings. This in-depth analysis really highlights the need to consider specific bamboo species when choosing propagation methods. The varied growth responses suggest that a one-size-fits-all approach may not be effective and that adjustments might be necessary to tailor the growth conditions optimally for each species. These insights are incredibly valuable for horticulturists and agriculturists who are working to refine bamboo propagation techniques, whether for boosting nursery production or for ambitious reforestation projects. Such detailed understanding can pave the way for more effective cultivation strategies, enhancing both the efficiency and sustainability of bamboo growth efforts.

## 4. Discussion

The findings of this study reveal that using the plant growth regulator Indole-3-butyric acid (IBA) significantly enhances both root number and root length in *Dendrocalamus longispathus* and *Bambusa tulda.* These results not only confirm our own observations but also align closely with previous research, highlighting the effectiveness of IBA in promoting root development across different bamboo species. For example, Kaushal et al. (2011) found that *B. tulda* treated with 200 ppm of IBA for 8 hours developed 20 roots with a root length of 15.8 cm. This finding is consistent with our results, which also show a significant increase in both root number and length with the application of IBA. Another study by Ilorkar et al. (2021) reported a rooting percentage of 49.97% after 90 days using 2500 ppm of IBA. This suggests that higher concentrations of IBA, when applied over a longer period, can significantly enhance the rooting process. This likely occurs because IBA mimics the natural auxin hormone in plants, promoting cell elongation and division, which are critical processes in root formation.

Similarly, Sahoo et al. (2020) examined the effects of various IBA concentrations (0 ppm, 100 ppm, and 200 ppm) on *B. tulda*. Their results showed that even at lower concentrations, IBA positively influenced root development, with a root length of 10.37 cm and a root number of 7 at 200 ppm. This clear positive correlation between IBA concentration and root growth reinforces the idea that IBA is effective in stimulating root development (Chowdhury et al., 2024), even at relatively low dosages. One possible explanation for this is that lower concentrations of IBA may be sufficient to initiate the rooting process, especially in species that are more responsive to auxin-like compounds. The ability of lower concentrations to produce significant root growth suggests that the plant's own hormonal balance, combined with the applied IBA, creates an optimal environment for root induction.

A study on *Dendrocalamus asper* by Hossain et al. (2018) produced results similar to our observations with *D. longispathus*. Hossain and colleagues found that using 0.8% IBA resulted in the highest root number of 120 and a root length of 980 cm after 6 months. This demonstrates that higher concentrations of IBA, maintained over a longer period, can lead to substantial improvements in root growth. The prolonged exposure to higher concentrations of IBA might increase the duration of the hormone's effect on the root cells, thereby allowing more extensive root development. The extended period of root formation could be due to sustained cell division and differentiation driven by the higher auxin concentration, leading to the observed increase in root number and length.

Furthermore, for nodal and tip cuttings of *B.* vulgaris, the highest root number and root length were recorded after one month of treatment with 0.8% IBA. This consistency with our study highlights the effectiveness of IBA in promoting root development across various bamboo species and cutting types. The broad applicability of IBA as a plant growth regulator is further supported by these findings, indicating its potential utility in diverse horticultural and forestry practices (Imran et al., 2024). The consistent results across different species suggest that IBA's mechanism of action is robust and adaptable to various genetic backgrounds and environmental conditions.

The outcomes of our study are supported by these previous studies, demonstrating that IBA's role as a plant growth regulator is both significant and versatile. It enhances root growth across different species and under various experimental conditions, making IBA a valuable tool for horticulturists and foresters aiming to improve root development in bamboo species. This versatility could be attributed to IBA's ability to modulate a range of physiological processes involved in root initiation and development, such as gene expression related to root cell division, elongation, and differentiation. Bamboo is crucial for both ecological and economic reasons, playing an essential role in soil stabilization, carbon sequestration, and as a renewable resource for numerous industries. Therefore, improving root development through the application of IBA can contribute to more robust and sustainable bamboo cultivation practices (Imran et al., 2023). Enhanced root systems not only improve the plant's ability to anchor itself and access nutrients and water but also increase its resilience to environmental stressors, such as drought or poor soil conditions. This could lead to higher survival rates in reforestation and afforestation projects, making IBA-treated bamboo a valuable resource in these initiatives.

The application of IBA significantly promotes root growth in *Dendrocalamus longispathus* and *Bambusa tulda*, as evidenced by increased root numbers and lengths. This is consistent with findings from various studies, which also demonstrate IBA's efficacy across different bamboo species and conditions. The positive correlation between IBA concentration and root development underscores the importance of this plant growth regulator in enhancing bamboo cultivation. Given the ecological and economic benefits of bamboo, the use of IBA could be a key strategy in optimizing bamboo growth and productivity. By improving the efficiency of vegetative propagation through IBA application, it may be possible to meet the growing demand for bamboo in various industries, while also contributing to environmental conservation efforts.

## 5. Conclusion

In conclusion, our comprehensive analysis of the treatments and cuttings types pertaining to *D. longispathus* and *B. tulda* underscore significant differences in root number and length dynamics across treatments. For *D. longispathus*, nodal leaf cuttings (NLC) generally exhibited higher mean root lengths compared to tip cuttings (TC), particularly evident at earlier time points. However, for *B. tulda*, while initial differences were less pronounced, TC cuttings tended to display slightly higher mean root lengths over the course of the experiment. These findings highlight the species-specific responses to different propagation methods and emphasize the importance of considering such nuances in cultivation and conservation practices. Moreover, the variability observed across time points underscores the dynamic nature of root development processes and suggests the need for longitudinal studies to fully elucidate growth patterns. Overall, our findings contribute valuable insights to the field of botanical research, informing strategies for optimizing propagation techniques and fostering the sustainable management of these important bamboo species.

**Author Contribution:** Mohd Imran Hossain Chowdhury was responsible for the methodology , investigation , and writing the original draft. Chinmoy Das and Md. Faridul

Alam Shoron took care of the review , editing , visualization , formal analysis , and data curation. The conceptualization, methodology , project administration , resources , formal analysis , writing (review and editing) , and supervision were managed by Mohd Imran Hossain Chowdhury , Mohammad Siddiqur Rahman. All the authors reviewed and approved the final manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Availability of Data and Materials:** Data will be available on a formal request from the corresponding authors.

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