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Rooting of *Eucalyptus* Cuttings with IBA

Original Articles

Improving Clonal Propagation of *Eucalyptus grandis x urophylla* with Indole-3-Butyric Acid

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Abstract

The *Eucalyptus grandis* × *E. urophylla* hybrid is characterized by its rapid growth and high productivity, which has led to an increasing demand for its propagation in nurseries. To preserve these desirable traits, it is essential to establish clonal gardens and apply effective clonal propagation methods. With the aim of advancing knowledge on asexual propagation techniques, this study evaluates the influence of indole-3-butyric acid (IBA) and a commercial formulation combining IBA and naphthaleneacetic acid (NAA) on the rooting and root development of cuttings of this hybrid. To achieve this, cuttings were collected from a clonal garden and subjected to five rooting treatments: T0 (control) with 0 ppm IBA; T1 with 1,000 ppm IBA; T2 with 1,500 ppm IBA; T3 with 2,000 ppm IBA; and T4, a commercial formulation containing 4,000 ppm NAA and 1,000 ppm IBA. The experiment was conducted under a completely randomized design (CRD) with 90 experimental units. After treatment application, the cuttings were placed in a greenhouse for 30 days. At the end of this period, rooting percentage, mortality rate, number of roots, root length, and root dry weight were assessed. The results indicated that T2 and T4 exhibited the best performance in terms of rooting and root development. Furthermore, comparison with other studies revealed that excessively high concentrations of IBA can exert toxic effects on cuttings. Overall, the study concludes that IBA, either alone or in combination with NAA, positively influences the rhizogenesis process of *Eucalyptus grandis* × *E. urophylla* cuttings, increasing rooting percentage by up to 62% compared to the control.

Keywords: asexual propagation, cloning; cuttings, forest nursery, rhizogenesis, rooting agents, root development.

Introduction

According to the *Global Forest Resources Assessment 2020* (FAO, 2021), there are 131 million hectares of commercial forest plantations (CFPs) worldwide, accounting for 3% of the total forest area. Although CFP area increased between 2010 and 2020, the annual expansion rate declined compared to previous decades. In the Americas, South America holds 20.1 million hectares, and North and Central America, along with the Caribbean, 15.2 million hectares—reflecting a slowdown influenced primarily by the United States, Brazil, and other countries such as Chile, Colombia, Peru, and Uruguay.

In Peru, *Forest and Wildlife Law N° 29763* define forest plantations as human-created ecosystems where one or more forest species - either native or introduced - are established to produce timber forest products (TFP), non-timber forest products (NTFP), or provide ecosystem services, or a combination of these (Servicio Nacional Forestal y de Fauna Silvestre, 2015). Beyond supplying raw materials and goods, CFPs contribute to reducing pressure on timber extraction from natural forests (Pirard et al., 2016), mitigating climate change through carbon sequestration and storage (Osuri et al., 2020), providing provisioning and supporting ecosystem services (Zeng et al., 2021) and enhancing biodiversity conservation (Silva et al., 2019).

Peru currently has 1,088,470 hectares of CFPs (FAO, 2021). However, these figures may be imprecise, as they are based on the number of seedlings distributed, without differentiating land tenure (state, communal, or private). Despite progress in establishment, plantation productivity remains low due to poor silvicultural practices, limited genetic improvement, insufficient soil management, and the lack of site selection criteria (Guariguata et al., 2017). To advance the development of CFPs in Peru, a comprehensive forest policy is essential, focusing on strategic economic corridors, securing legal tenure for public lands, and aligning species selection with market demand (Servicio Nacional Forestal y Fauna Silvestre, 2021). Research, innovation, and technological development are also key to enhancing CFP sustainability.

One significant initiative has been the introduction of the *Eucalyptus grandis × E. urophylla* hybrid in Amazonian regions such as Ucayali, Huánuco, Junín, Pasco, and San Martín (Servicio Nacional Forestal y Fauna Silvestre, 2021). This hybrid is widely used in clonal propagation within modern nurseries due to its fast growth and high productivity. Vegetative propagation enables the preservation of genetic quality, reduces dependency on seeds of unknown origin, and ensures continuous year-round clonal production (Cachique et al., 2011). These techniques are fundamental for forest genetic improvement and for boosting CFP productivity in the Peruvian Amazon by promoting uniform wood characteristics, high-quality stem formation, greater resistance to biotic and abiotic stressors, faster growth rates, and more efficient management (Abedini, 2005; Navarrete-Luna & Vargas-Hernández, 2005).

Despite the high potential of these techniques, asexual propagation of commercial species remains underexplored in Peru, particularly for the *Eucalyptus grandis × E. urophylla* hybrid. To promote environmentally and economically sustainable clonal forestry programs that contribute to the livelihoods of Amazonian communities, this study evaluates the effect of indole-3-butyric acid (IBA) and a commercial formulation combining IBA and naphthaleneacetic acid (NAA) on the rooting and root development of *Eucalyptus grandis × E. urophylla cuttings*. The primary objective is to determine the optimal auxin treatment for enhancing rooting percentage and root system development to support the sustainable propagation of this hybrid in the Peruvian forest sector.

Methods

Study Area. This study was conducted in the greenhouse of TEC FOREST S.A.C., located in the district of San Martín de Pangoa, province of Satipo, Junín region, Peru. The site is geographically positioned at 11° 25' 24.72" S, 74° 29' 17.20" W (-11.42353 S, -74.4881 W) at an altitude of 792 meters above sea level (m a.s.l.) (Figure 1). The mean temperature in the study area ranges between 19°C and 35°C. The precipitation regime is seasonal, with a rainy season from October to April and a dry season from May to September (Servicio Nacional de Meteorología e Hidrología del Perú, 2024). Within the greenhouse, temperatures ranged from 25°C to 30°C, and the relative humidity varied between 60% and 70%.

Plant Material. The plant material used consisted of cuttings from mother plants of the hybrid clone *Eucalyptus grandis × E. urophylla* (code TF-001), originally imported from Brazil. The mother plants were seven months old, with an average height of 1.50–1.80 m, ensuring the collection of juvenile propagules representative of an early ontogenetic stage. Their average diameter at breast height (DBH) was 10 cm. This clone is part of a group of eight genotypes selected through a technological innovation program in forest plantations and was initially established in a clonal trial plot in Oxapampa to evaluate its adaptation under Peruvian conditions. Successive clonal propagations were carried out in this plot to ensure the production of high-quality vegetative material, which was later used in experimental trials across different sites in the Peruvian central jungle, ranging from 300 m a.s.l. in Iscozacín to 3,000 m a.s.l. in Palca, Tarma. The production of these clones was managed in the clonal garden of TEC FOREST S.A.C., using plant material sourced from the district of Palca, province of Tarma, department of Junín (Machacuay &

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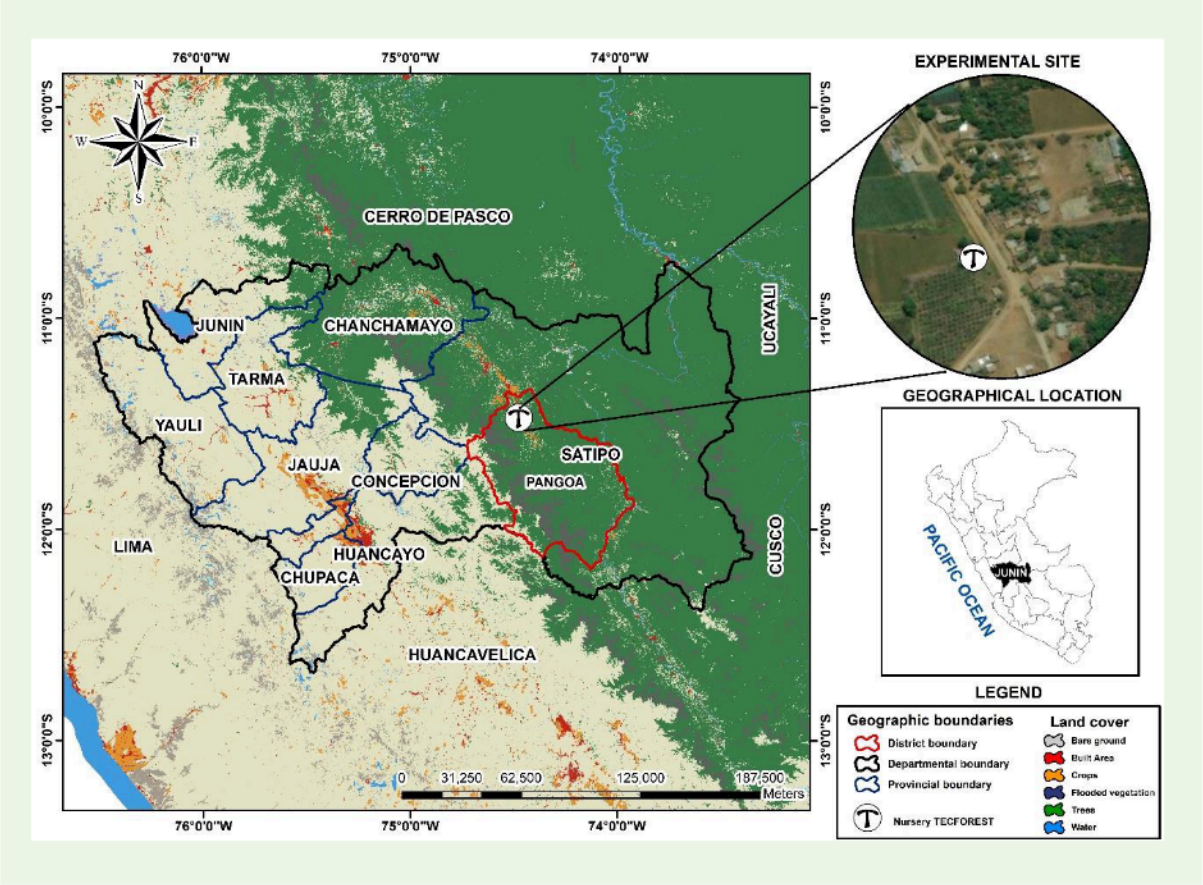

Figure 1. Geographical location of the experimental research area of the clonal propagation study of *Eucalyptus grandis* × *E. urophylla* with indole-3-butyric acid. Forest nursery of TEC FOREST S.A.C., San Martín de Pangoa district, Junín department, Peruvian central jungle.

Llancari, 2020). The collected cuttings consisted of juvenile orthotropic shoots measuring 8–10 cm in length and 3–4 mm in basal diameter, obtained from mother plants maintained in this clonal garden.

Experimental stage. The experiment began in January 2021 with the fertilization of the clonal garden at TEC FOREST S.A.C. using a 20-20-20 NPK formula to stimulate new shoot development in the *Eucalyptus grandis* × *E. urophylla* TF-001 clone and improve rooting potential. Two weeks later, apical cuttings were collected using sterilized pruning shears, making a beveled cut below a node or bud, then immediately placed in water to maintain turgor and transported to the nursery.

At the nursery, 50% of the basal leaf area was removed, and the cuttings were disinfected by immersing them in a 0.1% fungicidal solution for 10 seconds (Swarts et al., 2018). Five treatments were applied: T0 (control, 0 ppm IBA), T1 (1,000 ppm IBA), T2 (1,500 ppm IBA), T3 (2,000 ppm IBA), and T4 (commercial mix with 4,000 ppm NAA + 1,000 ppm IBA). For T1–T3, IBA was dissolved in distilled water and applied by immersing the base of each cutting for 60 seconds. For T4, the powdered hormone mix was applied directly to the base, and excess was removed before planting.

Cuttings were inserted 2 cm deep into T-51 tubes (51 cm^3 volume) filled with a moist substrate of composted pine bark and expanded vermiculite. The substrate was manually compacted to eliminate air pockets and ensure proper base-substrate contact. Tubes were then placed in trays and arranged inside a greenhouse.

Traits evaluation. At 30 days after cutting establishment, the following variables were assessed: rooting percentage (%), mortality percentage (%), number of roots (units), dry root biomass (mg), and length of the longest root. Rooting and mortality percentages were calculated based on the total number of cuttings per treatment. Root development

was evaluated by counting the number of roots per rooted cutting and measuring the longest root with a millimeter ruler. Dry root biomass was determined by oven-drying the roots at 70 °C until constant weight and weighing them on an analytical balance.

Experimental design and statistical analysis. The experiment was conducted under a completely randomized design (CRD). Each experimental unit consisted of a single *Eucalyptus grandis* × *E. urophylla* cutting. For each treatment, 90 cuttings were used and individually randomized within the greenhouse rooting benches, ensuring no grouping or blocking structure. Thus, each replicate corresponded to one cutting, and the total number of experimental units was 450. The treatments corresponded to different concentrations of IBA. Rooting variables (rooted, callused, and dead) were analyzed using generalized linear models (GLMs) with a binomial distribution, while variables related to root development were subjected to analysis of variance (ANOVA). Mean comparisons were performed using Fisher's LSD test (α = 0.05) with the *agricolae* package (Mendiburu, 2023). Additionally, a principal component analysis (PCA) was conducted using the *FactoMineR* package (Husson et al., 2024) to explore the multivariate structure of the variables. All analyses were performed using R version 4.4.2 (R Core Team, 2024).

Results

Rooting. Significance differences were observed in the rooted cuttings variable between the control treatment and those where rooting agents were applied ($P < 0.001$; Figure 2). The commercial treatment (4,000 ppm NAA + 1,000 ppm IBA) showed the highest rooting percentage (69%), followed by the 1,500 ppm IBA treatment, which reached 44%. Both treatments significantly outperformed the control, which recorded only 7% rooting. Among the IBA treatments,

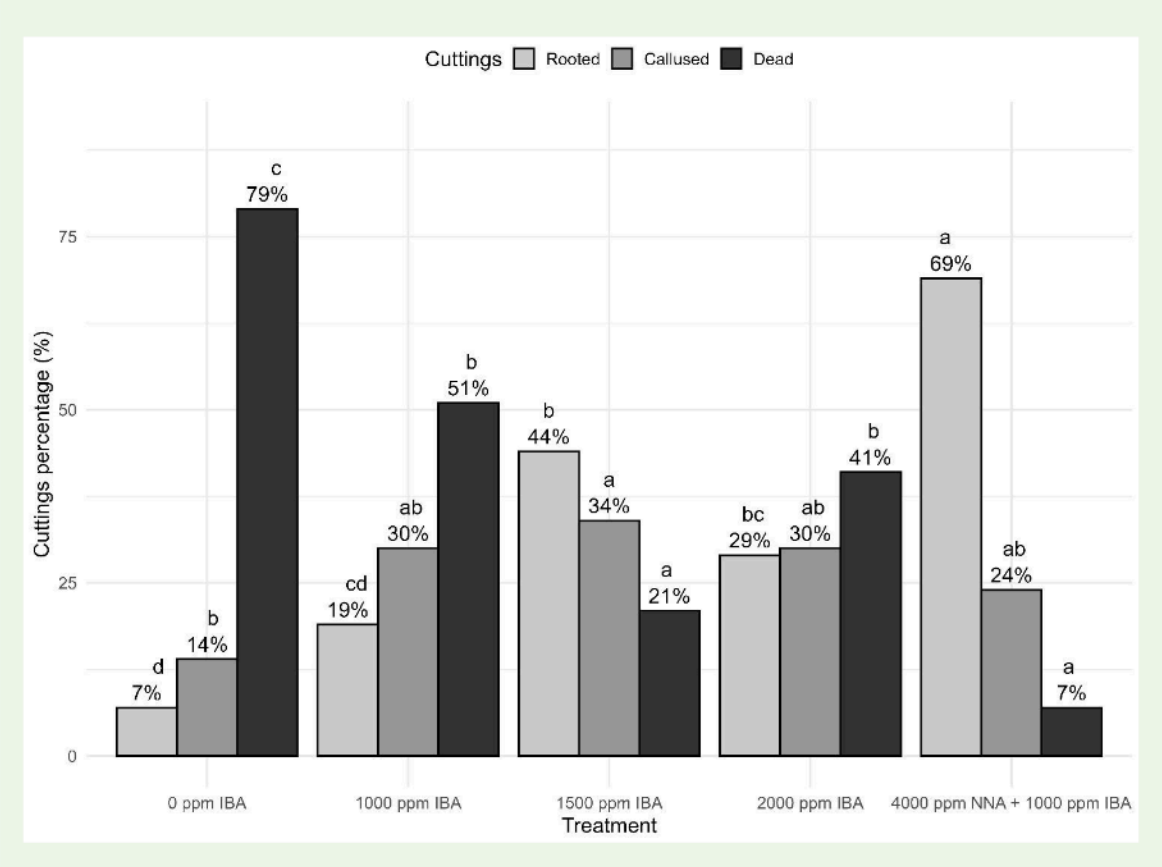

Figure 2. Status of *E. grandis* × *E. urophylla* cutting in response to the application of different concentrations of indole-3-butyric acid (IBA) at the end of the rooting period. Results of Fisher's least significant difference (LSD) test. Groups are assigned according to mean difference probability (α = 0.05). Treatments with the same letter within each cutting status (rooted, callused, or dead) are not significantly different (p-value < 0.05, n = 450).

1,500 ppm exhibited a higher rooting percentage than 1,000 ppm (19%) and 2,000 ppm (29%).

For the callused cuttings variable, significant differences were observed between the control treatment (0 ppm IBA) and the treatments with different IBA concentrations ($P < 0.01$). The control treatment had the lowest percentage of callus formation (14%), whereas the IBA-treated cuttings averaged 30%.

Regarding mortality, an inverse trend was observed compared to the previous traits with, significant differences among treatments ($P < 0.001$). The control treatment showed the highest mortality rate (79%), while the lowest mortality rates were recorded in the commercial treatment (4,000 ppm NAA + 1,000 ppm IBA) with 7% and the 1,500 ppm IBA treatment (21%). Among the IBA treatments, 1,500 ppm exhibited the lowest mortality rate, followed by 2,000 ppm (41%) and 1,000 ppm (51%). These results highlight the commercial treatment as the most effective in enhancing rooting and reducing mortality, followed by the 1,500 ppm IBA treatment.

Root system development. The statistical analysis of root number using ANOVA ($\alpha = 0.05$) revealed no significant differences among treatments ($P = 0.92$). However, trends indicated improved root development in treatments with IBA application, with up to six roots per cutting, compared to the control (0 ppm IBA), which showed a maximum of three roots. The commercial formulation (4,000 ppm NAA + 1,000 ppm IBA) recorded the highest mean (2.27 roots per cutting), while the control treatment had the lowest (1.83 roots per cutting). These results suggest a potential positive effect of the NAA + IBA combination on root initiation, despite the lack of statistically significant differences (Table 1).

The ANOVA analysis of root length ($\alpha = 0.05$) revealed no statistically significant differences among treatments ($P = 0.11$); however, notable differences in mean values were observed. The control (0 ppm IBA) and 1,000 ppm IBA treatments recorded the shortest average root lengths (3.43 cm and 4.10 cm, respectively), while the commercial formulation (4,000 ppm NAA + 1,000 ppm IBA) exhibited the highest average (6.92 cm). Maximum root length reached 19.00 cm in hormone-treated cuttings, compared to 5.00 cm in the control. Most roots, however, ranged between 1.00 and 10.00 cm, indicating a tendency toward short root formation under the given conditions (Figure 3A).

In contrast, the analysis of root dry weight showed highly significant differences among treatments ($P = 0.001$). The control, 1,000 ppm IBA, and 2,000 ppm IBA treatments showed the lowest mean values (7.36 mg, 7.10 mg, and 7.98 mg, respectively). Conversely, cuttings treated with 1,500 ppm IBA and the commercial formulation exhibited higher root dry biomass (12.12 mg and 13.98 mg, respectively), highlighting the positive effect of these doses on root system development (Figure 3B).

Principal component analysis (PCA) was conducted to evaluate the interaction among variables, with the first two components explaining 96.13% of the total variance—Dimension 1 accounting for 82.01% and Dimension 2 for 14.12% (Figure 4A). The PCA vectors revealed a strong positive correlation among root number, root length, and root dry weight. In relation to treatments, both 4,000 ppm NAA + 1,000 ppm IBA and 1,500 ppm IBA were closely aligned with the vectors for root length and dry weight, indicating that these doses effectively enhanced these traits. For root number, the highest values were observed in cuttings treated with 2,000 ppm IBA. In contrast, the control (0 ppm IBA) and the 1,000 ppm IBA treatments showed a negative association with all root development variables, suggesting limited effectiveness during the rhizogenesis process of *Eucalyptus grandis* × *E. urophylla* cuttings (Figure 4B).

Table 1. Results of Fisher's least significant difference (LSD) test for indole-3-butyric acid (IBA) dosage based on the number of roots variable. Groups are assigned according to mean difference probability ($\alpha = 0.05$). Treatments with the same letter are not significantly different (p-value < 0.05, n = 151).

Treatments	Mean	Standard desviation	Minimum	Maximum	Significance
T0	1.833	0.753	1	3	a
T1	2.176	1.131	1	4	a
T2	2.200	1.244	1	6	a
T3	2.192	1.167	1	5	a
T4	2.274	1.089	1	5	a

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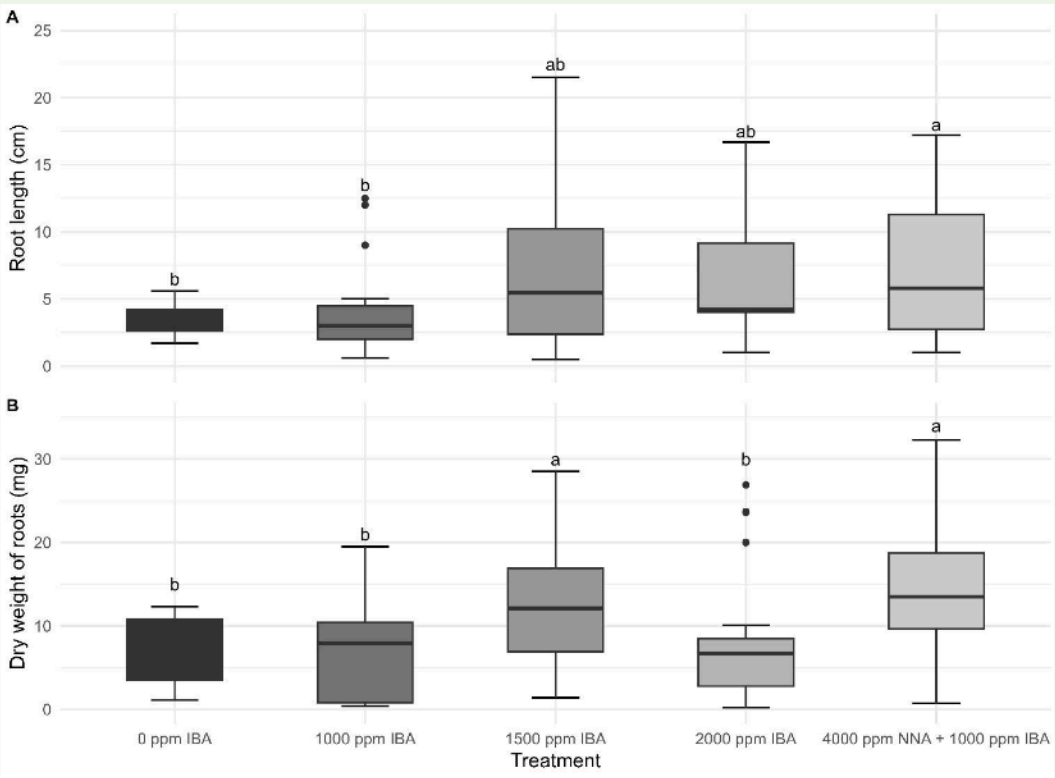

Figure 3. Root development of *Eucalyptus grandis* × *E. urophylla* cuttings for each applied dose of indole-3-butyric acid (IBA) at the end of the rhizogenesis process. (A) Root length (cm) (B) Root dry weight (mg). Results of Fisher's least significant difference (LSD) test. Groups are assigned according to mean difference probability (α = 0.05). Treatments with the same letter are not significantly different (p-value < 0.05, n = 151).

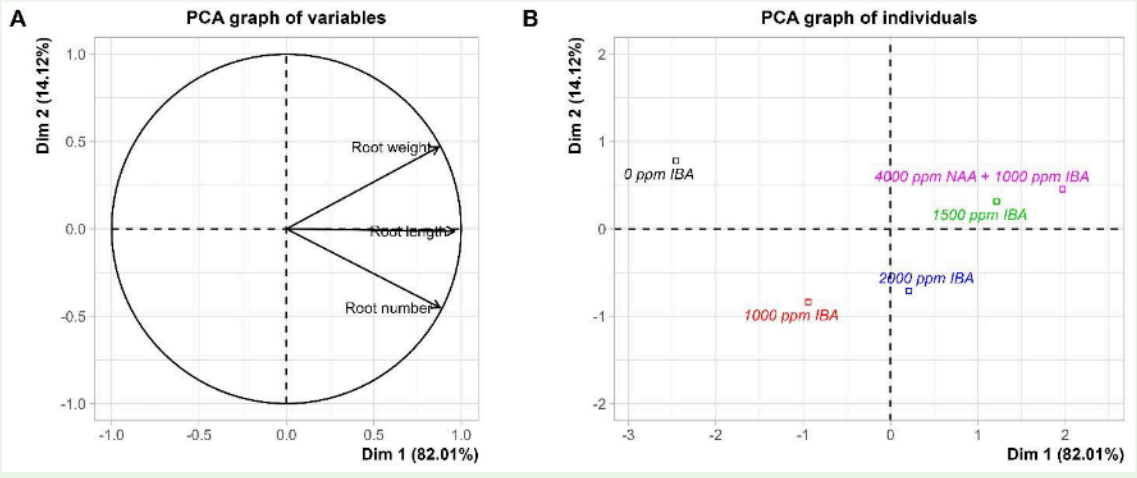

Figure 4. Principal Component Analysis (PCA) of root development variables based on the application of different doses of indole-3-butyric acid (IBA). (A) Evaluation of variables to determine the effect of the growth regulator on root system characteristics. (B) Treatment grouping based on IBA concentrations to identify the optimal dose for maximizing root development. The analysis was conducted on 151 observations (n = 151).

Discussion

Significant differences observed with the commercial treatment (4,000 ppm NAA + 1,000 ppm IBA) support previous findings indicating that, despite NAA's slightly higher toxicity compared to IBA, their combined application is more effective than using either hormone alone. This synergy promotes higher rooting success and greater root biomass (Peña-Baracaldo et al., 2018). Additionally, the superior performance of the 1,500 ppm IBA treatment over the 1,000 ppm and 2,000 ppm concentrations is consistent with the results of Carranza Patiño et al. (2022), highlighting 1,500 ppm IBA as a potentially optimal dose for enhancing the vegetative propagation of this hybrid species.

Rooting efficiency generally increases with auxin concentration up to an optimal threshold, beyond which higher doses become toxic and reduce rooting capacity (Nourissier & Monteuuis, 2008). This effect is attributed to excessive auxin stimulating ethylene synthesis, which inhibits root formation, while low concentrations may only induce callus formation without rooting (Alcantara-Cortes et al., 2019). In this study, 1,000 ppm auxin was insufficient to stimulate rooting, whereas 2,000 ppm appeared to exert a toxic effect, limiting root development. These results underscore the need to determine an optimal auxin dose that maximizes rooting without inducing toxicity. The rooting percentages observed were similar to those reported by Brondani et al. (2010), Nourissier & Monteuuis (2008), and Ayala et al. (2020) for hybrids such as *E. benthamii × E. dunnii, E. grandis × E. urophylla, E. grandis × E. tereticornis, and E. grandis × E. camaldulensis*. These studies emphasize that rooting capacity varies significantly with clonal genotype, which may explain the low rooting success observed in this trial.

The rooting percentages in this study were lower than those reported by Titon et al. (2003) and Bueno et al. (2008) in experiments with mini-cuttings of *E. grandis* and *E. grandis × E. urophylla*, respectively. This discrepancy may be attributed to the mini-cutting technique, which uses mother plants propagated from cuttings and allows for better nutrient control. The nutritional status of the donor plant is crucial for root formation, as auxin and carbohydrate concentrations directly influence the rooting process (Oliva-Cruz et al., 2005; Bautista-Ojeda et al., 2022). This observation aligns with findings from Gallo et al. (2017), who worked with clones of *E. grandis × E. urophylla* and *E. urophylla × E. globulus.* Their study concluded that endogenous auxin content in shoots directly impacts rhizogenesis, emphasizing the importance of proper genetic material management and optimal mother plant conditions.

The results also indicated that IBA application at appropriate concentrations significantly reduced mortality. This aligns with studies by Bautista-Ojeda et al. (2022) and Carranza Patiño et al. (2022), which demonstrated that auxin application at optimal doses enhances cutting survival during rhizogenesis. The mortality rates observed in this study were similar to those reported by Brondani et al. (2010) and Rivera Melo et al. (2021), who tested different IBA concentrations for rooting induction in *Eucalyptus benthamii × E. dunnii* and *Pinus hartwegii,* respectively. These studies found that higher IBA concentrations increased mortality rates, reinforcing the hypothesis that excessive IBA can cause phytotoxicity, negatively affecting cutting viability.

However, the mortality rates in this study slightly differed from those reported by Muñoz (2018), who recorded 20- 30% mortality in *Eucalyptus grandis × E. urophylla* cuttings. The lower mortality rates observed by Muñoz may be attributed to the use of the mini-cutting technique, which provides more controlled growth conditions. This technique allows for the development of a more efficient root system, improving water and nutrient uptake and ultimately enhancing survival rates. Conversely, Felice et al. (2024) found no significant impact of IBA concentration on the mortality of apical mini-cuttings of *E. camaldulensis*. These authors suggested that factors such as plant maturity, genetic variability, and environmental conditions play a crucial role in rooting response and cutting survival.

Differences were observed between IBA-treated cuttings and the control (0 ppm IBA) regarding root number. This trend has been reported in various species, where higher IBA doses generally increase the average number of roots per cutting (Vásquez Inuma et al., 2018). This effect may be attributed to IBA's role in redistributing metabolites from leaves and stems to the cutting base, promoting rhizogenesis. Additionally, carbohydrates, which play a key role in root formation, contribute to increased root production (Alcantara-Cortes et al., 2019). However, statistical analysis showed no significant differences among treatments. Similar results were reported by Borges et al. (2011) in their study on mini-cuttings of *E. urophylla × E. globulus* and *E. grandis × E. globulus.* In contrast, Basauri Torres et al. (2019) demonstrated that IBA powder application significantly increased root number in *Guazuma crinita* mini-cuttings. This suggests that IBA's effect may vary across species and clones, potentially depending on its ability to mobilize metabolites to the cutting base.

The root length values obtained in this study are consistent with those reported by Muñoz (2018) and Carranza Patiño et al. (2022), who documented averages of 8.10 cm and 9.60 cm, respectively, for *Eucalyptus grandis × urophylla*, without detecting statistically significant differences among IBA doses. In contrast, although no significant differences were found among IBA concentrations in our study, clear differences were observed in the mean values when comparing the commercial formulation (4,000 ppm NAA +

1,000 ppm IBA) with the control and 1,000 ppm IBA treatments, with the commercial treatment exhibiting the highest mean root length. This variation may be associated with environmental and nutritional factors—such as irradiation, temperature, and nitrogen availability—that influence auxin activity, transport, and interaction with carbohydrates (De Almeida et al., 2017; Vilasboa et al., 2019; More et al., 2021). The synergistic action of NAA and IBA, combined with these conditions, likely explains the superior mean values observed in the present study.

Regarding root dry weight, statistically significant differences were detected between the control (0 ppm IBA), 1,000 ppm IBA, and 2,000 ppm IBA treatments compared with 1,500 ppm IBA and the commercial formulation. These findings align with Gallo et al. (2017), who reported decreasing root dry weight as IBA concentration increased in micropropagated clones of *E. grandis* × *urophylla* and E. *urophylla* × *globulus*. These differences may be attributed to the role of auxins such as IBA in promoting cell division and elongation, both essential for root development. Very low concentrations may be insufficient to induce effective rooting, whereas excessively high doses may cause phytotoxicity, reducing root formation and growth. Additionally, elevated concentrations of IBA may trigger negative interactions with endogenous cytokinins, disrupting the hormonal balance required for rhizogenesis and inhibiting cell elongation and differentiation in root tissues (Druege et al., 2019).

The high variability observed in root length and root dry weight, particularly in treatments T2 and T4, aligns with previous reports on clonal propagation systems. Auxin-induced rhizogenesis frequently produces heterogeneous responses among cuttings, even when derived from uniform clonal gardens, due to differences in physiological status, ontogenetic gradients of juvenility, carbohydrate allocation, and intrinsic genotypic regulation of auxin transport and sensitivity (Díaz-Sala, 2020). Such variability has been documented in *Eucalyptus* and other woody species, and is considered a normal feature of vegetative propagation under greenhouse conditions. Therefore, the observed dispersion in certain treatments reflects biological variability inherent to auxin-mediated rooting processes.

The findings of this study underscore the importance of selecting an appropriate IBA concentration to optimize root induction while avoiding phytotoxic effects. The commercial treatment (4,000 ppm NAA + 1,000 ppm IBA) exhibited the highest efficiency, reinforcing its potential for enhancing the vegetative propagation of *Eucalyptus grandis* × *E. urophylla* in commercial forestry programs in the Central Jungle of Peru. The results highlight that rooting responses are influenced by auxin concentration and environmental conditions. Although this study evaluated a single hybrid genotype, previous literature indicates that clonal variability can affect rooting capacity. Therefore, future studies should include multiple genotypes to better understand how genetic background may interact with auxin treatments to influence clonal propagation efficiency.

Conclusions

The application of auxins improved the rooting and root system development of *Eucalyptus grandis* × *E. urophylla cuttings*. The treatment with 1,500 ppm IBA achieved a rooting percentage of 44%, while the commercial formulation containing 4,000 ppm NAA + 1,000 ppm IBA reached 69%, representing increases of 37% and 62%, respectively, compared to the control (7%). Root length also improved notably, with the commercial formulation recording an average of 6.92 cm, compared to 3.43 cm in the control. In terms of root dry biomass, both the 1,500 ppm IBA treatment (12.12 mg) and the commercial formulation (13.98 mg) showed improvements compared to the control (7.36 mg). However, the number of roots per cutting did not differ statistically among treatments ($P = 0.92$), although trends suggested greater root initiation under hormone application. Overall, these results demonstrate that the combined use of IBA and NAA enhances rooting efficiency, root elongation, and biomass accumulation more effectively than IBA alone, confirming the potential of auxin-based formulations to optimize clonal propagation of *Eucalyptus grandis* × *E. urophylla* in the Peruvian central jungle.

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Author contributions

Conceptualization, S.C-N., G.M., and J-E.C.; methodology, S.C-N.; formal analysis, S.C-N. and F.L-I.; investigation, S.C-N.; data curation, S.C-N. and F.L-I.; writing—original draft preparation, G.M., J-E.C., F.L-I., and S.C-N.; writing—review and editing, F.L-I., G.M., and J-E.C.; visualization, S.C-N. All authors have read and agreed to the published version of the manuscript.

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