



## Efficiency of air layering in three tropical pine taxa with different auxin concentrations

### Eficiencia de acodo aéreo en tres taxones de pinos tropicales con diferentes concentraciones de auxina

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#### Abstract

Air layering is an effective tool for cloning adult trees that are difficult to propagate, but roots are not always successfully generated; therefore, it is necessary to apply hormones exogenously and define their dosage. The objective of this study was to evaluate the effect of Indole-3-butyric acid (IBA) concentration on the rooting of layers in three taxa of tropical pines. Twenty trees were selected in a provenance trial, and the experiment was established in a completely randomized design with treatments arranged factorially: (1) taxon factor: *Pinus caribaea* and the hybrids *P. caribaea* × *P. tecunumanii* and *P. elliottii* × *P. caribaea*; (2) IBA concentration factor: 10, 20, 30, and 40 g kg<sup>-1</sup> of substrate. Survival (%), rooting (%), bud growth (cm), and root number were evaluated in the three taxa. The data were analyzed using Aligned Ranks Transformations. The two factors and their interaction showed no statistical differences ( $p < 0.05$ ) for any variable. The survival of the layered branch in the three taxa was >50%. The maximum rooting value and the number of roots generated were recorded in *P. caribaea* (31.25% and 0.78) and *P. caribaea* × *P. tecunumanii* (28.57% and 1.17). IBA concentrations of 10, 20, 30, and 40 g kg<sup>-1</sup> promoted 35, 25, 15, and 25% rooting of the layer. Propagation was achieved in all tested taxa, but other auxin concentrations are needed to increase the success of the layers.

**Keywords:** Auxin, adventitious rooting, hybrid, *Pinus caribaea* Morelet, *Pinus caribaea* × *Pinus tecunumanii*, *Pinus elliottii* × *Pinus caribaea*.

## Resumen

El acodo aéreo es una herramienta efectiva para clonar árboles adultos de difícil propagación, pero no siempre se logra generar raíces; por ello es necesario aplicar hormonas de forma exógena y definir su dosis. El objetivo del presente estudio fue evaluar el efecto de la concentración del ácido Indol-3-butírico (AIB) en el enraizamiento de acodos en tres taxones de pinos tropicales. Se seleccionaron 20 árboles en un ensayo de procedencias y el experimento se estableció en un diseño completamente al azar y los tratamientos en arreglo factorial: (1) factor taxón: *Pinus caribaea* y los híbridos *P. caribaea* × *P. tecunumanii* y *P. elliottii* × *P. caribaea*; (2) factor concentración de AIB: 10, 20, 30 y 40 g kg<sup>-1</sup> de sustrato. En los tres taxones se evaluó la supervivencia (%), el enraizamiento (%), el crecimiento de la yema (cm) y el número de raíces. Los datos se analizaron mediante Transformaciones de Rangos Alienados. Los dos factores y su interacción no presentaron diferencias estadísticas ( $p < 0.05$ ) para ninguna variable. La supervivencia de la rama acodada en los tres taxa fue >50 %. El valor máximo de enraizamiento y el número de raíces generadas se registró en *P. caribaea* (31.25 % y 0.78) y *P. caribaea* × *P. tecunumanii* (28.57 % y 1.17). Las concentraciones de AIB de 10, 20, 30 y 40 g kg<sup>-1</sup> promovieron 35, 25, 15 y 25 % de enraizamiento del acodo. Se logró la propagación en todos los taxones probados, pero se requieren otras concentraciones de auxina que aumenten el éxito de los acodos.

**Palabras clave:** Auxina, enraizamiento adventicio, híbrido, *Pinus caribaea* Morelet, *Pinus caribaea* × *Pinus tecunumanii*, *Pinus elliottii* × *Pinus caribaea*.

## Introduction

The *Pinus* L. genus, with around 120 species, has adapted to diverse environments (Gernandt & Pérez-de la Rosa, 2014). *Pinus caribaea* var. *hondurensis* (Sénécl.) W. H. Barrett & Golfari is a tropical conifer of global interest due to its fast growth and straight stem; it can reach 6 to 8 m high in three years and is used for the production of timber, paper and resin (Asociación Mexicana de Plantadores Forestales A. C. [Ameplanfor A. C.], 2016; Torres-Ávila et al., 2020). *P. caribaea* Morelet can hybridize through genetic exchange with other pine species (Central America and Mexico Coniferous Resources Cooperative [Camcore], 2009). The *P. elliottii* var. *elliottii* × *P. caribaea* var. *hondurensis* hybrid was established in Australia in 1955 and possesses favorable characteristics that have surpassed its parent species in growth, form and wind resistance (Nunes et al., 2018). In Mexico, thousands of hectares of this hybrid have been planted for timber and resin production (Cabrera-Ramírez et al., 2022). *Pinus caribaea* can also hybridize with *Pinus tecunumanii* F. Schwerdtf. ex Eguiluz &

J. P. Perry, resulting in individuals with improved form and faster growth. Furthermore, its productivity can be increased by planting it in appropriate ecological niches (Ameplanfor A. C., 2016).

In Mexico, provenance trials are being conducted with tropical pine species to select those best adapted for establishing plantations for resin extraction and timber commercialization (Puc-Kauil et al., 2024; Torres-Ávila et al., 2020). Due to the importance of this group of fast-growing pines, cloning protocols for phenotypically outstanding trees should be explored to allow for their conservation and propagation. Although various vegetative propagation techniques exist, the most favorable one for each species and purpose should be selected (Hartmann et al., 2014).

Rooting cuttings is not feasible in pine trees older than two years because the capacity for adventitious rooting decreases due to their physiological maturity and cellular specialization (Bautista-Ojeda et al., 2022). Furthermore, grafting techniques are difficult, mainly due to the lack of tropical pine rootstocks and the limited information on interspecific graft compatibility. Therefore, air layering can be a viable initial alternative for conserving and propagating, on a smaller scale, trees of interest from the *Pinus* genus older than five years and for obtaining clones adapted to the donor region (Vacek et al., 2012). However, the effectiveness of this technique can vary with the species (Stuepp et al., 2018), as described by Singh and Ansari (2014) for five tropical species.

Air layering consists of inducing adventitious roots on branches while they are still attached to the parent plant. Once rooted, the branch is cut from the tree and placed in substrate to continue its growth as an independent plant (Hartmann et al., 2014). Success depends on several factors such as the type and concentration of auxin, species, physiological and hormonal states, tree age, girdling location, branch position on the tree, environmental factors, date of execution, and substrate (Kamila & Panda, 2019).

Exogenous application of IBA to air layering promotes induction and accelerates the rate of adventitious rooting in many species, and also increases the number of roots (Noori et al., 2019). In air layers of *Syzygium samarangense* (Blume) Merr. & L. M.

Perry and *Psidium guajava* L., high concentrations of IBA favored rooting (Baghel et al., 2016; Khandaker et al., 2022); while in *Prosopis chilensis* (Molina) Stuntz and *Prunus lusitanica* subsp. *azorica* (Mouill.) Franco, a better response was obtained with low doses of auxin (Moreira et al., 2009; Tarnowski, 2021), and in *Brosimum alicastrum* Sw. and *Pinus greggii* Engelm. ex Parl. it was indistinct (Becerra & Plancarte, 1993; Ortigoza-García et al., 2025). In this context, it is important to evaluate the auxin concentration that guarantees the highest probability of rooting in air layers of tropical pine species. The objective of this study was to evaluate the rooting response, survival, number of roots, and bud growth of air layers as a result of IBA concentration, taxon, and their interaction. The hypothesis was that a specific IBA concentration will generate a better air layering response, and that taxa respond differently in the evaluated variables, suggesting that there may be an optimal IBA concentration for each taxon that favors the air layer's rooting performance.

## **Materials and Methods**

### **Provenance trial**

The layering was carried out on trees from an eight provenance trial of two pure species of tropical pines and two hybrids: (1) *P. caribaea* from two provenances (Australia and Venezuela), (2) *P. elliottii* Engelm. (Argentina), (3) The *P. elliottii* var. *elliottii* × *P. caribaea* var. *hondurensis* hybrid from four provenances (Australia, China and two provenances from Argentina) and (4) The *P. caribaea* var. *hondurensis* × *P. tecunumanii* hybrid (Australia). The trial was established in 2016 at the *Instituto Tecnológico Superior de Venustiano Carranza* (ITSVC, for its acronym in Spanish), in

*Venustiano Carranza* municipality, *Puebla* state ( $20^{\circ}28'19.6''$  N and  $97^{\circ}41'56.4''$  W), at 340 masl, in a randomized complete block design with six blocks, each containing 49 trees ( $7 \times 7$ ) from each provenance. The plantation covers 1.7 ha, with a spacing between plants of  $3.5 \times 2.3$  m. Climate is warm and humid (Cw) with  $24^{\circ}\text{C}$  average annual temperature and 1 450 mm average annual precipitation (Puc-Kauil et al., 2024; Servicio Meteorológico Nacional [SMN], 2024).

## Tree selection

In October 2023, diameter at breast height (*DBH*; cm) was measured using a diameter tape (model 283D/5m Forestry Supplier®), total height (*TH*; m), and clear stem height (*CSH*; m) were measured using an electronic clinometer (model HEC-MP Haglöf®, Sweden). Based on this data, 20 trees were selected that stood out in these variables: eight of *P. caribaea*, five of *P. elliottii*  $\times$  *P. caribaea*, and seven of *P. caribaea*  $\times$  *P. tecunumanii* (Table 1). *P. elliottii* was excluded from the air layering study because it exhibited the least growth in the trial (Puc-Kauil et al., 2024).

**Table 1.** Characteristics ( $\pm$ standard error) of eight-year-old trees of the tropical pine taxa selected for air layering.

<b>TAXON</b>	<b>TH (m)</b>	<b>DBH (cm)</b>	<b>CSH (m)</b>
<i>Pinus caribaea</i> Morelet	$11.0 \pm 0.5$	$17.1 \pm 1.1$	$2.3 \pm 0.1$
<i>Pinus elliottii</i> $\times$ <i>Pinus caribaea</i>	$9.1 \pm 0.9$	$17.1 \pm 0.6$	$2.3 \pm 0.1$
<i>Pinus caribaea</i> $\times$ <i>Pinus tecunumanii</i>	$13.0 \pm 0.3$	$18.9 \pm 0.9$	$3.4 \pm 0.3$

*TH* = Total height; *DBH* = Diameter at breast height at 1.30 m; *CSH* = Clean stem height.

## Substrate preparation

Four treatments were prepared by mixing 4 kg of peat moss (Kekkilä Professional®, Finland) with different amounts of powdered IBA (Radix 10,000®, Mexico) (10, 20, 30, and 40 g kg<sup>-1</sup> of substrate). Each mixture was stirred and moistened with 4 L of pure water until homogeneous. Then, 150 g substrate were placed in 20 transparent polyethylene bags measuring 8×15 cm per treatment, marked with a distinctive color and tied with raffia to prevent moisture loss (Figure 1A). The mixing of IBA into the substrate and its prior bagging was a variation of the air layering technique generally used to maintain the auxin in contact with the girdled area throughout the rooting process and facilitate its application in the canopy of the trees.



A = Peat moss substrate with different concentrations of IBA; B = Ascent of the tree; C = Branch girdling; D = Placement of bag with substrate; E = Air layering; F = Separation of the layer from the mother plant; G = Transplanting of the layer.

**Figure 1.** Air layering process of tropical pines.

## Air layering

In November 2023, air layers were performed on branches in the upper third of the tree canopy (Figure 1B), 40 cm from the branch tip towards the trunk. The branches for air layering had an average diameter of 4 cm at the base and  $1.4 \pm 0.08$  cm at the girdling point. A 2 cm wide ring-shaped cut was made with a knife to remove the bark and expose the xylem (Figure 1C). Bags containing substrate were opened in the middle and placed so that the substrate completely covered the girdled area; finally, they were tied with raffia to prevent moisture loss (Figure 1D and 1E). Four air layers (one for each IBA treatment) were performed on each tree of each of the study taxa at the same height.

## Separation of the air-layer from the mother plant

At three months, the air layers were cut with a curved saw, 5 cm from the air layer, to separate them from the mother plant (Figure 1F). The air layers were then lowered with a string and labeled for transport to the *ITSVC* nursery, located 120 m away. The air layers were submerged in water for 24 hours to rehydrate. The following day, the bag covering the air layer was removed, and rooting was assessed. The air layer was transplanted into a 4 L black polyethylene bag, which was filled with substrate until the neck of the air layer was covered (Figure 1G). The substrate consisted of perlite (10 %), organic matter from the topsoil (60 %), and soil from the "A" horizon (30 %), both from the local vegetation. The air-layered cuttings were placed in a nursery under 70 % light-retention shade netting and watered every three days for one month to acclimate.

## Experimental design

The trial was established using a completely randomized factorial design (3×4). Two factors were tested: (1) Taxon (*P. caribaea*, *P. elliottii* × *P. caribaea* and *P. caribaea* × *P. tecunumanii*) and (2) IBA concentration (10, 20, 30, and 40 g kg<sup>-1</sup> of substrate), for a total of 12 treatments, with eight replications for *P. caribaea*, five for *P. elliottii* × *P. caribaea*, and seven for *P. caribaea* × *P. tecunumanii*.

In each air layer, the following were evaluated: (1) Survival as live (1) or dead (0), regardless of the presence of roots. (2) Rooting as rooted (1) and unrooted (0). Layers were considered rooted if they developed roots longer than 2 cm, and unrooted if they did not, regardless of their survival status. The survival percentage and rooted layers was then calculated. (3) Terminal bud growth (cm) was measured with a measuring tape (model FH-3M Truper®, Mexico) starting from the 40 cm length at the time of layering. (4) The number of roots was determined by direct counting before transplanting the layer to the bag. These variables were evaluated when the layers were separated from the mother plants.

The Shapiro-Wilk and Kolmogorov-Smirnov tests were performed to verify the distribution of the residuals. The survival and rooting variables were transformed using the arcsine function to reduce the effect of extreme observations (Burbidge et al., 1988). Since the assumption of normal distribution for all variables was rejected, the analysis of variance using Aligned Range Transformations (ART) was chosen, along with multiple comparisons between factor levels using the ART structure, to determine the effect of the taxon, the concentration of IBA, and their interaction on the response variables (Saste et al., 2016). The analyses were performed using the R® statistical package version 3.5.3 with the agricolae and ARTTool libraries (R Core Team, 2020). The following statistical model was considered:

$$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + \epsilon_{ijk}$$

Where:

$Y_{ijk}$  = Value of the response variable corresponding to the  $i^{th}$  level of  $T$  ( $i=P. caribaea$ ,  $P. elliottii \times P. caribaea$ , and  $P. caribaea \times P. tecunumanii$ ),  $j^{th}$  level of  $A$  ( $j=10, 20, 30$ , and  $40 \text{ g kg}^{-1}$  of IBA in the substrate), and  $k=1, 2, 3, \dots$  replications

$\mu$  = Overall mean

$T_i$  = Effect of taxon

$A_j$  = Effect of auxin IBA concentration

$TA_{ij}$  = Effect of taxon-IBA concentration interaction

$\epsilon_{ijk}$  = Experimental error

## Results and Discussion

### Rooting and survival of layers

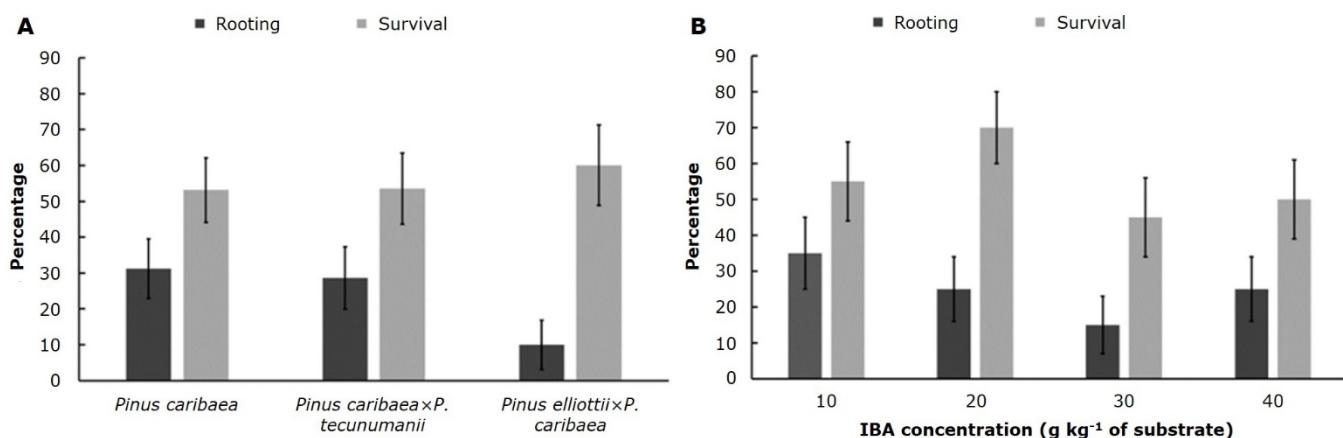
There were no significant differences ( $P>0.05$ ) in rooting and survival due to taxon, auxin dose, or the interaction between these factors (Table 2). The similar response of the layers in the three taxa could be related to their taxonomic closeness, since  $P. caribaea$  and the two species with which it hybridized are grouped in the same subsection and are expected to have evolved in a similar way (Gernandt et al., 2005).

**Table 2.** Results of the Aligned Rank Transformation analysis of rooting and survival of layers in tropical pines.

Factor	DF	Mean square	F-value	P>F
Rooting				
Taxon	2	597.6	1.71	0.17
IBA concentration (g kg <sup>-1</sup> )	3	815.4	1.12	0.33
Taxon×IBA concentration	6	389.36	0.73	0.62
Survival				
Taxon	2	10.2	0.02	0.98
IBA concentration (g kg <sup>-1</sup> )	3	712.8	1.24	0.30
Taxón× IBA concentration	6	172.6	0.30	0.93

DF = Degrees of freedom; IBA = Indole-3-butyric acid.

The highest rooting rate was observed in *P. caribaea* (31.25 %), followed by the *P. caribaea* × *P. tecunumanii* hybrid (28.57 %), while *P. elliottii* × *P. caribaea* only reached 10 % (Figure 2A). The two taxa with the highest rooting rates also showed better performance in height and diameter growth within the provenance trial (Puc-Kauil et al., 20-24), which may indicate that they have been better adapted to the region's conditions, thus favoring the rooting of the air layer. In this regard, more vigorous trees generate greater metabolic resources that benefit adventitious rooting due to the accumulation of carbohydrates in the area near the girdle (Amissah & Bassuk, 2004). However, in addition to the efficiency in the production of photosynthates, it is important to consider the amount of endogenous auxins of the trees at the time of layering and that vary according to the physiology of each species (Uribe et al., 2008).



A = Taxon factor; B = IBA concentration factor. Error bars represent the standard error.

**Figure 2.** Percentage of rooting and survival of air layers in tropical pines.

The success of air layering can differ among species within the same genus; air layers of *Quercus bicolor* Willd. rooted at 65 %, compared to 50 % in *Q. microphylla* Née (Amissah & Bassuk, 2004). Even the rooting response to air layering can vary among varieties of the same species, as reported by Afzal et al. (2017) described for *Olea europaea* L.

Some groups of species have a better response to rooting by layering than others; for example, in mangrove species such as *Laguncularia racemosa* (L.) C. F. Gaertn. and *Conocarpus erectus* L., they have reached up to 90 % (Benítez-Pardo et al., 2002); in fruit trees, such as *Psidium guajava*, close to 60 % (Baghel et al., 2016); while, in conifers, the response has been lower, as in *Pinus greggii* with 20-40 % (Becerra & Plancarte, 1993).

The rooting response of *P. caribaea* and the *P. caribaea* × *P. tecunumanii* hybrid can be considered significant, as it is difficult for adult individuals of this genus to develop adventitious roots. Furthermore, through this technique, clones were obtained from the best trees in the trial, with provenances adapted to the region and with a functional root system.

The survival rate of the layered branch showed no significant differences and ranged from 50 to 60 % (Figure 2A), suggesting that the same factors likely caused the death

of all the tested taxa. This suggests that the layering technique may negatively affect the survival of some layered branches.

In another study with five species of tropical trees, the survival rate of the layered branch was 60 % (Singh & Ansari, 2014), which was similar to the result of this study. Although the layered branch is expected to remain alive and continue the flow of water and nutrients through the xylem, sometimes the stress generated by the damage to the vascular tissue caused by the cuts made during girdling can damage the branch to the point of killing it (Hartmann et al., 2014). The survival of the layered branch may also be related to the nutritional status of the tree, so the more vigorous ones have a greater chance of surviving while the rhizogenesis process occurs in the layering (Vacek et al., 2012).

No significant differences were found among the tested IBA doses; however, the highest rooting percentage (35 %) occurred with the lowest auxin concentration (10 g kg<sup>-1</sup>) (Figure 2B). In a similar way, in *Brosimum alicastrum* air layers, the rooting percentage was not significant between air layers with and without IBA (Ortigoza-García et al., 2025). These results suggest that it is possible to obtain acceptable rooting and survival rates in the evaluated species and hybrids using low doses of auxin. Although exogenous application of high doses of IBA generally stimulates rapid translocation and movement of photosynthates to promote root development, in some cases it has a negative effect (Khandaker et al., 2022). This response may be related to the endogenous auxin levels produced by the species, which vary according to their physiological state and time of year. If these levels are high, excessive accumulation could occur and inhibit rooting of the air layer (Mesén et al., 1997). Some tree species do not tolerate high doses of auxin, such as *Prosopis chilensis* and *Prunus lusitanica* L. (Moreira et al., 2009; Tarnowski, 2021).

In 15-year-old *P. greggii* trees, air layering rooting rates of 20 to 40 % were reported using different doses of IBA, with no differences observed compared to the control (Becerra & Plancarte, 1993). Another study reported rooting rates of up to 93.6 % in *P. elliottii* air layers using a 1.2 % auxin concentration (Hoekstra, 1957). This reinforces the idea that in some conifer species, high doses of auxin are not necessary

to promote root formation in air layering. However, it is important to note that this propagation technique appears to yield better results using juvenile material, as demonstrated in *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M. C. Johnst. with rooting rates of 69.2 and 31.5 % in five- and fifteen-year-old trees, respectively (Ramírez-Malagón et al., 2014). However, further testing of IBA concentrations is necessary to increase rooting rates in the evaluated pine taxa, which is the central aim of this research.

### Number of roots and terminal bud growth

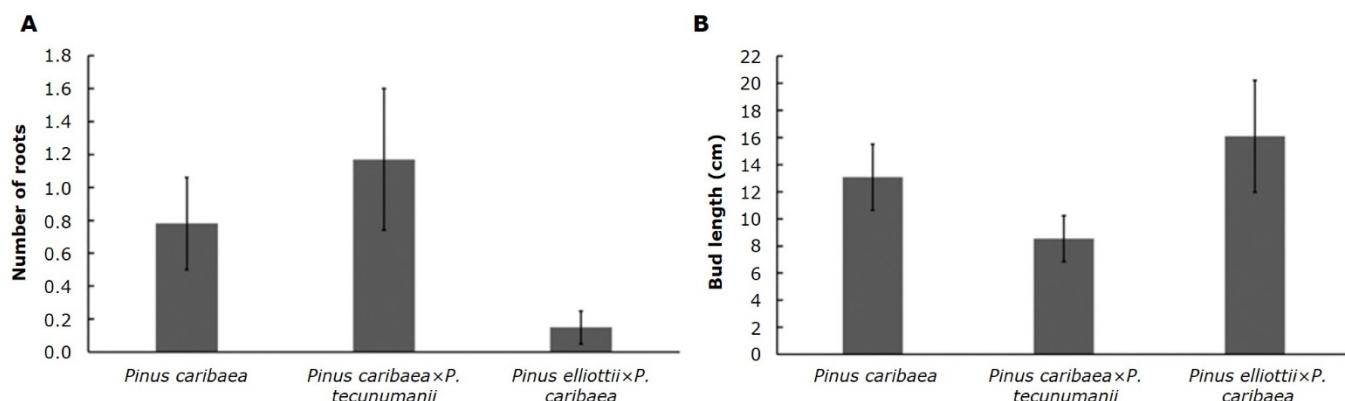
The number of adventitious roots generated and the growth of the terminal bud did not show significant differences ( $P < 0.05$ ) due to any of the individual factors, nor their interaction (Table 3).

**Table 3.** Results of the Aligned Rank Transformation analysis of the number of roots and terminal bud growth in air layers of three tropical pines.

Factor	DF	Mean square	F-value	P>F
Number of roots				
Taxon	2	1 304.4	2.48	0.09
IBA concentration (g kg <sup>-1</sup> )	3	240.6	0.30	0.82
Taxon×IBA concentration	6	576.4	1.10	0.37
Terminal bud growth				
Taxon	2	366.3	0.60	0.55
IBA concentration (g kg <sup>-1</sup> )	3	105.8	0.17	0.91
Taxon×IBA concentration	6	37.0	0.06	0.99

DF = Degrees of freedom; IBA = Indole-3-butyric acid.

*P. caribaea* × *P. tecunumanii* and *P. caribaea* recorded the highest average values for the number of roots generated, with 1.17 and 0.78, respectively (Figure 3A). This hybrid and species surpassed the root generation of *P. elliottii* × *P. caribaea* air layers (0.15 roots on average) by 87 and 80 %, respectively, demonstrating the latter's lower capacity to develop adventitious roots and coinciding with the lowest rooting percentage (10 %). At the phenotypic level within a taxon, the number of roots developed by the air layer varied significantly from 12.6 to 21.7 in *Myrciaria dubia* (Kunth) McVaugh (Liao-Torres et al., 2012). There are no publications on the number of roots generated in pine air layers, but the averages obtained in the present study were lower than those of other woody species such as *Lasiococca comberi* Haines (2.2 a 14.5), *Syzygium samarangense* (14.3), *Pistacia vera* L. (2.2), and *Brosimum alicastrum* (8 a 12) (Kamila & Panda, 2019; Khandaker et al., 2022; Noori et al., 2019; Ortigoza-García et al., 2025).



A = Average values of number of roots; B = Average values of bud length. Error bars represent the standard error.

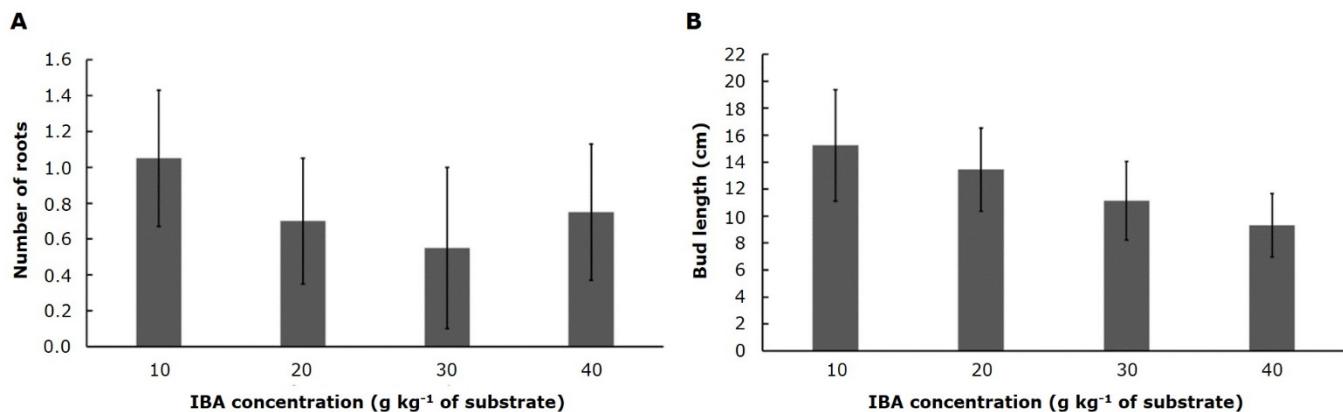
**Figure 3.** Average values of number of roots and bud length of air layers for three tropical pines.

The low root count for the three taxa may be a result of the advanced age of the trees used for cloning, as species of the *Pinus* genus decrease their rooting capacity with age (Bautista-Ojeda et al., 2022; Stuepp et al., 2018). In *Pinus elliottii*, the air layers developed a greater number of roots, up to 33.8 in young ortets (Hoekstra, 1957). This variable is highly relevant, since a robust root system ensures greater survival of the air layer when separated from the parent plant and transplanted (Liao-Torres et al., 2012). In this regard, the air layers of *P. caribaea* and *P. caribaea* × *P. tecunumanii* might have a greater chance of success during acclimatization, compared to those of *P. elliottii* × *P. caribaea*.

On the other hand, air layers of *P. caribaea* and the *P. elliottii* × *P. caribaea* hybrid tend to develop greater terminal bud growth and fewer roots, indicating a variation in the response pattern for these variables compared to *P. caribaea* × *P. tecunumanii* (Figure 3). This relationship between the number of roots and bud growth may also reflect competition between root and shoot development during propagation (Mesén et al., 1997). In the case of *P. elliottii* × *P. caribaea*, the low percentage of rooting and number of roots suggests that its resources were primarily invested in bud growth; whereas the other species and hybrid distributed them in the growth of both apical meristems (shoot and root). The development of the bud and needles in the layered branch are important because they provide photosynthate, endogenous auxins (IAA) and other metabolites that are transported by the phloem and accumulate in the upper part of the ring, to stimulate rooting (Hartmann et al., 2014).

Although there were no significant differences ( $P<0.05$ ) between the IBA concentrations, the greatest number of roots (1.05) and terminal bud length (15.25 cm) was observed at the lowest concentration (10 g kg<sup>-1</sup>) (Figure 4A and 4B), suggesting that high doses of auxin may cause toxicity or inhibit rooting in some species of the genus *Pinus* (Bautista-Ojeda et al., 2022). Therefore, an optimal threshold for exogenous auxin concentration should be considered to promote adventitious root production; for *Lasiococca comberi*, the optimal dose was 5 000 ppm IBA, and rooting of the air layer was reduced at doses lower or higher than this concentration (Kamila & Panda, 2019). Therefore, for the evaluated pine species and

hybrids, it will be necessary to test other concentrations of IBA to identify the one that best promotes adventitious rooting of the air layer.



A = Number of roots generated; B = Length of the terminal bud. Error bars represent the standard error.

**Figure 4.** Growth variables of air layers by IBA concentration.

## Interaction between factors

The interaction between the factors (taxon×IBA concentration) did not show significant differences for any variable (Tables 2 and 3). The interaction in rooting, survival, number of roots, and branch growth ranged from 0 to 48 %, 37.5 to 80 %, 0 to 2.1 cm, and 6.6 to 21.9 cm, respectively. The maximum values were obtained with the following combinations: in rooting, *P. caribaea* × *P. tecunumanii* and 10  $\text{g kg}^{-1}$  of IBA (48 %). For survival, *P. elliottii* × *P. caribaea* with 30  $\text{g kg}^{-1}$  of IBA (80 %). For the number of roots, it was *Pinus caribaea* with 20  $\text{g kg}^{-1}$  of IBA (2.1). Regarding bud

length, *P. elliottii* × *P. caribaea* with 20 g kg<sup>-1</sup> of IBA (21.9 cm). Although the analysis did not detect differences, these average maximum values can provide a preliminary indication of the IBA concentration that might favor each of the three taxa for the evaluated variables.

## Conclusions

The taxa used in this study showed similar performance in the rooting of the air layer, a response that may be related to their taxonomic proximity. With the tested doses of IBA applied to the air layering substrate, it is possible to obtain up to 35 % rooting. Further studies are suggested to determine the optimal auxin concentration that increases adventitious rooting in air layers of this species and tropical pine hybrids. Furthermore, the study demonstrated that this asexual propagation method is a viable alternative for conserving and multiplying phenotypically outstanding adult pine trees on a small scale and obtaining ramets adapted to local conditions.

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## **Conflict of interest**

The authors declare no conflict of interest.

## **Contribution by author**

Esther Paredes Díaz: manuscript writing, research design and supervision; Benito González Jiménez: interpretation of results, drafting, revision, and restructuring of the manuscript; Alberto Pérez Luna: modeling, statistical analysis, and structuring of results; Marcos Jiménez Casas: drafting, revision, and proofreading of the manuscript; Norberto Silva Pérez: methodology, execution, and supervision of the field experiment.

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