Manejo nutrimental de árboles de pino híbrido y uso de ácido *indolbutírico* para su clonación por estacas

Nutritional management of hybrid pine trees and use of *indole butyric* acid for cloning by cuttings

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Resumen

Las estacas de árboles jóvenes de pino presentan bajas tasas de enraizamiento, lo que dificulta su clonación; sin embargo, la fertilización y podas programadas promueven la producción de estacas juveniles. La aplicación exógena de auxinas favorece la formación de raíces. En este trabajo se analiza el efecto del manejo nutrimental de los árboles donadores, de cuatro años de edad, del híbrido *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* y del ácido indolbutírico (AIB) en el enraizamiento de estacas. En un diseño de bloques completos con arreglo factorial, se evaluó la aplicación de cinco nutrimentos o mezclas de ellos a los árboles. Los tratamientos y dosis por árbol fueron: testigo, nitrógeno (80 g), fósforo (20 g), magnesio (10 g) y nitrógeno/fósforo (80/20 g). Además, se probaron dos dosis de AIB (0 y 3 000 ppm) para el enraizamiento. La supervivencia de estacas, el porcentaje de enraizamiento y la morfología de raíces, se registraron después de 90 días. El manejo nutrimental y el AIB mejoraron tanto la morfología de raíces primarias y secundarias, como el enraizamiento de estacas en más de 30 %, respecto a sus testigos. Lo anterior permitió una mayor supervivencia de estacas durante la formación de raíces. Con el tratamiento de N+P se obtuvo la mejor respuesta, superior a 60 %, en todas las variables evaluadas. La clonación de árboles jóvenes del híbrido, por el enraizamiento de estacas, es posible con el apoyo de un programa de fertilización, aplicado como parte del manejo del árbol donador y el uso de AIB.

Palabras clave: Auxina, enraizamiento, diagnóstico nutrimental, nutrición, planta donadora, propagación vegetativa.

Abstract

Young tree cuttings generally show low rooting rates, thus making cloning difficult. Fertilization and programmed pruning of the young trees help production of juvenile cuttings. The exogenous application of auxins promotes roots formation. This work analyzes the effect of nutrient management of four-year-old donor trees of the hybrid *Pinus elliottii* var. *elliotti* × *Pinus caribaea* var. *hondurensis*, as well as that of the auxin Indolbuturic acid (IBA) on rooting of cuttings. In a complete block design with factorial arrangement, we evaluated the application of five nutrients or combinations of them to trees. The treatments and doses per tree were: control, nitrogen (80 g), phosphorus (20 g), magnesium (10 g) and nitrogen/phosphorus (80/20 g). Additionally, we tested two doses of IBA (0 and 3 000 ppm) on cutting rooting. Cutting survival, rooting percentage and root morphology were recorded after 90 days. Nutrient management and IBA improved morphology of primary and secondary roots, as well as rooting of the cuttings by more than 30 %, compared to the control treatment. This allowed greater survival of cuttings during the root formation process. Treatment with N+P gave rise to the best response, which was over 60 %, in all variables evaluated. Cloning of young trees of the hybrid by the technique of cutting rooting is possible with the support of a program of fertilization applied as a management practice for donor plant and the use of IBA as well.

Key words: Auxins, rooting, nutritional diagnosis, nutrition, donor plant, vegetative propagation.

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Introduction

Hybrids of *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* developed in Australia outperform their parent species in growth, shape and wind resistance (Nikles, 2000). In Mexico, thousands of hectares have been planted with this hybrid for resin and wood production (Conafor, 2016). Superior hybrid genotypes must be cloned in order to accelerate the production of plants required by the industry.

Plant cloning allows maximizing production, quality and uniformity in plantations. In trees, the cloning process is difficult because their physiological maturation stage affects the production of cuttings and their rooting capacity (Majada *et al*., 2011). To promote the formation of juvenile cuttings with good rooting response, in various *Pinus* species, the technique includes rejuvenation through programmed pruning (Riov *et al*., 2020).

Resprouting is a characteristic of some forest species that enables them to recover their aerial biomass after damage; this response depends, to a great extent, on the carbon reserves existing in the tree tissues than enables them to produce the necessary amount of shoot buds (Smith *et al*., 2018). This regrowth capacity is harnessed, through pruning of the mother plant, to obtain cuttings and to clone genotypes of *Pinus thunbergii* Parl., *Picea abies* L. Karst. and *Pinus patula* Schltdl. *et* Cham. (Tikkinen *et al*., 2018; Matsunaga and Ohira, 2019; Escamilla-Hernández *et al*., 2020).

Vigorous cuttings more suitable for rooting are formed on well-fertilized donor plants (Hernández and Rubilar, 2012). Studies in *Pinus taeda* L. show that treatment with fertilizers increased their levels of carbon reserves (carbohydrates), which improved production and rooting of cuttings (Rowe *et al*., 2002). Furthermore, fertilization of mother plants with adequate amounts of

nitrogen and phosphorus influences shoot production and rapid adventitious rooting (Moe and Andersen, 1988).

Most of the cuttings from *Pinus* species are difficult to root, so the use of synthetic auxins has been resorted to (Coimbra *et al.*, 2016). The most commonly used auxin is Indolebutyric Acid (IBA), due to its stability and solubility (Tilahun *et al.*, 2019). In Mexican pines, IBA improves the percentage of rooted cuttings by more than 45 % in *Pinus leiophylla* Schiede ex Schltdl. & Cham. (Cuevas *et al.*, 2015), and 90 % for *P. jaliscana* Pérez de la Rosa (Aparicio-Rentería *et al.*, 2008).

In this work, cloning was tested by means of one of the most practical and low-cost methods, which is the rooting of cuttings from selected genotypes of the *P. elliottii* var. *elliottii* × *P. caribaea* var*. hondurensis* hybrid that have shown superior growth and stem straightness. Given that the donor trees have reached certain physiological maturity, their ability to resprout for the production of cuttings, and their subsequent rooting, are limited. Therefore, the objective was to evaluate the effect of the nutritional management of four-year-old donor trees of the hybrid *P. elliottii* var. *elliottii* × *P. caribaea* var. *hondurensis*, and the use of IBA, on the rooting of cuttings, in order to vegetatively propagate the best genotypes and establish, in the future, clonal plantations of the hybrid.

Materials and Methods

Location of the experiment

The experiment was established in the greenhouse at the Jefatura de Ingeniería Forestal del *Instituto Tecnológico Superior de Venustiano Carranza* (ITSVC), located between the geographical coordinates 20°28'19.6" N and 97°41'56.4" W, at an altitude of 333 m, in *Lázaro Cárdenas*, *Puebla*.

Plant material and nutritional analysis

In July 2019, 25 pest-free trees, with best growth and stem straightness were selected from a four-year-old plantation of the hybrid *P. elliottii* var. *elliottii* × *P. caribaea* var. *hondurensis* located within the facilities of the ITSVC. A sample of foliage (approximately 200 g of green material) was obtained from each selected tree to analyze nutritional status. The samples were placed in paper bags and transported to the *Laboratorio del Posgrado en Ciencias Forestales* of *Colegio de Postgraduados*, where they were washed with running and distilled water and dried in a Shel-Lab oven (model 1675-S) for 48 hours at 70 °C. Subsequently, three samples of 60 g each of dry matter were ground and processed for chemical analysis at the *Salvador Alcalde Blanco Laboratorio del Programa de Posgrado en Edafología* at *Colegio de Postgraduados*. For each foliar sample, the concentrations of phosphorus, potassium, calcium, magnesium, iron, copper, zinc, manganese and boron were determined by wet digestion extraction using Merck® sulfuric acid, Merck® perchloric acid, and Merck ® hydrogen peroxide (1.3:0.7:1, v:v:v:v). The extracts were analyzed with a plasma optical emission spectrometer (*Agilent* 725 Series ICP-OES). Nitrogen concentration was determined in a 10 mL aliquot of the extract described above, by distillation of the sample and assessed by titration with sulfuric acid (Alcántar and Sandoval, 1999).

The foliar analyses were interpreted using the critical concentrations cited by Dickens *et al.* (2016) for *Pinus elliottii* Engelm. ex Parl. and those suggested by Xu *et al.* (2000) for *Pinus elliottii* × *Pinus caribaea*.

Application of fertilization treatments

In accordance with the foliar nutrient concentrations (Table 1), five fertilization treatments were prescribed, based on solutions prepared with tap water: T1 control (water only), T2 Nitrogen (80 g urea per tree), T3 Phosphorus (20 g phosphoric acid per tree), T4 Magnesium (10 g magnesium sulfate per tree), and T5 Nitrogen/Phosphorus (80 and 20 g urea and phosphoric acid per tree, respectively).

Table 1. Foliar nutrient concentrations in selected *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* trees and critical foliar concentrations cited for *Pinus elliottii* (Dickens *et al.*, 2016) and for the hybrid (Xu *et al.*, 2000).

Elements	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
	%					**Ppm**				
LC ES	0.82*	0.14	0.44	0.25	0.10*	59.6	2.50	18.2	88.3	6.4
CC *PE* *	1.00	0.09	0.25	0.08	0.06	15.0	1.50	10.0	20.0	4.0
CC *PE* × *PC***	0.96	0.06	0.06	0.10	0.11	-	2.24	16.1	55.0	-

* Deficiencies; LC = Leaf concentration; ES = Experimental site; CC = Critical concentration; PE = *Pinus elliottii*; PC = *Pinus caribaea*.

In November 2019, the experiment was conducted under a completely randomized design; each treatment was administered arbitrarily to five trees. A total of 25 trees were considered in the experiment. The solutions were applied within a trench of approximately 1 m in diameter around each tree.

After one month from fertilization, all individuals were pruned. Pruning was carried out at the tip of eight branches located in the second third of the crown of each tree, using scissors with a telescopic handle. The purpose of this action was to induce the production of adventitious shoots. During the experiment, continuous weeding was carried out in order to prevent competition for nutrients.

Collection and preparation of cuttings

By February 2020, the shoots (cuttings) had reached a length greater than 5 cm, and were harvested. At the base of each cutting, a bevel cut was made with a scalpel disinfected with alcohol (70 %). Then, the stakes were submerged in a (Adama México) captan solution (2 mL L^{-1}) for 5 minutes.

Planting and application of IBA

The basal section (2 cm) of each cutting was immersed in the IBA solution (Radix$^®$ 3 000) for 20 seconds; for the control, only running water was used for the same period of time. Cuttings were then planted in a substrate mixture of peat moss, agrolite, and vermiculite (3:1:1). Prior to planting, the substrate was brought to field capacity with running water. The trial was established in rooting chambers 120 cm long by 60 cm wide and 15 cm high, which were built with wooden boards and polycarbonate sheets. During the experiment, a relative humidity of 80 % and an average temperature of 20 °C were recorded in the chambers.

Experimental design and evaluated variables

The experiment was set up under a completely randomized block design with factorial arrangement; each rooting chamber represented one block. IBA was considered as factor A with two levels (0 and 3 000 ppm), while factor F represented the fertilization (of mother or donor trees) with five levels (control, nitrogen, phosphorus, magnesium, and the combination of nitrogen and phosphorus), for a total of ten treatments in each block. The experiment was replicated four times with each experimental unit consisting of eight cuttings, so that the total number of cuttings was 320.

In April 2020, 11 weeks after planting, the number of live cuttings, number of rooted cutting, number of primary and secondary roots, length of primary roots and number of cuttings with callus formation were recorded. These were used to determine: the percentage of cutting survival, percentage of rooted cuttings, number of primary roots, length of the main root, number of secondary roots, and percentage of cuttings with callus formation. Cuttings were considered dead when more than half of their tissue, starting from the base, was necrotic at the time of evaluation. Cuttings were considered rooted when they presented at least one 0.5 cm long root.

Data analysis

With the average values of the response variables, the statistical analysis was carried out using the Mixed procedure in the statistical analysis package SAS (Statistical Analysis System$^{©}$) version 9.4 (SAS, 2013). In order to verify the assumptions of the parametric variance analysis, the Shapiro Wilk test for normality and the Levene and Barlett test for homogeneity were used. Then, the variables expressed in percentage (rooting, callus and survival) were transformed with the arc-sine function of the square root of the original value expressed in decimal fraction, since the data in the original scale did not meet the criteria of normality and homogeneity of variance. A similar procedure was carried out for the variables number of rooted cuttings, number of primary and secondary roots, and length of primary roots, which showed a Poisson-type distribution; in this case, the square root of the original value was used. After analysis, the average values per treatment were retransformed to the original units. The variance analysis model used was as follows:

$$Y_{ijk} = \mu + B_i + A_j + F_k + AF_{jk} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = ijk^{th} observation in i^{th} block containing the jth level of factor A and the kth level of factor F

μ = Overall average

B_i = Effect of the ith block

A_j = Effect of the ith level of factor A (auxin)

F_k = Effect of the ith level of factor F (fertilization)

AF_{jk} = Interaction between the ith level of factor A and factor F

ε_{ijk} = Experimental error

For variables that showed significant differences, Tukey's mean comparison test (α= 0.05) was applied to determine statistical differences between the levels of the factors under study.

Results and Discussion

Effects of IBA and fertilization of the mother plant

IBA and fertilization treatments had significant effects on survival of the cuttings, number of rooted cuttings, main root length, number of primary roots (except for IBA), and number of secondary roots; whereas, only the rooted cuttings showed interaction between treatments (Table 1). On the other hand, neither treatment influenced the observed callus development in live un-rooted cuttings (Table 2).

Table 2. P values of the rooting variables in cuttings obtained from the *Pinus elliottii* var*. elliottii* × *Pinus caribaea* var*. hondurensis* hybrid.

Source of variation	%SC	%Callus	%RC	PRL	NTR	NSR
IBA	0.0091	0.2369	0.0001	0.002	0.0512	0.0055
F	0.0001	0.8170	0.0001	0.001	0.0001	0.0001
IBA × F	0.0511	0.7549	0.0085	0.110	0.0920	0.0599

IBA = Indolebutyric acid; F = Fertilization; %SE = Percent survival of cuttings; %Callus = Percent of cuttings with callus formation; %RC = Percent of rooted cuttings; PRL = Principal root length; NTR = Number of tap roots; NSR = Number of secondary roots.

In general, the cuttings maintained a survival rate above 50 %, which is an acceptable percentage for propagation by this technique (Table 3). IBA increased cutting survival by 25 % and also increased the rooting rate by just over 35 %, compared to the controls (Table 3). According to these results, it can be deduced that auxin has a direct influence on root promotion, particularly in those cuttings that probably required stimulation from the external hormonal regulator for their development. The presence of roots in the cuttings allows them to reactivate their physiological functions and form a seedling. Otherwise, unrooted cuttings survive only until the reserves in their tissues are exhausted, and then die. This coincides with what has been pointed out by Fachinello *et al.* (2005): the survival of the cutting depends not only on its quality (nutritional status, vigor and juvenility) but also on the rapid formation of adventitious roots, which are responsible for providing water and nutrients.

Table 3. Mean values (± standard error) of percent survival of cuttings, rooted cuttings and cuttings with callus formation, by dose of Indolebutyric Acid (IBA) and fertilizer, in cuttings of the hybrid of *Pinus elliottii* var*. elliottii* × *Pinus caribaea* var*. caribaea*.

Treatments	Survival of cuttings (%)	Rooted cuttings (%)	Cuttings with callus (%)
IBA			
0	52.5±4.3b	32.8±5.1b	15.0±3.6a
3000	70.0±8.1a	53.6±6.3a	21.2±5.5a
Fertilizer			
T	34.5±4.8d	12.5±9.6d	18.7±3.6a
N	54.7±8.8bc	42.2±9.3bc	12.5±2.9a
P	76.6±8.7ab	62.6±3.1ab	17.2±2.6a
Mg	51.6±9.8bc	31.5±6.8dc	20.3±4.7a
N+P	89.1±7.3a	67.2±2.4a	21.8±5.1a

*Different letters indicate significant differences ($P \leq 0.05$).

The use of IBA in the promotion of adventitious roots in cuttings is well documented; this hormone accelerates the developmental and root formation process in the shortest possible time, thus ensuring the survival of the cutting (Endres *et al.*, 2007; Gratieri-Sossella *et al.*, 2008; Latsague-Vidal *et al.*, 2010).

A small percentage of cuttings showed the proliferation of callus, an undesirable effect, since these failed to develop adventitious roots; however, its formation was not linked to the treatments (Table 3). Callus cells lack connection to conductive tissues, are fragile and remain undifferentiated at the level of the root structure (Heaman and Owens, 2011). For some species, such as sequoia, the development of callus is considered an indispensable initial stage that precedes root formation (Navroski *et al.*, 2014). In this work, no factor promoting callus production in the cuttings was determined, but the environment may be relevant in this process.

IBA had no significant effect on the number of primary roots, but it did influence secondary root production and primary root length (Figure 1). When compared to

the untreated controls, the cuttings treated with IBA increased the production of secondary roots by more than 30 %, while the length of the main root increased by 35 % (Figure 1).
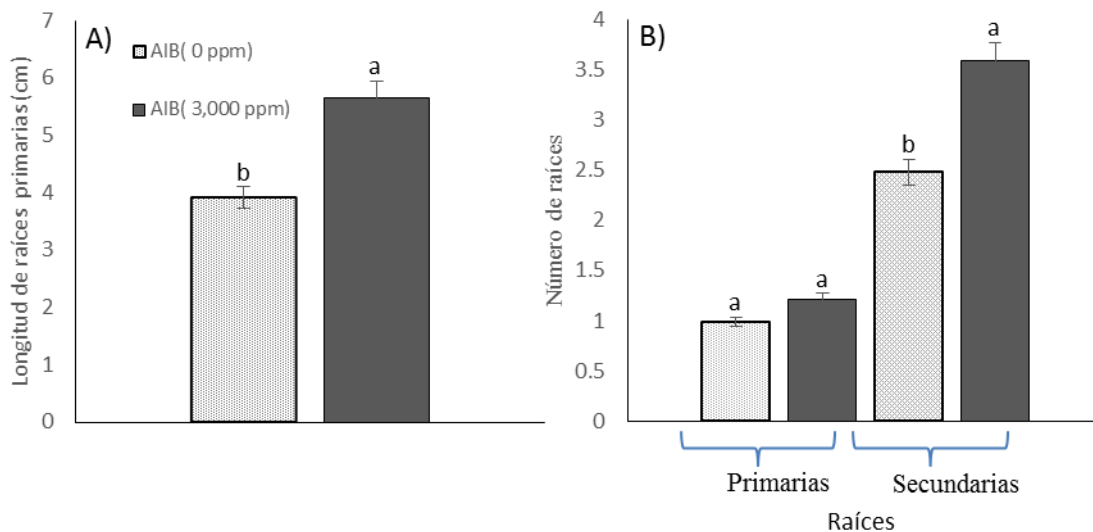
Longitud de raíces primarias = Length of primary roots; *Number of roots* = Número de raíces; *Primarias* = Primary; *Secundarias* = Secondary.

A) Length of primary roots, B) Number of primary and secondary roots. Within a root type, different letters in the error bars indicate significant differences between treatments ($\alpha=0.05$).

Figure 1. Effect of Indolebutyric Acid (IBA) dosage on root production in *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* hybrid cuttings.

In conifers that are difficult to root, IBA is an excellent promoter of adventitious roots. In cuttings of *Pinus leiophylla* Schiede ex Schltdl. *et* Cham., IBA not only induces rooting of the cuttings but also elongates the primary roots and increases the number of primary and secondary roots (Cuevas *et al*., 2015); a similar effect was found in the present study. However, little or no effect of IBA on root morphology has been documented; therefore, the variation in the responses to

IBA is considered to depend on the applied dose, as well as on the predisposition of the cutting to rooting (De Oliveira *et al.*, 2020).

Auxins facilitate rooting by stimulating cambium activity and mobilization of reserves to the site of root initiation, where cells divide and differentiate in order to generate primordia (Dhillon *et al.*, 2011; Brondani *et al.*, 2012). Therefore, the application of IBA on cuttings is desirable to improve root morphology and quality, which in turn is important for plant growth and development, since it increases the area of nutrient and water absorption (Davis and Jacobs, 2005).

Fertilization treatments favored the survival of cuttings in a differentiated manner, but always above 50 % (Table 3). Donor plants treated with N+P generated cuttings with a survival rate of more than 85 %, while the cuttings of control plants did not reach 40 %. These results demonstrate the importance of having a donor plant in good nutritional condition to produce quality cuttings, and are in agreement with other authors, in the sense that adequate fertilization of the donor plants influences the level of carbohydrates, auxins and other compounds in the tissues of the cuttings, which keep them alive while initiating the process of rhizogenesis (Kanmegne *et al.*, 2017).

Fertilization treatments promoted root formation. The highest number of rooted cuttings was recorded with the N+P and P-only treatments, which increased rooting by more than 80 %, with respect to the control (Table 3).

In *Pinus radiata* D. Don, N-based fertilization of donor hedges had a positive effect on the induction of adventitious roots on cuttings (Hernández and Rubilar, 2012). Nitrogen is a constituent of proteins and nucleic acids; moreover, its participation in plant growth is essential; when N is absorbed by the plant in the form of nitrate (NO_3^-) or ammonium (NH_4^+), it is incorporated into the meristems for plant growth and development. In the cambium of the cuttings, N is required for the formation of adventitious roots (Emer *et al.*, 2019).

Phosphorus is an element that enhances plant metabolism and favors absorption; combined with N and an optimal dose of auxin, it favors a better absorption of nutrients and root development in the cuttings (Henry and Blazich, 1992). A significant relationship was observed between P concentration and rooting potential, which reinforces the hypothesis that P is important in post-transplant survival via the promotion of root extension in the field (Planelles, 2004; Oliet *et al.*, 2005). In general, the nutritional status of the plant affects the processes of production of new roots (Villar *et al.*, 2004; Oliet *et al.*, 2006).

The length of primary roots and the number of primary and secondary roots were also significantly affected by fertilization treatments (Figure 2). In particular, the N+P treatment increases by more than 80 % the number of primary and secondary roots in the cuttings, with respect to the control without fertilization; whereas the other fertilization treatments outperformed the control by more than 35 % (Figure 2 A). Similar increases were obtained in the length of the primary root of the cuttings (Figure 2 B).
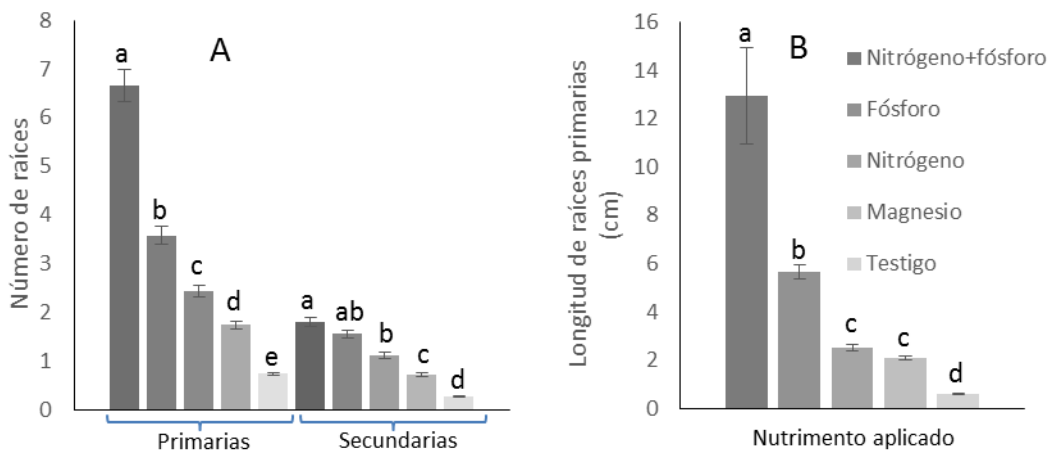
Number of roots = Número de raíces; *Longitud de raíces primarias* = Length of primary roots; *Primarias* = Primary; *Secundarias* = Secondary; *Nutrimento aplicado* = Applied nutrient; *Nitrógeno+Fósforo* = Nitrogen+ Phosphorus; *Magnesio* = Magnesium; *Testigo* = Control.

A) Number of primary and secondary roots present in cuttings, B) Length of primary roots of cuttings.

Figure 2. Effect of fertilizer on root production in *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* hybrid cuttings.

The length of the main root and the number of primary and secondary roots conform the architecture of the plant root system. Therefore, they are important parameters for the plant produced from a cutting. Fertilization of the mother plant contributes to the carbohydrate content of the cuttings, which are in high demand during the formation of adventitious roots (Otiende *et al.*, 2017). In this work, fertilization improved these parameters considerably. It is likely that, in the untreated cuttings, the available carbohydrates did not cover the requirement for the generation of new roots; in cuttings photosynthesis does not provide sufficient carbohydrates for the processes involved in rhizogenesis (Kanmegne *et al.*, 2017).

As for the interactions of the IBA dosage by fertilizer type, significant differences ($P<0.05$) were obtained only for the percentage of rooted cuttings (Table 1). The percentage of rooted cuttings from fertilizer-treated trees increased with the IBA treatment, while those from unfertilized (control) trees decreased (Figure 3).
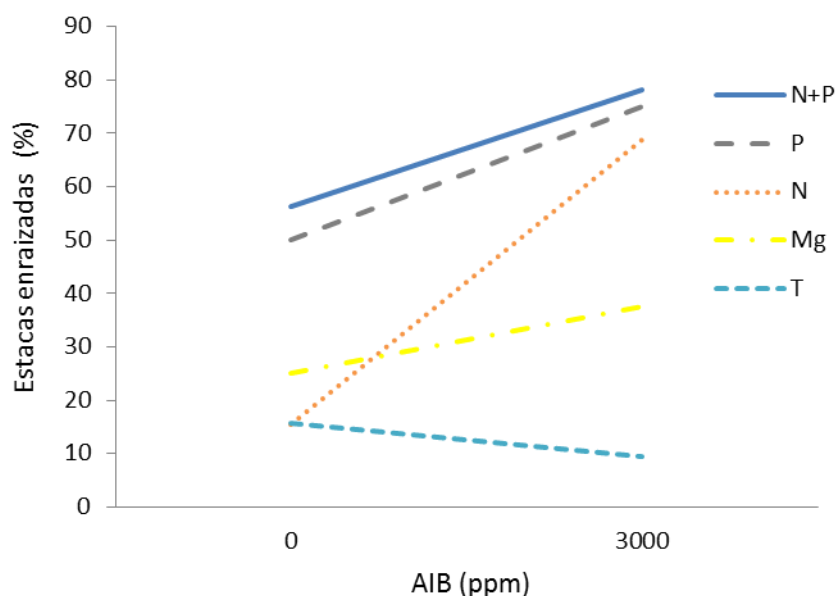
Estacas enraizadas = Rooted cuttings.

Figure 3. Interaction between mother plant fertilization treatments and IBA dosage in rooted cuttings of the hybrid *Pinus elliottii* var*. elliottii* × *Pinus caribaea* var*. hondurensis*.

This interaction implies that rooting capacity depends not only on the exogenous application of IBA, but also on the quality and vigor of the cutting; therefore, the nutritional status of the cutting is of paramount importance in the process of rhizogenesis for the hybrid in question. Nutrient mobilization within the cutting is known to be limited during the root initiation phase, but not during growth and development.

In this case, unfertilized cuttings were not potentiated with auxin, as were the fertilized cuttings. Probably, in the former, the low level of carbohydrates or other metabolic compounds in the tissue limited the rhizogenesis process and reduced the capacity to form roots. Thus, from a nutritional point of view, root initiation depends, to a large extent, on the initial nutrient levels found in the portion of the cutting where they will form. Hence the fundamental role of mother plant nutrition on the subsequent rooting of cuttings (Mesén *et al.*, 2001; Leakey, 2014).

The formation of adventitious roots is a process directed by growth regulators, but it is affected by multiple factors that include, among others, the nutritional state of the cutting; thus, mineral nutrition is important in the conformation of organic structures, activation of enzymatic reactions, and osmoregulation, among others (Geiss *et al.*, 2018).

Conclusions

The nutritional management of mother trees and the application of IBA to the cuttings improve the rooting process, thereby increasing the probability of survival. In particular, at the study site, fertilization of mother trees with N+P is recommended, since this treatment generates cuttings with better rooting characteristics. It is possible to clone tree saplings of the hybrid *Pinus elliottii* var*. elliottii × Pinus caribaea* var*. hondurensis* by the rooting of cuttings supported by a fertilization program and the use of IBA.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by autor

Rosmeri Cabrera Ramírez: research development, data collection, statistical analysis, structure and manuscript design; Marcos Jiménez Casas: design, supervision of the experiment, analysis of the results, writing and correction of the manuscript; Miguel Ángel López López: design, supervision of the experiment and correction of the manuscript; José Pastor Parra Piedra: supervision and correction of the manuscript.

References

Alcántar, G. G. y M. V. Sandoval. 1999. Manual de análisis químico de tejido vegetal. Guía de muestreo, preparación, análisis e interpretación. Publicación especial No. 10 de la Sociedad Mexicana de la Ciencia del Suelo A. C. Chapingo, Edo. de Méx., México. 156 p.

Aparicio-Rentería, A., C. Jimenez H. y C. M. Ruiz O. 2008. Multiplicación clonal de pinos a través del uso de estacas: una alternativa para mantener ganancias genéticas forestales. Foresta Veracruzana 10:53-58. https://www.redalyc.org/articulo.oa?id=49711434007 (24 de noviembre de 2020).

Brondani, G. E., I. Wendling, A. E. Brondani, M. A. Araujo, A.L. Lopes da S. and A. Natal G. 2012. Dynamics of adventitious rooting in mini-cuttings of *Eucalyptus benthamii* x *Eucalyptus dunnii*. Acta Scientiarum. Agronomy 34(2): 169-178. Doi: 10.4025/actasciagron.v34i2.13059.

Comisión Nacional Forestal (Conafor). 2016. Situación actual del germoplasma utilizado en los programas de plantaciones forestales comerciales en el Sureste de México. Diagnóstico. 109 p. https://www.gob.mx/cms/uploads/attachment/file/246716/Situacion_actual_de_ germoplasma_utilizado_en_los_proyectos_de_PFC_en_el_sureste.pdf (4 de noviembre de 2020).

Coimbra C., I. M., O. Alves L., F. De Assis O. e I. Wendling. 2016. Enraizamiento de estacas juveniles de Bertholletia excelsa con diferentes concentraciones de ácido indol-butírico. Agrociencia 50 (2): 227-238. http://www.scielo.org.mx/pdf/agro/v50n2/1405-3195-agro-50-02-00227.pdf (14 de diciembre 2020).

Cuevas, C., J. C., M. Jiménez C., J. Jasso M., P. Pérez R., J. López U. y A. Villegas M. 2015. Propagación asexual de *Pinus leiophylla* Schiede ex Schltdl. *et* Cham. Revista Chapingo. Serie Ciencias Forestales y del Ambiente 21(1): 81-95. Doi:10.5154/r.rchscfa.2014.08.033.

Davis, A. S. and D. F. Jacobs. 2005. Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests 30(2): 295-311. Doi: 10.1007/s11056-005-7480-y.

De Oliveira, P., M., A. Camargo Â., M. C. Navroski, L. M. de Oliveira, D. Felippe and C. Moraes. 2020. Mini-cuttings rooting of *Sequoia sempervirens* at different IBA concentrations abd clones. Floresta 50(2): 1279-1286. Doi:http://dx.doi.org/10.5380/rf.v50i2.62579.

Dhillon, R., M.Hooda, J.Pundeer, K. Ahlawat and I. Chopra. 2011. Effects of auxins and thiamine on the efficacy of techniques of clonal propagation in *Jatropha curcas* L. Biomass and bioenergy 35(4): 1502-1510. Doi:10.1016/j.biombioe.2010.12.017.

Dickens, E. D., D. J. Moorhead, L. A. Morris and D. Kissel. 2016. Proper Soil Sampling and Analysis for Nutrient Needs Determination in Loblolly, Longleaf, and Slash Pine Stands. http://bugwoodcloud.org/bugwood/fertilization/Soil_Sampling_Pine_Stands_Dec_2016.pdf.

Emer, A. A., E. D. Avrella, C. F.Sidnei and G. Schafer. 2019. Nitrogen fertilization for ministrains of *Campomanesia aurea* O. Bergand its influence on productivity and rooting of minicuttingsat different seasons of the year. Revista Brasileira de Ciências Agrárias 4(2): 1-8. Doi:10.5039/agraria.v14i2a5632.

Endres, L., P. M. Guimarães M., C. Moura do S. and N Nunez F. de S. 2007. Enraizamento de estacas de Pau-Brasil (*Caesalpinia echinata* Lam.) tratadas com ácido indol butírico e ácido naftaleno acético. Ciência Rural 37(3):886-889. Doi: 10.1590/S0103-84782007000300046.

Escamilla-Hernández, N., A. Aldrete, J. J. Vargas-Hernández, Á. Villegas-Monter y M. Á. López-López. 2020. Propagación vegetativa de *Pinus patula* SCHIEDE ex SCHLTDL. et CHAM. en diferentes sustratos. Revista Fitotecnia Mexicana 43(2): 215-221. Doi:10.35196/rfm.2020.2.215.

Fachinello, J. C., A. Hoffmann, J. C. Nachtigal and E. Kersten. 2005. Propagação vegetativa por estaquia. Propagação de plantas frutíferas. Informação Tecnológica. Embrapa. Brasília, Brasil. pp. 69-109.

Geiss, G., L. Gutierrez and C. Bellini. 2018. Adventitious root formation: new insights and perspectives. Annual Plant Reviews 37: 127-156. DOI:10.1002/9781119312994.apr0400.

Gratieri-Sossella, A., C. Petry and A. A. Nienow. 2008. Propagação da corticeira do banhado (*Erythrina crista-galli* L.)(Fabaceae) pelo processo de estaquia. Revista Árvore 32(1): 163-171. Doi: 10.1590/S0100-67622008000100018.

Henry, P. and F. A. Blazich. 1992. Nitrogen Nutrition of Containerized Eastern Redcedar. II. Influence of Stock Plant Fertility on Adventitious Rooting of Stem Cuttings. Journal of the American Society for Horticultural Science 117(4):568-570. Doi: 10.21273/JASHS.117.4.568.

Heaman, J. C. and J. N. Owens. 2011. Callus Formation and Root Initiation in Stem Cuttings of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Canadian Journal of Forest Research 2(2):121-134. Doi: 10.1139/x72-023.

Hernández, A. and R. Rubilar. 2012. Efecto de la fertilización nitrogenada y fosforada en el desarrollo y fenología de brotes de setos de *Pinus radiata*. Bosque 33(1): 53-61. Doi: 10.4067/S0717-92002012000100006.

Kanmegne, G., H. D. Mbouobda, C. N. Mbakop and D. N. Omokolo. 2017. The influence of stockplant fertilization on tissue concentrations of nitrogen, carbohydrates and amino acids and on the rooting of leafy stem cuttings of *Cola anomala* K. Schum (Malvaceae). New Forests 48: 17-31. Doi: 10.1007/s11056-016-9553-5.

Latsague-Vidal, M., P. Sáez-Delgado, E. Hauenstein-Barra and F. Peña-Cortés. 2010. Propagación vegetativa de *Myrceugenia exsucca* y *Blepharocalyx cruckshanksii*, especies dominantes del bosque pantanoso de la Depresión Intermedia de la región de La Araucanía, Chile. Bosque 31(3): 247-251. Doi: 10.4067/S0717-92002010000300009.

Leakey, R. R. B. 2014. Plant Cloning: Macropropagation. *In*: Neal Van Alfen (ed) Encyclopedia of Agriculture and Food Systems. Elsevier Publishers. Vol. 4. San Diego, CA, USA pp. 349-359. https://www.researchgate.net/publication/269395064_Leakey_RRB_2014_Plant_cloning_Macro-propagation_In_Encyclopedia_of_Agriculture_and_Food_Systems_Vol_4_349-359_van_Alfen_N_et_al_eds_Elsevier_Publishers_San_Diego_USA/link/5a11c0db0f7e9bd1b2c0f830/download (12 de eenro de 2021).

Majada, J., A. C. Martínez, I. Feito, A. Kidelman, I. Aranda and R. Alía. 2011. Mini-cuttings: an effective technique for the propagation of *Pinus pinaster* Ait. New Forests 41(3): 399-412. Doi: 10.1007/s11056-010-9232-x.

Matsunaga, K. and M. Ohira. 2019. Effect of cutting size on rooting ability and first year growth of *Pinus thunbergii* in nursery containers. Journal of Forest Research 24(6): 356-364. Doi: 10.1080/13416979.2019.1675252.

Mesén, F., R. Leakey and A. Newton. 2001. The influence of stockplant environment on morphology, physiology and rooting of leafy stem cuttings of *Albizia guachapele*. New Forests 22: 213-227. Doi: 10.1023/A:1015668011884.

Moe, R. and A. S. Anderson. 1988. "Stock plant environment and subsequent adventitious rooting". *In*: Davis, T. D., B. E. Haissig and N. Sankhla (eds.). Adventitious Root Formation in Cuttings. Dioscorides Press. Portland, OR, USA. pp. 214-234.

Navroski, M. C., M de Oliveira P., A. F. Hess, R. Silvestre, A. C. Ângelo, A. J. Fazzini and A. A. Alvarenga. 2014. Resgate e propagação vegetativa de *Sequoia sempervirens*. Floresta 45: 383-392. Doi:10.5380/rf.v45i2.35407.

Nikles, D. G. 2000. Experience with some Pinus hybrids in Queensland, Australia. *In*: Dungey, H. S., M. J. Dieters and D. G. Nikles (eds.). Proceedings of QFRI/CRC-SPF symposium: hybrid breeding and genetics of forest trees. Department of Primary Industries, Brisbane. Noosa, Queensland, Australia. Pp. 27–43.

Oliet, J. A., R. Planelles, F. Artero and D. F. Jacobs. 2005. Nursery fertilization and tree shelters affect long-term field response of *Acacia salicina* Lindl. planted in Mediterranean semiarid conditions. Forest Ecology and Management 215(1-3): 339-351. Doi: 10.1016/j.foreco.2005.05.024.

Oliet, J. P., A. Valdecantos D., J. Puértolas S. and R. Trubat D. 2006. Influencia del estado nutricional y el contenido en carbohidratos en el establecimiento de las plantaciones. *In*: Peñuelas-Rubira, J. L., A. Vilagrosa C., R. Savé I. M. y J. Puértolas S. (eds). Calidad de planta forestal para la restauración en ambientes mediterráneos: estado actual de conocimientos. Ministerio de Medio Ambiente y Medio Rural y Marino, Serie Forestal. Madrid, España. pp. 89-118.

Otiende, M. A., J. O. Nyabundi, K. Ngamau and P. Opala. 2017. Effects of cutting position of rose rootstock cultivars on rooting and its relationship with mineral nutrient content and endogenous carbohydrates. Scientia Horticulturae 225: 204-212. Doi:10.1016/j.scienta.2017.07.009.

Planelles G., R. 2004. Efectos de la fertilización NPK en vivero sobre la calidad funcional de plantas de *Ceratonia siliqua* L. Teis doctoral. Universidad Politécnica de Madrid. Madrid, España. 150 p.

Riov, J., H. Fox, R. Attias, G. Shklar, L. Farkash-Haim, R. Sitbon, Y. Moshe, M. Abu-Abied, E. Sadot and R. David-Schwartz. 2020. Improved method for vegetative propagation of mature *Pinus halepensis* and its hybrids by cuttings, Israel Journal of Plant Sciences 67(1-2): 5-15. Doi: https://doi.org/10.1163/22238980-20191118.

Rowe, D. B., F. A. Blazich and C. D. Raper. 2002. Nitrogen nutrition of hedged stock plants of loblolly pine. I. Tissue nitrogen concentrations and carbohydrate status. New forests 24: 39-51. Doi: 10.1023/A:1020555013964.

Statical Analysis System (SAS). 2013. SAS Statical Analysis System V.9.4. Computer software. Institute Inc. Cary, NC, USA. 5121 p.

Smith, M. G., S. K. Arndt, R. E. Miller, S. Kasel and L. T. Bennett. 2018. Trees use more non-structural carbohydrate reserves during epicormic than basal resprouting. Tree Physiology 38(12): 1779–1791. Doi: 10.1093/treephys/tpy099.

Tikkinen, M., S. Varis, H. Peltola and T. Aronen. 2018. Norway spruce emblings as cutting donors for tree breeding and production. Scandinavian Journal of Forest Research 33(3): 207-214. Doi: 10.1080/02827581.2017.1349925.

Tilahun, A., B. Manahlie, G. Abebe and G. Negash. 2019. Effect of cutting position and indole butyric acid (auxin) concentration on rooting response of *Araucaria heterophylla*. African Journal of Biotechnology 18(4): 86-91. Doi: 10.5897/AJB2018.16450.

Villar, S., P., R. Planelles, E. Enrıquez and J. P. Rubira. 2004. Nursery cultivation regimes, plant functional attributes, and field performance relationships in the Mediterranean oak *Quercus ilex* L. Forest Ecology and Management 196(2-3): 257-266 pp. Doi: 10.1016/j.foreco.2004.02.061.

Xu, Z. H., P. G. Saffigna, G. D. Farquhar, J. A. Simpson, R. J. Haines, S. Walker, D. O. Osborne and D. Guinto. 2000. Carbon isotope discrimination and oxygen isotope composition in clones of the F1 hybrid between slash pine and Caribbean pine in relation to tree growth, water-use efficiency and foliar nutrient concentration, Tree Physiology 20(18): 1209-1217. Doi:10.1093/treephys/20.18.1209.