https://doi.org/10.18004/investig.agrar.2024.diciembre.2602820 e-ISSN: 2305-0683 ISSN: 1684-9086

SCIENTIFIC ARTICLE

Incidence of Substrate Type and Hormone Application on Oil Carob (*Pongamia pinnata* L. Pierre) Multiplication by Root Cuttings in Different Seasons

Incidencia del tipo de sustrato y aplicación de hormona en la multiplicación del algarrobo aceitero (*Pongamia pinnata* L. Pierre) por esquejes de raíz en diferentes épocas del año

Isaura Cantero García[1], Manuel Marino Enciso Gómez[1], María Esther Riveros Pineda[2] y Juan Venancio Benítez Núñez[2]*

[1] Universidad Nacional de Asunción, Facultad de Ciencias Agrarias, Carrera de Ingeniería Forestal, San Lorenzo, Paraguay.
[2] Universidad Nacional de Asunción, Centro Multidisciplinario de Investigaciones Tecnológicas. San Lorenzo, Paraguay.

***Author for correspondence:**
jbenitez@rec.una.py

Conflicts of Interest:
The authors declare that they have no conflicts of interest

Author's contribution:
All authors participated significantly in the conception and design of this study, as well as in the analysis and interpretation of the data. In addition, they contributed to the revision of the manuscript and approved its final version. All assume full responsibility for the content of the document.

Financing:
None

Publication period:
July-December 2024

History:
Received: 07/11/2024;
Accepted: 23/12/2024

Responsible Editor:
Arnaldo Esquivel-Fariña
Universidad Nacional de Asunción, Facultad de Ciencias Agrarias. San Lorenzo, Paraguay.

ABSTRACT

Pongamia pinnata L. Pierre is a multipurpose oil tree species, a source of renewable biofuel, in addition to being a recuperator of degraded soils due to its capacity to fix nitrogen. The most common form of propagation is through seeds, but there is little information available on its vegetative propagation, with asexual propagation being essential to maintain the characteristics of the mother plant and avoid variability. The objective of this work was to evaluate the incidence of substrate type, rooting hormone application and time of year on the vegetative propagation capacity of *P. pinnata* by means of root cuttings. The experiment was carried out in the Forest Nursery of the Faculty of Agricultural Sciences, National University of Asunción. A completely randomized design with factorial arrangement (3×2×2) was used, composed of 3 types of substrates, 2 times of the year, with and without application of IBA hormone. The variables evaluated were: percentage of survival and rooting, number of rootlets, rootlet length and rooting index. The data were subjected to analysis of variance (ANOVA) and in cases where significant differences were found, means were compared using Tukey's test at 5% probability. The results indicate that *Pongamia* cuttings are not significantly influenced by the application of the IBA hormone and the type of substrate. However, they are significantly influenced by the time of year, with spring-summer being the best time for propagation by root cuttings, as demonstrated in this study.

Keywords: legume, rooting, vegetative propagation, growth regulators

RESUMEN

Pongamia pinnata L. Pierre es una especie arbórea oleaginosa, de uso múltiple, fuente de biocombustible renovable, además de ser recuperadora de suelos degradados por su capacidad para fij r nitrógeno. La forma más común de propagación es a través de semillas, pero existe poca información disponible sobre su propagación vegetativa, siendo la propagación asexual esencial para mantener las características de la planta madre y evitar la variabilidad. El objetivo de este trabajo fue evaluar la incidencia del tipo de sustrato, la aplicación de hormona de enraizamiento y la época del año sobre la capacidad de multiplicación vegetativa de *P. pinnata* mediante esquejes de raíz. El experimento se llevó a cabo en el Vivero Forestal de la Facultad de Ciencias Agrarias, Universidad Nacional de Asunción. Se utilizó un diseño completamente al azar con delineamiento factorial (3×2×2), compuesto por 3 tipos de sustrato, 2 épocas del año, con y sin aplicación de hormona AIB. Las variables evaluadas fueron: porcentaje de enraizamiento y sobrevivencia, número de raicillas, longitud de raicillas e índice de enraizamiento. Los datos fueron sometidos a un análisis de varianza (ANAVA) y, en los casos en que se encontraron diferencias signific tivas, se realizó una comparación de medias por el Test de Tukey al 5%. Los resultados indican que los esquejes de *Pongamia* no se ven influenci dos signific tivamente por la aplicación de la hormona AIB y el tipo de sustrato, pero sí por la época, siendo primavera-verano la mejor época para la multiplicación por esquejes de raíz demostrada en este estudio.

Palabras clave: leguminosa, enraizamiento, propagación vegetativa, reguladores de crecimiento

INTRODUCTION

The oil carob tree (*Pongamia pinnata* L. Pierre) is a tree used in India and neighboring countries as a source of traditional medicines, green manure, timber, animal fodder, fuel, biopesticides, and fish poison. Recently, interest in this species has focused on its potential as a source of biofuel, as its seeds contain around 40% oil. However, *Pongamia* has multiple applications beyond biofuel production (Islam, Chakrabarty, Yaakob, Ahiduzzaman, y Kalam Mohammad Mominul Islam, 2021; Degani et al., 2022).

It is a legume species that can form symbiotic associations with mycorrhizal fungi, has been shown to be tolerant to drought, salinity, and heavy metals in the soil, and is a promising species to mitigate climate change (Degani et al., 2022). This species is still little researched in South America, where there is a wide variety of potential soil and climate conditions for its cultivation, considering its plasticity. In Paraguay, there are few studies on the production of this species. Some scientific articles have been found regarding the species on propagation by seed (Rika Kubota, Enciso Gómez, y Vera de Ortiz, 2015) and another on vegetative propagation by means of mini root cuttings (Cantero García, Benitez Núñez, Enciso y Samudio Oggero, 2021).

Vegetative or asexual propagation arises as an alternative production method for plants with the same genotype as the mother plant, since cuttings have buds with the potential to develop new shoots (Hartmann et al., 1998).

A determining factor is the season of the year; Hartmann and Kester (1998) mention that root formation in vegetative propagation through cuttings may depend on certain inherent factors such as the physiological condition of the mother plant and climatic variables. Another factor is the substrate, which must be an adequate growing medium that facilitates good aeration, moisture retention, temperature regulation, and the supply of nutrients, generating favorable conditions for callus formation and root emission in the cuttings (Hartmann, Kester, Davies y Geneve, 2011). To propagate any species vegetatively, there are factors that determine the rooting phase, among these factors are the use of auxins and the propagation medium (substrates) (Hartmann et al., 2011).

On the other hand, hormones have the function of regulating the development and growth of plants (Chambilla, 2018). Auxins are plant growth regulatory hormones that, in very small doses, regulate the physiological processes of plants. Among the auxins is the synthetic auxin known as indolebutyric acid (IBA) that helps stimulate the formation and development of roots when applied to the base of cuttings (Cuellar, 2018).

The aim was to evaluate the incidence of substrate type, rooting hormone application, and season of the year on the vegetative multiplication capacity of *P. pinnata*.

MATERIALS AND METHODS

The experiment was carried out at the Forest Nursery of the School of Forest Engineering, Faculty of Agricultural Sciences, National University of Asunción, located at the University Campus in San Lorenzo City, Central Department.

Root cuttings measuring 10 cm long and 0.5 to 2 cm in diameter at the proximal end were used, taken from a six-year-old plantation located in the experimental plot of the Faculty of Agricultural Sciences, University Campus of San Lorenzo City, Central Department, Paraguay.

The experiment was carried out in two seasons of the year. The first study was conducted in autumn-winter (SE1) 2014, between May, June, and July, and the second in spring-summer (SE2) 2015 between September, October, November, and early December. The trial lasted 90 days in each season, totaling 180 days.

The experimental design was completely randomized (CRD) with a 3 × 2 × 2 factorial arrangement, consisting of 3 substrates, 2 seasons (autumn-winter and spring-summer), and with or without application of IBA hormone (commercial hormone Raizol® 0.2% Indolebutyric Acid [Agrofield]). There were six treatments and six replications per treatment. Each experimental unit consisted of two root cuttings, with 12 cuttings corresponding to each treatment.

The roots were cut at a bevel at the proximal end (closest to the crown of the plant) and straight at the distal end (farthest from the crown). Root cuttings were obtained from 18 randomly selected trees, with three to four cuttings taken from each tree, totaling 72 units. As the cuttings were taken, they were wrapped in paper and placed in a plastic bag, soaked with water to minimize dehydration during transport to the nursery.

The substrates were prepared with different proportions of coarse sand, cattle manure, and worm humus. The preparation and mixing of the substrates were done manually, and the mixtures were distributed in 8 cm × 15 cm pots (3,014 cm^3). The substrates used in the experiment were: Substrate 1 (S1): coarse sand and cattle manure (50:50); Substrate 2 (S2): agricultural soil and worm humus (50:50); and Substrate 3 (S3): agricultural soil, cattle manure, and worm humus (50:25:25).

According to the substrate analysis (Table 1), S1 has the highest organic matter content among the substrates used and an acidic pH. Regarding nutrients, it contains medium amounts of essential macronutrients (P, Ca^{2+}, Mg^{2+}, K^+) and low amounts of exchangeable Na^+ and Al^{3+}+H^+. On the other hand, S2 shows low organic matter content, slightly acidic pH, and medium quantities of essential macronutrients (P, Ca^{2+}, Mg^{2+}, K^+), while exchangeable Na^+ and Al^{3+}+H^+ are in very low quantities. S3 also has slightly acidic pH, medium organic matter content, medium quantities of essential macronutrients (P, Ca^{2+}, Mg^{2+}, K^+), and low quantities of exchangeable Na^+ and Al^{3+}+H^+.

The treatments were subjected to the same conditions of temperature, humidity, and cultural care, including weed elimination (manual) and pathogen control, which

Table 1. Substrate analysis results of the experimental area. San Lorenzo, 2015.

Substrates	pH	MO	P	Ca^{+2}	Mg^{+2}	K^{+}	Na^{+}	Al^{+3}+H	Texture Class
		%	…..	mg/kg	…….	$cmol_c$/kg	……	%	
Substrate 1	5,40	2,39	64,54	4,21	1,25	0,22	0,06	0,31	Sandy
Substrate 2	5,80	1,12	109,72	3,21	1,81	0,22	0,02	0,00	Sandy
Substrate 3	5,60	1,48	85,74	3,01	1,35	0.13	0,03	0,00	Sandy

consisted of preventive applications of the fungicide Mancozeb 75 WG® (Shanghai E-Tong Chemical Co., Ltd.) composed of ethylene (bis) dithiocarbamate, at a dose of 1 g/L of water. Applications were made twice: first before planting the cuttings and second at 40 days after experiment installation. After installation, irrigation was carried out daily by hand.

At 90 days after planting in each season, the following variables were evaluated: cuttings with shoots and without callus; cuttings with shoots and without roots; cuttings with shoots and with callus; and presence of shoots with callus formation at the distal end of the cutting.

The cutting survival percentage was calculated by counting cuttings per treatment according to the assigned categories (A: Cuttings without shoots with callus; B: Cuttings with shoots without callus; C: Cuttings with shoots with rootlets; and D: Cuttings with shoots with callus), dividing by the total number of cuttings per treatment and multiplying by 100.

The rooting percentage was determined by counting the number of cuttings with rootlets per treatment, dividing by the total number of cuttings planted per treatment, and multiplying by 100.

The average number of rootlets was calculated by counting the rootlets emerging from the distal end of the cuttings and calculating the mean per treatment.

The average rootlet length was determined by measuring each rootlet and calculating the mean per treatment. These data were obtained by carefully separating each cutting from the substrate by washing with abundant water to prevent root detachment. A destructive sampling method was used.

The rooting index was determined using the method proposed by Díaz, Viera y Vargas (1995), which assigns survival categories and weights to identify the substrate that produces the highest quality plants (Table 2).

Table 2. Categories and Weights of Cutting Survival**.**

Categories	Symbol	Weights
Cuttings without shoots with callus	A	1
Cuttings with shoots without callus	B	2
Cuttings with shoots with callus	D	3
Cuttings with shoots with roots	C	4

Data adapted from Díaz et al. (1995)

To find the rooting index (RI) the following formula was applied:

$$RI: \frac{(C \times 4) + (D \times 3) + (B \times 2) + (A \times 1)}{\text{Total number of cuttings planted}}$$

Where: RI = Rooting index; C = Number of cuttings with shoots and roots; D = Number of cuttings with shoots and callus; B = Number of cuttings with shoots without callus; A = Number of cuttings without shoots with callus. The results were analyzed using analysis of variance (ANOVA) and means were compared using Tukey’s test at 5% probability using InfoStat® statistical software.

RESULTS AND DISCUSSION

Analysis of the studied factor

Season factor

Regarding the seasonal effect (Table 3), statistically significant differences were observed across all treatments, with the best responses obtained in spring-summer, showing higher percentages of survival and rooting, rooting index, rootlet length, and number of rootlets compared to autumn-winter.

The results obtained coincide with the research of Soto, Jasso-Mata, Vargas, González y Cetina (2006) on *Ficus benjamina* L., where the spring season resulted in a higher rooting percentage (76.9%), as well as increased number of roots, root volume, and plant height during the rooting period.

Hartmann and Kester (1998) state that the best rooting results are obtained from root cuttings collected in late winter or early spring, when roots have abundant nutrient reserves.

The harvesting time of the material is also a factor that influences rooting. The flowering period can be antagonistic to rooting. Better results are often obtained when cuttings are collected before or after this process (Hartmann et al., 2011). Boutherin and Bron (2005) state that cuttings should be collected during the vegetative dormancy of the mother plant in late winter or early spring, when it has sufficient stored nutrient

The results indicate that adventitious root production behavior is species-specific and can also be influenc d by environmental factors such as light, temperature, photoperiod, and the physiological state of the plant (Hartmann and Kester, 1998).

Table 3. Effect of substrate type, with and without indolebutyric acid (IBA) application, on the seasonal vegetative multiplication of *P. pinnata*

Variables	Season (SE)		Sustrate (S)			Hormone (AIB)	
	SE1	SE2	S1	S2	S3	CH	SH
Survival (%)	58,23 a	84,60 b	68,63 a	72,80 a	72,83 a	73,48 a	69,35 a
Rooting (%)	36,11 a	61,11 a	43,75 a	50,00 a	52,08 a	47,22 a	50,00 a
Number of rootlets	1,67 a	2,9 b	2,67 a	2,31 a	1,94 a	2,42 a	69,35 a
Length of rootlets	4,81 a	18,7 b	13,76 a	12,34 a	9,21 a	13,53 a	10,01 a
Rooting index	2,07 a	3,05 b	2,45 a	2,53 a	2,70 a	2,55 a	2,57 a

SE1: autumn-winter, SE2: spring-summer. S1: soil, cattle manure (50:50), S2: soil, worm humus (50:50), S3: soil, cattle manure, worm humus (50:25:25). CH: with hormone, SH: without hormone. Different letters indicate significant differences (p 0.05).

Substrate factor

For the substrate factor (Table 3), there were no significant differences among all variables analyzed; however, a numerical difference was observed with the substrate composed of soil, cattle manure, and worm humus (50:25:25), which showed higher percentages of survival and rooting.

Cantero García et al. (2021), evaluating substrates in *Pongamia pinnata* plants, found no statistical differenc s for rooting percentage, number of rootlets, rootlet length, number of shoots, shoot length, and number of cuttings with callus. Using worm castings, they obtained the best rooting percentage and highest number of cuttings with callus formation, as well as the highest number and length of roots and shoots. These results are similar to those of Ormeño, Ovalle, Terán y Rey (2013), who, working on guava seedling development, found that the best treatment was the combination of 20% manure tea substrate plus 10% vermicompost liquid humus, as it resulted in better development, greater height, and a higher number of secondary roots, providing plants with better conditions for field t ansplanting.

Hormone factor

Regarding the hormone factor (IBA), Table 3 shows no significant differences among all variables; however, numerically, hormone application resulted in a higher percentage of survival and greater rootlet length, while treatments without IBA showed better results in rooting percentage, number of rootlets, and rooting index. Regarding other related studies, according to Azcón and Talón (2008), rootlet formation occurs with the resumption of cambial mitotic activity in spring, along with the activation of IAA (3-indoleacetic acid) biosynthesis after winter dormancy. Kesari, Krishnamachari y Rangan (2009) reported positive effects using different concentrations and combinations of auxins for adventitious rooting of *P. pinnata* stem cuttings; however, auxin concentrations higher than 7 mM inhibited cutting rooting.

Table 4. Average statistical significance of p-values for rooting percentage (RP), rootlet number (NR), and rootlet length (RL) in Pongamia root cuttings.

Effect	GL	p - valor			
		PS (%)	PE (%)	NR (Unidad)	LR (cm)
A: Season	1	0,0037*	0,0211*	0,0111*	0,0071*
B: Sustrate	2	0,2102 ^{ns}	0,3945 ^{ns}	0,1317 ^{ns}	0,1769 ^{ns}
C: Hormone	1	0,7386 ^{ns}	0,3778 ^{ns}	0,4750 ^{ns}	0,9798 ^{ns}
AxB	2	0,5576 ^{ns}	0,3479 ^{ns}	0,9356 ^{ns}	0,9513 ^{ns}
AxC	1	0,5052 ^{ns}	0,5558 ^{ns}	0,9448 ^{ns}	0,4543 ^{ns}
BxC	2	0,4238 ^{ns}	0,2591 ^{ns}	0,0393 ^{ns}	0,6614 ^{ns}
AxBxC	2	0,5892 ^{ns}	0,3479 ^{ns}	0,4290 ^{ns}	0,5501 ^{ns}
Error	60				
CV (%)		52,74	77,43	85,29	106,46

GL: degrees of freedom *: significant at 0.05, ns: not significant, PS: surv al percentage, PE: rooting percentage, NR: number of rootlets, LR: rootlet length.

Our results are similar to those reported by Rodríguez et al. (2015), who observed that IBA application improves root system quality by increasing root number and length, although without significant effects on *Ugni molinae* ($p > 0.05$).

Analysis of interaction of factors

The degrees of freedom and statistical significanc (p-values) for the evaluated variables are shown in Table 4.

The analysis determined that season (Factor A) had a direct effect on *Pongamia* root cuttings ($p < 0.05$). Season 2 (spring-summer) was favorable compared to season 1 (autumn-winter), which showed lower values. This may be due to reduced physiological activity of the cuttings during cold season dormancy. Hartmann and Kester (1998) state that root formation in vegetative propagation by cuttings may depend on certain inherent factors, such as the physiological state of the mother plant and climatic variables. For the other factors studied, substrate (Factor B) and hormone application (Factor C), there was no interaction between factors ($p < 0.05$).

CONCLUSIONS

The multiplication of *P. pinnata* (L.) Pierre from root cuttings was successful under the experimental conditions, providing an additional method for asexual plant production, as propagation is normally conducted using aerial parts.

Based on the rooting index as the best indicator of vegetative propagation, it is concluded that oil carob tree root cuttings are not influenced by the application of commercial hormone Raizol® (0.2% IBA) or substrate types. However, they are aff cted by planting season, with spring-summer being the most suitable period for clonal propagation through root cuttings.

ACKNOWLEDGEMENTS

The authors want to thank to Griselda Meza for the translation of the manuscript

BIBLIOGRAPHIC REFERENCES

Azcón, B. J. y Talón, M. (2008). *Fundamentos de la fisiología vegetal.* Barcelona: McGRAW-HILL.

Boutherin, D. y Bron, G. (2005). *Reproducción de las plantas hortícolas*. Barcelona: OMEGA.

Cantero García, I., Benítez Núñez, J., Enciso, M., y Samudio Oggero, A. (2021). Propagación vegetativa de *Pongamia pinnata* (L.) Pierre (Algarrobo aceitero) por mini esquejes de raíz. *Steviana, 13*(1), 5–12. Recuperado de: https://doi.org/10.56152/StevianaFacenV13N1A1_2021

Cuellar, N. (2018). *Manual práctico de reforestación.* Bogotá: Grupo Latino editores.

Chambilla, L. (2018). Efecto de las hormonas de enraizamiento en esquejes de álamo (*Populus deltoides*) bajo riego por capilaridad. *Apthapi, 4* (1), 1009-1020.

Degani, E., Prasad, M., Paradkar, A., Peña, R., Soltangheisi, A., Ullah, I., Warr, B. y Tibbett, M. (2022). Una revisión crítica de las múltiples aplicaciones de *Pongamia pinnata*: desde la remediación de tierras y el secuestro de carbono hasta los beneficios socioeconómicos. *Revista de Gestión Ambiental., 324,* 1-20. Recuperado de https://www.sciencedirect.com/science/article/pii/S0301479722018709?via%3Dihub

Díaz, Y., Viera, J. y Vargas, G. (1995). Possibility of vegetative propagation by cuttings of Pachecoa venezuelensis Burkart. *Revista Agronomía Tropical, 45*(4): 551-559

Hartmann, H. y Kester, D. (1998). *Propagación de plantas: principios y prácticas*. México, Continental.

Hartmann, H., Kester, D., Davies, F. y Geneve, R. (2011). *Plant propagation: Principles and Practice*. Eighth edition. New Jersey, USA. Prentice Hall. 915 p.

Kesari, V., Krishnamachari, A., y Rangan, L. (2009). Effect of auxins on adventitious rooting from stem cuttings of candidate plus tree *Pongamia pinnata* (L.), a potential biodiesel plant. *Springer, 23*(3), 597-604. Recuperado de: https://www.researchgate.net/publication/226137906

Rika Kubota, V., Enciso Gómez, M. M. y Vera de Ortiz, M. (2015). Influencia del tamaño de maceta y la composición de sustrato sobre la calidad de *Pongamia pinnata* (L.) Pierre. *Investigación agraria, 17*(1), 65-71. https://www.agr.una.py/revista/index.php/ria/article/view/280/283

Islam, A.K.M.A., Chakrabarty, S., Yaakob, Z., Ahiduzzaman, M. y Kalam Mohammad Mominul Islam, A., (2021). Koroch (*Pongamia pinnata*): un recurso prometedor sin explotar para los trópicos y subtrópicos. En: Cristina Gonçalves, A., Sousa, A., Malico, I. (Eds.), *Biomasa forestal: de los árboles a la energía*. IntechOpen, Londres. Recuperado de https://doi.org/10.5772/intechopen.93075

Ormeño, M., Ovalle, A., Terán, N. y Rey, J. (2013). Evaluación de diferentes abonos orgánicos en el desarrollo de plantas de guayaba y calidad de los suelos en vivero. *Agronomía Tropical, 63*(1-2), 73-84. Recuperado de: https://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0002-192X2013000100008

Rodríguez, M., Carrillo, R., Chacón, M., Hormazábal, N., Tampe, J. & Tighe, R. (2015). Enraizamiento in vitro y ex vitro de microtallos de *Ugni molinae* Turcz., una especie nativa de Chile. Gayana. *Botánica, 72*(1), 14-20.

Soto, L., Jasso-Mata, J., Vargas, J., González, H. y Cetina, V. (2006). Efecto de diferentes dosis de AIB sobre el enraizamiento de *Benjamina ficus* L. en diferentes épocas del año. *Revista Ra Ximhai. 2*(3):795-814. Recuperado de https://www.redalyc.org/pdf/461/46120312.pdf