



## Vegetative propagation of *Manilkara bidentata* (A.DC.) A.Chev. using mini-tunnels in the Peruvian Amazon region

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### Abstract

**Aim of study:** *Manilkara bidentata* (A.DC.) A.Chev. ('quinilla') is a Neotropical tree highly threatened by extensive agricultural practices and climate change, resulting in a substantial reduction of natural stands and seed availability. Commercially, the propagation through seeds of this species is severely impeded by a low germination rate. Vegetative propagation could be an alternative tool for overcoming this limitation. This study aims to evaluate the vegetative propagation of *M. bidentata* by rooted cuttings using mini-tunnels in the Amazon.

**Area of study:** National University of San Martín, Tarapoto, Region of San Martín, Peru. Forested areas at three localities in the Picota province and two localities in the Bellavista province, San Martín Region, were visited for the selection of plus trees and collection of epicormic shoots from stumps.

**Materials and methods:** the study was performed on leaf areas of 0, 50, and 100% with indole-3-butyric acid (IBA) treatments of 0, 3000, and 6000 ppm, for 9 treatments combinations. The experiment consisted of 3 mini-tunnels, with 3 growth trays established on each mini-tunnel, and 16 cuttings established on each tray (and per treatment), for a total of 144 cuttings.

**Main results:** after 55 days, the best scores in rooting rates (75%), number of roots (3.88), root length (3.26 cm), and budding percentage (94%) were obtained for the combination of 50% leaf area left with 3000 ppm of IBA.

**Research highlights:** we propose the technique of mini-tunnels as a tool for the cloning, rescue, and germplasm conservation of *M. bidentata*.

**Keywords:** mini-tunnels; Peruvian Amazon; quinilla; rooting; vegetative propagation.

**Abbreviations used:** indole-3-butyric acid (IBA).

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## Introduction

*Manilkara bidentata* (A.DC.) A.Chev. ('quinilla' in Spanish) is a forest tree species belonging to the family Sapotaceae used in the Peruvian Amazon for its wood. Besides climate change, this species is highly threatened by extensive agricultural and grassland establishment, leading to its genetic erosion through the substantial reduction of natural stands and seed availability. *M. bidentata* is a shade-tolerant perennial and dioecious species with epigeal, limited, and irregular germination (Reynel *et al.*, 2003), and a low number of aggregated seedlings in the understory growing near the maternal tree (Uriarte *et al.*,

2005). Thus, seedling emergence is more dependent on seeds availability, space, and resources under the maternal tree canopy, than on the seeds' germination capacity. Still, seeds may present some type of dormancy associated to the hardness of the seed coat growing at low light levels.

There is an urgent need to plant and protect *M. bidentata* in the Peruvian Amazon, but so far this has been done mainly using seeds and heterogeneous and low-quality germplasm. Unlike seed propagation, clonal propagation allows selecting bigger, more vigorous genotypes. Vegetative propagation emerges as an alternative tool to overcome dispersal limitations and massively replicate selected (high-quality) genotypes in *M. bidentata* (Vallejos-Torres

*et al.*, 2014; Solis *et al.*, 2019), hopefully allowing the rescue of this economically important species. Auxin application can be further improved with the use of mini-tunnels, technique so far not proven in *M. bidentata*, and which can allow the efficient transformation of hundreds of cuttings into plants using relatively few space and time (Vallejos-Torres *et al.*, 2014, 2020). In Peru, rooting of cuttings in this type of mini-tunnels has proven very successful in a number of tropical species as *Calycophyllum spruceanum* (Vallejos-Torres *et al.*, 2014), *Coffea arabica* (Vallejos-Torres *et al.*, 2020), *Plukenetia volubilis* (Solis *et al.*, 2019; Ruiz-Solsol & Mesén 2010) *Hevea brasiliensis* (Vallejos-Torres *et al.*, 2021), and *Simarouba amara* (Soudre *et al.*, 2010).

It is well established for each species that there is an optimal leaf area for rooting, as it reaches a maximum when cuttings/seedlings are photosynthetically active, leading to root development and producing elongation assimilates. This has been proven in several tropical trees (Díaz, 1991). Auxins and co-factors are generated in young leaves and cuttings, then transported to the stem base where they induce rooting (Morales *et al.*, 2014). Despite this, in some Amazonian trees the relationship between leaf area and rooting is not so clear (Díaz, 1991). A combination of an unequal hormonal distribution in the plant material and a need for reducing its transpiration (Wu *et al.*, 2015) could result in this unclear relationship, which needs further and specific exploration in *M. bidentata*. The objective of this study was to determine the effects of IBA dose (0, 3000, and 6000 ppm) and leaf area (0%, 50%, and 100%) in the vegetative propagation of *M. bidentata* rooted cuttings using plastic mini-tunnels.

## Materials and Methods

### Study site and preparation of plant material

This study was conducted in the nursery of the Plant-Tissue Culture Laboratory, National University of San Martín, Tarapoto, San Martín Region, Peru, in July-August, 2019 (06°35'28"S, 76°18'47"W; altitude: 330 m.a.s.l.; mean annual temperature: 24.6°C; mean monthly precipitation: 63.1 mm; 45% average humidity) (El Porvernir weather station, 2019). Forested areas at the localities of Winge, Huañipo, and Leoncio Prado in the Picota province (6°58'22.7899"S, 76°15'40.6191"W; 276 m.a.s.l.), and at the localities of San Rafael and Carhuapoma in the Bellavista province (6°59'06.4255"S, 76°32'55.6043"; 286 m.a.s.l.) (San Martín Region) were visited for the selection of plus trees, and for the collection of 10-15 cm (in length) epicormic shoots from stumps that were cut and/or burned by farmers. 20 shoots were collected by each ortet; 10 ortets were collected on each locality between 6:00 am to 8:00 am to avoid hydric

stress due to cold transportation (ice and freeze gel at the bottom followed by layers of wet paper and the shoots).

The 10-15 cm hardwood epicormic shoots had an average diameter of 6.5 mm and were trimmed carefully just above a node (straight cut) in 6-8 cm long terminal shoot cuttings, with leaf are left intact (reduction 0%), halved (reduction 50%), or totally removed (reduction 100%). Cuttings were placed in containers with water to avoid physiological stress, soaked in a sodium hypochlorite solution diluted in sterile water (2%) for 5 minutes, and then disinfected in a fungicide solution (thiophanate methyl 2 g/L) for 3 minutes. The auxin used was the indole-3-butyric acid (IBA) (96%, Merck) -dissolved in absolute ethanol as a solvent, in three concentrations: 0 ppm, 3000 ppm, and 6000 ppm. IBA application was precisely directed to the cuttings base with a 10 µl micropipette, ensuring that all cuttings received the same solution amount (10 µl), allowing an exact control of the IBA amount and concentration applied on each cutting. Alcohol was evaporated with a cold air stream for 30 s directed to each cutting base.

### Rooting substrate and mini-tunnels environment

Medium-size sand, sieved, and washed with running water (rinsed 4 times) and sodium hypochlorite (diluted in sterile water at 2%), was used as rooting substrate. The sand was sun-dried and sterilized in an autoclave (131°C x 15 lb pressure, for 2 hours). After applying the 10 µl IBA dose to the cuttings base, they were placed in growth trays (52 cm X 28 cm; 180 g) with the sterilized sand at an approximate depth of 2 cm. The rooting was carried out in a 48 m<sup>2</sup> greenhouse with 6 mini-tunnels, each with 3 nebulizers to prevent desiccation and hydric stress. Mini-tunnels operate with an automated irrigation/cooling system that maintains relative humidity above 75%, and a temperature of 28°C – 35°C. An automated irrigation system controlled by a timer allowed 1-minute irrigation every 2 hours (from 7 am to 5 pm) with a frequency of 5 times/day, supplying a total of 21 L of water/day per mini-tunnel. Mini-tunnels were made of a galvanized metal frame with dimensions 1 m x 3 m x 0.60 m with 1 m-long support legs and covered with white transparent plastic which allows the passage of diffuse sunlight.

After 55 days, when four 3.5 cm roots per cutting were obtained in average (determined by randomly picking cuttings), the established trays in mini-tunnels were removed to extract the *M. bidentata* rooted cuttings. The experiment consisted of 3 mini-tunnels, with 3 growth trays established on each mini-tunnel, and 16 cuttings established on each tray, for a total of 144 cuttings. The rooted cuttings were acclimatized in a nursery with a shade system allowing 20% sunlight. Micro-spray irrigation was made 4 times per day (eight 200 ml sprinklers were

used, supplying a total of 6.4 L of water/day) to maintain the turgidity of the rooted cuttings, which achieved their hardening after 100 days.

## Experimental design and data analyses

The experimental design adopted was a full factorial of the 2 experimental factors investigated, *i.e.* IBA concentrations (0, 3000, and 6000 ppm; each one in a separate mini-tunnel) and leaf surface (0, 50, and 100%), corresponding to 9 combinations (each one in a separate tray). 16 cuttings were used for each combination, for a total of 144 cuttings. Data were analyzed in SPSS by the Tukey test with a significance level of  $p < 0.05$  to determine the nature of the differences between treatments and combinations. ANOVAs were performed to examine the effects of IBA dose, leaf area, and their interaction in the different parameters measured. Prior to analysis, the number of roots was transformed to  $\sqrt{(x + 1)}$ , and the percentages of rooting and budding were transformed to  $\arcsin \sqrt{\%}$  of rooting. Before and after the transformations, the homogeneity of variances and the normality of the residuals were checked using the Bartlett test and graphical checks, respectively.

## Results and Discussion

The treatment combination of 3000 ppm of IBA with a 50% of leaf area had a greater number of roots (3.88 roots

per cutting), rooting (75%), root length (3.26 cm), and budding (94%), compared to the other combinations (Table 1a). When doing ANOVAs, just budding was affected by IBA dose and leaf area (but not their interaction), while the other variables were not (Table 1b). As in other studies, we found that the auxin dose reached a plateau (saturation), after which rooting decreased, possibly due to the toxic effects of overdose. Possibly, with the above-mentioned combination, a greater photosynthetic, hormonal, and/or co-factor balance and movement of carbohydrate reserves in the cuttings during the rooting process were promoted, at the same time covering the nutritional needs during this process. Rooting is maximized when cuttings are most photosynthetically active (Newton *et al.*, 1992; Mesén *et al.*, 1997). For *Plukenetia polyadenia* and *P. volubilis*, Solís *et al.* (2017, 2019) has obtained akin results using similar combinations of leaf area (50 – 75%) with IBA dose (2000 ppm). In *Simarouba amara*, a combination of 60 cm<sup>2</sup> of leaf area and a higher IBA dose (8000 ppm), did not lead to a higher rooting (64%) (Soudre *et al.*, 2010).

Our results are also consistent with those of other Amazonian trees (Mesén, 1998). *Calycophyllum spruceanum* was successfully rooted (99%) using 2 leaves cuttings and 3000 ppm of IBA (Vallejos-Torres *et al.*, 2014). Similar results were obtained for *Plukenetia volubilis* (Ruiz-Solsol & Mesén, 2010), *Cabralea canjerana* (Gimenes *et al.*, 2015), *Prunus africana* (Tchoundjeu *et al.*, 2002), *Gmelina arborea* (Díaz 1991), and *Cheilocostus speciosus* but using 500 mg/L of 1-naphthalenacetic acid (Ballesteros

**Table 1.** Effects of the interaction of different doses of indole-3-butyric acid (IBA) and leaf area levels in the vegetative propagation of *Manilkara bidentata* using mini-tunnels, after 55 days.

a. IBA dose (ppm)	Leaf area	Number of roots	Rooting (%)	Roots length (cm)	Budding (%)
0	0%	0.25 <sup>c</sup>	13.00 <sup>c</sup>	0.78 <sup>c</sup>	19.00 <sup>c</sup>
0	50%	0.37 <sup>c</sup>	19.00 <sup>c</sup>	2.09 <sup>ab</sup>	69.00 <sup>ab</sup>
0	100%	0.50 <sup>c</sup>	19.00 <sup>c</sup>	1.81 <sup>ab</sup>	56.00 <sup>abc</sup>
3000	0%	0.50 <sup>c</sup>	38.00 <sup>abc</sup>	2.44 <sup>ab</sup>	44.00 <sup>bc</sup>
3000	50%	3.88 <sup>a</sup>	75.00 <sup>a</sup>	3.26 <sup>a</sup>	94.00 <sup>a</sup>
3000	100%	2.38 <sup>ab</sup>	69.00 <sup>ab</sup>	1.31 <sup>b</sup>	69.00 <sup>ab</sup>
6000	0%	0.75 <sup>c</sup>	19.00 <sup>c</sup>	1.72 <sup>ab</sup>	69.00 <sup>ab</sup>
6000	50%	1.50 <sup>bc</sup>	50.00 <sup>abc</sup>	1.93 <sup>ab</sup>	75.00 <sup>ab</sup>
6000	100%	0.50 <sup>c</sup>	25.00 <sup>bc</sup>	2.03 <sup>ab</sup>	88.00 <sup>ab</sup>
CV (%)		3.16	11.9	3.14	6.83
b. ANOVA					
IBA dose		2.191 <sup>ns</sup>	0.721 <sup>ns</sup>	0.881 <sup>ns</sup>	8.337 <sup>**</sup>
Leaf area LA		2.917 <sup>ns</sup>	2.878 <sup>ns</sup>	0.042 <sup>ns</sup>	8.337 <sup>**</sup>
IBA dose X LA		0.311 <sup>ns</sup>	0.602 <sup>ns</sup>	0.695 <sup>ns</sup>	0.666 <sup>ns</sup>
R <sup>2</sup>		0.037	0.011	0.009	0.110

a: Tukey's test ( $p < 0.05$ ). Averages followed by different letters indicate statistically significant differences.  
b:  $F_{p\text{-value}}$  shown;  $p < 0.01$ : \*\*, ns: non-significant.

& Álvarez, 2017). De Souza *et al.*, (2014) reached 98-100% rooting of *Prosopis alba* cuttings under different IBA doses (3000 – 7500 mg/L). These studies tend to show the positive effect of leaf area on the stimulation of the rooting process (Moraes *et al.*, 2014) -in part related to photosynthetic activity during propagation, but there is not always a direct relationship between leaf area and rooting, especially in tropical species (Díaz, 1991). Thus, it is vitally important to know the optimal range of leaf area and its effect on forest species to achieve optimal rooting. Root induction is one of the most exploited physiological effects of auxins, driven by the stimulation of cell dedifferentiation. Auxins have been associated to an increase in carbohydrates and co-factors transport and DNA synthesis, resulting in higher cell division, and therefore higher rooting (Hartmann & Kester, 1983; Gaspar & Hofinger, 1988). This, in turn, strongly affects photosynthetic performance, spectral reflectance, water potential, cell tissue electrical conductivity, and chlorophyll content, all of which have great importance for plant survival (Wu *et al.*, 2015).

Just in a couple of studies the vegetative propagation of *M. bidentata* has been assessed. When auxins were not used and 2-4 cm cuttings were sown in a soil-sand-sawdust mix, rooting of this species just reached 40% (Rodríguez & Avella, 2005). In contrast, Cervantes Owaki (2011) obtained an 83% rooting in *M. bidentata* cuttings (obtained from commercial seedlings) growing on a sand medium with 0.8% of indole-3-butyric acid (IBA) under sub-irrigation chambers. Although our maximum rooting was somewhat lower (75%), Cervantes Owaki (2011) cut living seedlings to obtain the cuttings, thus killing one plant to obtain other -while our study was based on epicormic shoots from stumps that were already cut and/or burned by farmers. Given that the cutting and burning of *M. bidentata* is -unfortunately- a common practice (to expand paddocks and crops, and/or the wood to be used for fences and firewood), we think that it is better to take advantage of this situation. Another difference is the use of costly (Cachique *et al.*, 2011) sub-irrigation chambers, which, unlike mini-tunnels, allow the propagation of only a few dozen cuttings. Thus, our study can be a first step into the massive production of advantageous genotypes of *M. bidentata* in a relatively inexpensive manner.

Murillo *et al.* (2001) considers that 70% is the minimum rooting percentage required to justify a commercial clonal propagation program. In this study we optimally achieved (rooting of 75%) the vegetative propagation of *Manilkara bidentata* through the use of cuttings with a 50% of leaf area and 3000 ppm of IBA, successfully obtaining vigorous cuttings. This technique could allow the rescue and repopulation of this species in its natural habitat. The propagation by rooting through mini-tunnels can be used to conserve the germplasm of this species for future genetic improvement, rapidly multiplying superior

genotypes, thus avoiding the loss of plant material due to the expansion of the agricultural frontier.

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## References

- Ballesteros ED, Álvarez AH, 2017. Enraizamiento de esquejes de Caña Agría (*Cheilocostus speciosus*. J. Koenig). *Rev Colomb Biotec* 19: 133-139. <https://doi.org/10.15446/rev.colomb.biote.v19n2.70395>
- Cachique D, Rodríguez del Castillo ÁM, Ruíz-Solsol H, Vallejos G, Solis R, 2011. Propagación vegetativa del sacha inchi (*Plukenetia volubilis* L.) mediante enraizamiento de estacas juveniles en cámaras de subirrigación en la Amazonía Peruana. *Folia Amazónica* 20: 95-100. <https://doi.org/10.24841/fa.v20i1-2.348>
- Cervantes Owaki D, 2011. Propagación vegetativa de quinilla (*Manilkara bidentata*, A.DC.) mediante el enraizamiento de estaquillas utilizando cámara de subirrigación en el distrito de Morales provincia de San Martín. Bachelor Thesis, Universidad Nacional de San Martín-Tarapoto, Perú.
- De Souza JCAV, Bender AG, Tivano JC, Barroso DG, Mroginski LA, Vegetti AC, Felker P, 2014. Rooting of *Prosopis alba* mini-cuttings. *New Forests* 45: 745-752. <https://doi.org/10.1007/s11056-014-9429-5>
- Díaz E, 1991. Técnicas de enraizamiento de estacas juveniles de *Cedrela odorata* L. y *Gmelina arborea* Linn. Master thesis, Centro Agronómico Tropical de Investigación y Enseñanza - CATIE, San José, Costa Rica.
- Gaspar T, Hofinger M, 1989, Auxin metabolism during rooting. In: Adventitious root formation in cuttings; Davis TD, Haissig BE, Sankhla N (eds). pp: 171-131. Dioscorides Press, Portland, United States.
- Gimenes ES, Kielse P, Haygert KL, Fleig FD, Keathley DE, Bisognin DA, 2015. Propagation of *Cabralea canjerana* by mini-cutting. *J Horticult For* 7: 8-15.
- Hartmann HT, Kester DE, 1983. Plant propagation: principles and practices, 2nd Ed. Prentice-Hall, Englewood Cliffs, NJ, United States.
- Mesén F, Newton AC, Leakey RRB, 1997. The effects of propagation environment and foliar area on the rooting physiology of *Cordia alliodora* (Ruiz & Pavon) Oken cuttings. *Trees* 11: 404-411. <https://doi.org/10.1007/PL00009683>

- Mesén F, 1998. Enraizamiento de estacas juveniles de especies forestales: uso de propagadores de sub-irrigación. Centro Agronómico Tropical de Investigación y Enseñanza - CATIE, Turrialba, Costa Rica.
- Moraes CE, Fonseca RCM, Rui M, 2014. Influência das folhas no enraizamento de miniestacas de híbridos de eucalipto. *Nucleus* 11: 101-106. <https://doi.org/10.3738/1982.2278.995>
- Murillo O, Rojas JL, Badilla Y, 2001. Reforestación clonal. Instituto Tecnológico de Costa Rica, Cartago, Costa Rica.
- Newton AC, Muthoka PN, Dick JM, 1992. The influence of leaf area on the rooting physiology of leafy stem cuttings of *Terminalia spinosa* Engl. *Trees* 6: 210-215. <https://doi.org/10.1007/BF00224338>
- Reynel C, Pennington RT, Pennington TD, Flores C, Daza A, 2003. Árboles útiles de la Amazonía peruana: un manual con apuntes de identificación, ecología y propagación de las especies. Lima, Perú. 509 pp.
- Rodríguez R, Avella A, 2005. Propagación y diagnóstico de regeneración natural de algunas especies maderables empleadas por la comunidad indígena de Mocagua (PNN Amacayacu. Amazonas, Colombia). *Colombia Forestal* 9: 34-51. <https://doi.org/10.14483/udistrital.jour.colomb.for.2005.1.a03>
- Ruiz-Solsol H, Mesén F, 2010. Efecto del ácido indolbutírico y tipo de estaquilla en el enraizamiento de sacha inchi (*Plukenetia volubilis* L.). *Agron Costarricense* 34: 259-267. <https://doi.org/10.15517/rac.v34i2.3636>
- Solis R, Pezo M, Diaz G, Arévalo L, Cachique D, 2017. Vegetative propagation of *Plukenetia polyadenia* by cuttings: effects of leaf area and indole-3-butyric acid concentration. *Braz J Biol* 77: 580-584. <https://doi.org/10.1590/1519-6984.20415>
- Solis R, Gomales N, Pezo M, Arévalo L, Vallejos-Torres G, 2019. Rooting of sacha inchi (*Plukenetia volubilis*) juvenile cuttings in microtunnels. *Acta Agron* 68: 35-40. <https://doi.org/10.15446/acag.v68n1.72101>
- Soudre M, Vidal F, Mori J, Guerra H, Mesén F, Perez F, 2010. Propagación vegetativa de marupa (*Simarouba amara* Aubl.) mediante enraizamiento de estacas juveniles en propagador de subirrigación. *Folia Amazon* 19: 61-68.
- Tchoundjeu Z, Avana ML, Leakey RRB, Simons AJ, Asah E, Duguma B, Bell JM, 2002. Vegetative propagation of *Prunus africana*: effects of rooting medium, auxin concentrations and leaf area. *Agrofor Syst* 54: 183-192. <https://doi.org/10.1023/A:1016049004139>
- Uriarte M, Canham CD, Thompson J, Zimmerman JK, Brokaw N, 2005. Seedling recruitment in a hurricane-driven tropical forest: light limitation, density-dependence and the spatial distribution of parent trees. *J Ecol* 93: 291-304. <https://doi.org/10.1111/j.0022-0477.2005.00984.x>
- Vallejos-Torres G, Gonzales-Polar LET, Arévalo-López LA, 2014. Enraizamiento de brotes de capirona *Calycophyllum spruceanum* (Benth.) Hook. f. ex Schum., en la amazonía peruana. *Revista Forestal Mesoamericana Kurú* 11: 55-59. <https://doi.org/10.18845/rfmk.v11i27.1778>
- Vallejos-Torres G, Arévalo LA, Ríos O, Cerna A, Marín C, 2020. Propagation of rust-tolerant *Coffea arabica* L. plants by sprout rooting in microtunnels. *J Soil Sci Plant Nutr* 20: 933-940. <https://doi.org/10.1007/s42729-020-00180-7>
- Vallejos-Torres G, Ríos-Ramírez O, Corazon-Guivin MA, Reátegui E, Mesén Sequeira F, Marín C, 2021. Effects of number of leaflets and indole-3-butyric acid dose in the vegetative propagation by mini-tunnels of the rubber tree (*Hevea brasiliensis*). *J Rubber Res*. First online. DOI: 10.1007/s42464-021-00097-5 <https://doi.org/10.1007/s42464-021-00097-5>
- Wu CW, Lin KH, Lee MC, Peng YL, Chou TY, Chang YS, 2015. Using chlorophyll fluorescence and vegetation indices to predict the timing of nitrogen demand in *Pentas lanceolata*. *Hortic Sci Technol* 33: 845-853. <https://doi.org/10.7235/hort.2015.15043>