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Hydroponics and Natural Biostimulants Accelerate Candelilla Propagation for Arid-Zone Restoration

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Abstract. This study evaluated an innovative protocol for the accelerated vegetative propagation of *Euphorbia antisyphilitica* (candelilla), an endemic shrub threatened by unsustainable harvesting in Mexico's arid regions. Three propagation systems were compared, combining hydroponics, nursery, and open-field approaches with natural biostimulants and conventional rooting agents. Hydroponics with a natural biostimulant blend of *Rosmarinus officinalis, Lens culinaris* and *Cinnamomum zeylanicum* extracts yielded the fastest and most vigorous rooting, achieving functional root systems within four weeks, a reduction of over 90% compared to traditional timelines. Survival exceeded 99%, demonstrating the reliability of this approach. In contrast, indolebutyric acid proved ineffective in hydroponics due to its instability in aqueous media. Nursery propagation highlighted the importance of substrate selection, with native mountain soil outperforming commercial and agricultural substrates, likely due to its favorable pH and natural mycorrhizal associations. Overall, the integration of hydroponics and natural biostimulants provided the most sustainable and reproducible protocol, offering significant advantages for ecological restoration, ex situ conservation, and community-based management of arid ecosystems. These results establish a scientific foundation for scaling up vegetative propagation of candelilla and similar species, reducing dependence on wild populations while promoting restoration in degraded arid landscapes.

Keywords: *vegetative propagation, rooting success, natural extracts, ex-situ conservation, arid restoration.*

Introduction

Candelilla (*Euphorbia antisyphilitica Zucc.*) is an endemic species of the arid ecosystems of northern Mexico, recognized for its economic, ecological, and sociocultural importance. This xerophytic shrub produces a vegetal wax that constitutes a strategic raw material for multiple industries—cosmetic, pharmaceutical, and food—due to its physicochemical and functional properties. Recent studies have reviewed potential applications of the wax and its by-products, as well as improvements in extraction methods and value-added strategies, reaffirming its relevance as a non-timber forest product (Aranda-Ledesma *et al.,* 2022; Núñez-García *et al.,* 2022).

Despite its wide distribution, modeling and inventory approaches reveal the species' vulnerability to overharvesting and unsustainable collection practices, which threaten the long-term persistence of natural populations (Martínez-Sifuentes *et al*., 2023). These findings underscore the urgent need for efficient vegetative propagation and restoration protocols to reduce pressure on wild populations while supporting conservation and sustainable use.

Propagation of candelilla under controlled conditions represents a viable strategy to reduce harvesting pressure while promoting more sustainable production systems (Domínguez-Martínez *et al*., 2021). Nevertheless, traditional propagation methods based on direct reimplantation exhibit slow and highly variable rooting rates, often requiring between 12 and 40 months (Flores-del Ángel *et al*., 2013). Such limitations compromise nursery efficiency and delay large-scale implementation in conservation or commercial production programs.

In this context, there is a growing need for innovative protocols that enhance the vegetative propagation of *Euphorbia antisyphilitica*. Hydroponic systems such as the Nutrient Film Technique (NFT) and the application of natural biostimulants have shown promise in accelerating rooting and improving survival in several plant species (Nunes *et al.,* 2022; Rouphael and Colla, 2020). These approaches enable precise control of environmental conditions and reduce physiological stress, which is particularly critical in arid zones where plants are exposed to water scarcity and extreme temperatures. However, their potential remains unexplored for candelilla, representing a critical knowledge gap with direct implications for conservation and restoration strategies.

The objective of this study was to develop and validate an efficient protocol for the vegetative propagation of candelilla. We hypothesized that the integration of hydroponic systems with natural biostimulants would accelerate rooting and improve survival compared to conventional approaches. Beyond addressing a technical barrier in candelilla propagation, this work seeks to contribute a replicable methodology for the conservation and sustainable management of threatened arid-zone species in Mexico and other regions facing similar challenges.

Material and Methods

Study site and plant material

The study was conducted at the Universidad Politécnica de la Región Laguna (UPRL), San Pedro de las Colonias, Coahuila, Mexico (25°47′N; 103°11′W; 1,120 m a.s.l.), under controlled environmental conditions of temperature and humidity.

A total of 50 adult plants of *Euphorbia antisyphilitica Zucc*. (30–40 cm in height; 1.5–2.0 cm basal diameter) were collected from authorized harvesting areas in Francisco I. Madero, Coahuila (permit SGPA/DGVS/08742/19). From these, 1,200 stem cuttings (12.0 ± 0.5 cm length; 0.8–1.2 cm diameter) were prepared, disinfected, rinsed, and shade-dried for 48 h to promote basal callus formation.

Experimental design

A balanced factorial design was established to evaluate the effect of three independent factors: (i) Propagation system: nursery (substrate-based), hydroponics using the Nutrient Film Technique (NFT), and open field. (ii) Rooting treatment: control (water), indolebutyric acid (IBA), and natural biostimulant. (iii) Substrate: native mountain soil, agricultural soil, and commercial succulent mix.

In nursery and hydroponic systems, all three substrates and all three rooting treatments were fully tested. In the open field, only the two rooting treatments (IBA and biostimulant) were applied, given the absence of artificial substrates. Each experimental unit consisted of 50 cuttings, and treatments were replicated across blocks to ensure balanced representation.

Environmental conditions (substrate temperature, solution pH, and EC) were monitored with HOBO® and YSI ProDSS® sensors. Substrates were characterized following the standard characterization procedures outlined in the World Reference Base for Soil Resources (ISRIC, 2020).

Natural biostimulant

A natural biostimulant was prepared using *Rosmarinus officinalis, Lens culinaris*, and *Cinnamomum zeylanicum,* following standardized extraction and formulation protocols previously validated for plant growth promotion (Souri and Bakhtiarizade, 2019; Rouphael and Colla, 2020; Calvo *et al.,* 2014). The final solution was adjusted to pH 4.5–5.0 and stabilized with natural preservatives.

Full details of raw materials, extraction procedures, and formulation steps are provided in Supplementary Material S1 to ensure replicability.

Variables measured

During 16 weeks, the following variables were recorded: Survival (%; proportion of cuttings remaining viable), Root development (primary root length and number of secondary roots), Shoot growth (stem elongation), Physiological stress (visual scale 0–3).

At week 16, mycorrhizal colonization was determined using the gridline intersect method (Koske and Gemma, 1989): cleared roots were stained with trypan blue and examined at 200× magnification; 100 intersections per root system were scored for the presence of arbuscules or vesicles.

Chlorophyll content (SPAD) was measured with a handheld chlorophyll meter (Konica-Minolta SPAD-502) on the youngest fully expanded leaf of each cutting (Guillén-Enríquez *et al*., 2022).

Pearson correlations were computed across all morphological and physiological variables listed in this section.

Statistical analysis

Data were analyzed in Python v3.10. Normality and homoscedasticity were verified (Shapiro–Wilk and Levene). Percentage survival data were arcsine-square-root transformed before ANOVA to meet normality assumptions (Zar, 2010). A three-way ANOVA (Propagation system × Rooting treatment × Substrate) was applied to root development variables, with all factors treated as fixed. When significant effects were detected ($p < 0.05$), means were compared using Tukey's HSD.

In the open-field system, where substrate was not applicable, data were analyzed with a two-way ANOVA (system × treatment), maintaining consistency with the overall factorial structure. Pearson correlations were also calculated among morphological and physiological variables.

Results and Discussion

Root development and survival: comparative analysis across systems

The factorial ANOVA revealed significant effects of propagation system, rooting treatment, and substrate on root length, with a strong interaction between system and treatment (Table 1). This confirms that rooting success depends not only on the treatment applied but also on the propagation environment and the substrate where cuttings are established.

Table 1. Balanced factorial ANOVA results for primary root length.

Factor	df	Sum of squares	Mean square	F	p-value	Partial η²
Propagation system	2	1,247.30	623.65	287.41	<0.001***	0.681
Rooting treatment	2	891.7	445.85	205.63	<0.001***	0.578
Substrate	2	156.8	78.4	36.15	<0.001***	0.196
System × Treatment	4	234.5	58.63	27.06	<0.001***	0.285
Error	324	702.1	2.17			

The Tukey HSD post-hoc test revealed clear differences among treatments (Table 2). The full matrix of pairwise comparisons is provided in Table 2 to ensure transparency and to allow exact effect sizes to be reused in future meta-analyses or replication studies.

Table 2. Tukey HSD multiple comparisons for root length.

Comparison	Mean Difference	SE	p-value
NFT Bio vs NFT IBA	10.2 cm	0.8	<0.001***
NFT Bio vs Nursery Bio	8.8 cm	0.7	<0.001***
NFT Bio vs Open field	12.0 cm	0.9	<0.001***
NFT IBA vs Nursery Bio	-1.4 cm	0.6	>0.05
NFT IBA vs NFT Control	1.8 cm	0.6	>0.05

NFT hydroponics combined with the natural biostimulant significantly outperformed all other treatments in root length ($p < 0.001$), reaching an average of 12.0 ± 1.3 cm at 16 weeks (Figure 1). By contrast, open-field treatments failed to produce functional rooting (0.0 ± 0.0 cm).

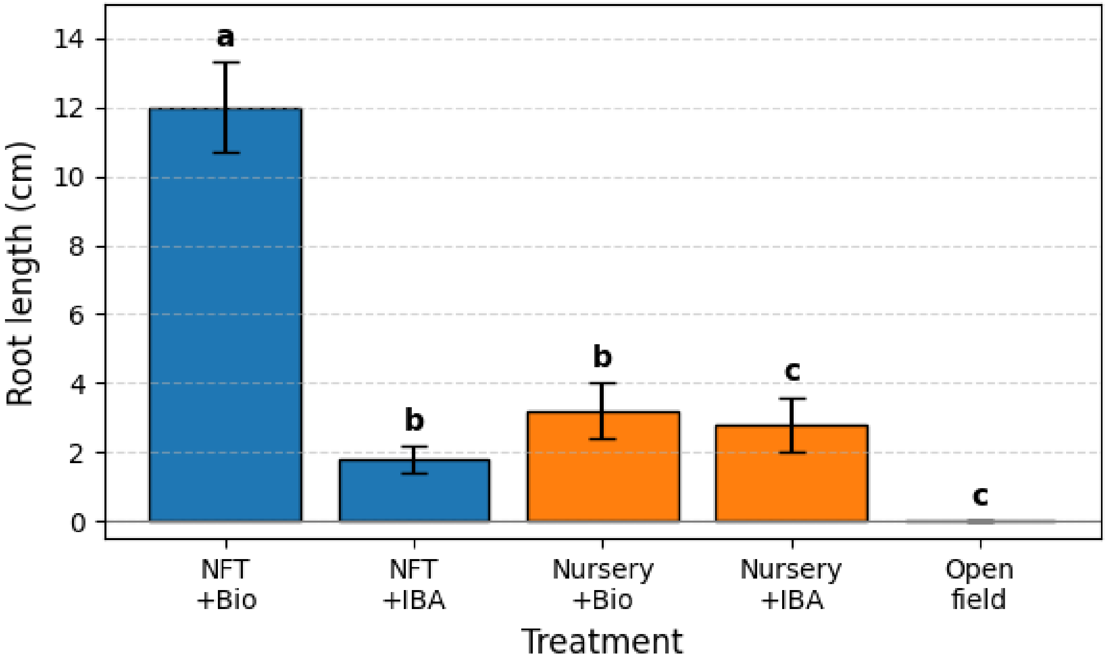

Figure 1. Root development by propagation system at 16 weeks (cm). Values are means ± SD (n = 50). Different lowercase letters above bars indicate significant differences (Tukey HSD, $p < 0.05$); NS = non-significant.

Survival and cutting viability

Survival rates varied significantly across systems and treatments (Table 3). NFT consistently showed the highest values, followed by nursery conditions, while open-field propagation was least effective. These results highlight the advantage of hydroponic systems, where stable water and nutrient supply reduce stress and the absence of soilborne pathogens minimizes losses (Jackson and Armstrong, 1999; Martínez-Ballesté and Mandujano, 2013).

Table 3. Survival of *Euphorbia antisyphilitica* cuttings after 16 weeks (back-transformed means)

System	**Biostimulant**	**IBA**	**Control**	**System mean**
NFT	99.1 ± 0.5^{a}	97.0 ± 1.1^{ab}	95.8 ± 1.4^{b}	97.3 ± 1.7^{A}
Nursery	94.2 ± 2.1^{c}	91.5 ± 2.8^{cd}	89.0 ± 3.2^{d}	91.6 ± 3.1^{B}
Field	84.5 ± 3.8^{e}	81.0 ± 2.1^{ef}	77.5 ± 4.1^{f}	81.0 ± 3.5^{C}

Different lowercase letters indicate significant differences among treatments within the same system; uppercase letters indicate differences among systems (Tukey HSD, $p < 0.05$, ANOVA on arcsine-square-root transformed data).

Across all treatments, NFT consistently outperformed nursery and open-field conditions (Table 3). This system-level superiority is linked to continuous oxygenation of the root zone and the absence of soil-borne pathogens, factors that recently have been shown to accelerate rhizogenesis in drought-adapted species (George *et al*., 2023).

The paradox of indolebutyric acid (IBA) in hydroponics

An unexpected result was the limited performance of IBA under NFT conditions. The direct comparison NFT AIB vs NFT Control yielded a mean difference of 1.8 cm (SE = 0.6, $p > 0.05$, Table 2), confirming that IBA did not outperform the hydroponic control under NFT conditions. Chemical instability of IBA in aqueous media (half-life < 48 h at neutral pH) explains its weak effect in NFT, whereas the natural biostimulant contains phenolic antioxidants (e.g., rosmarinic acid) that prolong biological activity under hydroponic conditions (Phenolic compounds from Rosemary, 2023). Although survival remained high, root elongation was minimal and not significantly different from the hydroponic control. In contrast, in nursery substrates IBA induced moderate rooting. This behavior can be explained by the chemical instability of IBA in aqueous solutions (Zaier *et al*., 2020). Conversely, the natural biostimulant provided more stable compounds such as rosmarinic acid, whose antioxidant activity enhances persistence and biological activity (Pérez-Fons *et al*., 2010).

Effect of substrate and role of mycorrhizae

The main effect of substrate detected in the ANOVA (Table 1) was confirmed by independent analysis of substrate characteristics (Table 4). Mountain soil outperformed agricultural soil and commercial mixes, showing lower cutting losses and higher mycorrhizal colonization. The presence of arbuscular mycorrhizae (*Glomus spp*.) likely contributed to nutrient uptake efficiency under alkaline conditions, reinforcing their ecological importance in *E. antisyphilitica* adaptation to arid environments (Smith and Read, 2008).

Table 4. Physical, chemical, and biological characterization of substrates (mean ± SD, n = 3)

Parameter	Mountain soil	Agricultural soil	Commercial mix	F	p-value
pH	7.8 ± 0.2^{a}	6.5 ± 0.3^{b}	6.0 ± 0.1^{c}	247.3	<0.001***
EC (dS m^{-1})	0.6 ± 0.1^{c}	1.2 ± 0.2^{a}	0.8 ± 0.1^{b}	89.7	<0.001***
Organic Matter (%)	1.2 ± 0.3^{c}	2.5 ± 0.4^{b}	3.8 ± 0.5^{a}	167.2	<0.001***
Porosity (%)	49 ± 3^{c}	55 ± 4^{b}	85 ± 5^{a}	312.8	<0.001***
Mycorrhizal colon. (%)	68.4 ± 5.2^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	891.5	<0.001***
Cutting losses (%)	9.0 ± 1.5^{c}	14.0 ± 2.1^{b}	17.1 ± 2.3^{a}	67.4	<0.001***

Different lowercase letters in the same row indicate significant differences among substrates (Tukey HSD, $p < 0.05$).

The superior performance of native mountain soil is linked to its alkaline pH (7.8) and high arbuscular mycorrhizal colonization (68 %), which may contribute to improved phosphorus uptake and drought tolerance in xerophytic shrubs (Wahab *et al*., 2023); nevertheless, other edaphic factors cannot be excluded.

Correlations among response variables

Correlations were calculated across the complete set of variables described in section 2.4 (N = 351). Correlation analysis revealed strong positive associations among root length, biomass, survival, chlorophyll content, and mycorrhizal colonization (Table 5). This indicates that early root development is closely linked to physiological performance and overall plant viability, confirming its role as a key predictor of propagation success.

Table 5. Pearson correlation matrix among response variables (N = 351)

Variable	**Root length**	**Root biomass**	**Survival**	**Chlorophyll (SPAD)**	**AMF colonization (%)**
Root length	1				
Root biomass	0.89***	1			
Survival	0.72***	0.68***	1		
Chlorophyll (SPAD)	0.67***	0.61***	0.78***	1	
AMF colonization (%)	0.58***	0.54***	0.71***	0.82***	1

***p < 0.001; correlations were performed on the complete set of variables described in section 2.4.

Reduction in rooting time and protocol feasibility

One of the most remarkable outcomes was the drastic reduction in rooting time achieved with NFT hydroponics and natural biostimulants. Functional rooting was obtained in only four weeks, compared to the year or more required by traditional methods (Figure 2). This acceleration results from the synergy of continuous oxygenation, the action of phenolic compounds and phytohormones, and the absence of soilborne pathogens (Jackson and Armstrong, 1999; Calvo *et al.,* 2014; Nunes *et al.,* 2022; Martínez-Ballesté and Mandujano, 2013). In addition, the modified Hoagland solution supplied macro- and micronutrients continuously, ensuring that rooting was not limited by nutrient deficiency, a common constraint in substrate-based or water-only systems (Resh, 2022). Although the present factorial design does not isolate individual factors (e.g., NFT without biostimulant or pathogen-free substrate-only controls), the observed acceleration is consistent with the combined effect documented here and provides a robust baseline for future experiments that statistically dissect synergistic interactions.

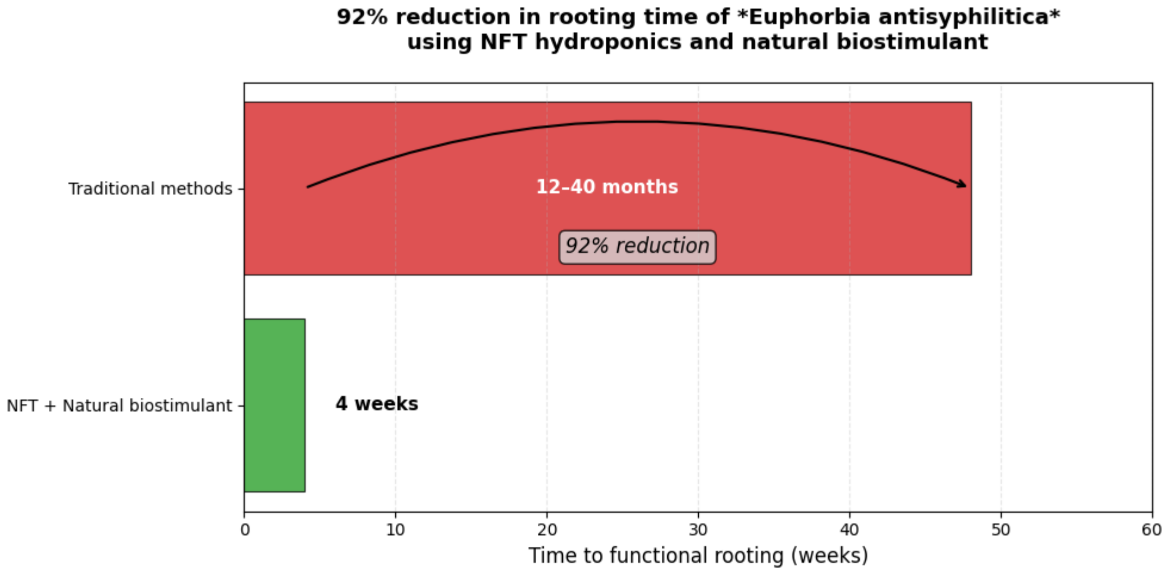

Figure 2. Reduction in rooting time between traditional methods and NFT hydroponics with biostimulants.

Implication for conservation and ecological restoration

The capacity to produce rooted seedlings of *E. antisyphilitica* within weeks instead of months or years represents a technological breakthrough with direct implications for conservation. This advance could enable large-scale restoration programs in the Chihuahuan Desert by facilitating mass production of viable plants, reconnecting fragmented populations, and enhancing long-term genetic viability (Young *et al*., 1996).

Study limitations and future directions

Despite the promising results, some limitations should be acknowledged. This study focused only on vegetative propagation and did not assess field performance after transplanting. Moreover, the protocol should be tested in other priority desert species such as Agave lechuguilla, Parthenium argentatum, and Larrea tridentata, to evaluate its broader applicability.

Conclusions

This study demonstrates an innovative and efficient protocol for the rapid propagation of *Euphorbia antisyphilitica*, combining NFT hydroponics with natural biostimulants. The approach drastically shortens rooting time and enhances seedling quality, offering a sustainable alternative to conventional propagation methods.

Although further validation under field and large-scale conditions is required, the methodology shows strong potential to support ex situ conservation, strengthen ecological restoration strategies in arid ecosystems, and offer opportunities for community-based production systems. By integrating controlled hydroponic environments with biologically active natural extracts, this protocol provides a scalable tool for conserving threatened desert species and promoting sustainable resource management in fragile landscapes.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Supplementary material S1 will be provided by corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Not applicable.

AUTHOR CONTRIBUTIONS

J.L. Ríos-Plaza: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft, Project administration. **E.M. García-Carrillo:** Investigation, Methodology, Resources, Writing – review & Editing.**A. Gonzales-Torres:** Investigation, Data Curation, Writing – Review & Editing. **M.G. Cervantez-Vázquez:** Investigation, Validation, Writing – Review & Editing. **T.J.Á. Cervantez-Vázquez:** Investigation, Validation, Writing – Review & Editing. **A.P. Galindo-Guzmán:** Investigation, Laboratory analyses, Writing – Review & Editing. **J.G. Luna-Ortega:** Conceptualization, Supervision.

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