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Effect of ROOT-HOR doses on the vegetative propagation of *Polylepis racemosa* (Ruiz & Pavon) in High-Andean ecosystems of Peru

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Abstract
The restoration of high-Andean ecosystems has become a priority due to increasing environmental degradation, biodiversity loss, and the decline of essential ecosystem services in the mountainous regions of Peru. Among native tree species, *Polylepis racemosa* Ruiz & Pavon is recognized for its ecological importance in protecting soils, regulating water resources, and conserving high-altitude biodiversity. However, restoration programs are constrained by the limited availability of vigorous seedlings and the low efficiency of vegetative propagation. This study evaluated the effect of different doses of the ROOT-HOR biostimulant on the vegetative propagation of P. racemosa under high-Andean nursery conditions in Ayacucho, Peru. A randomized complete block design was established with six treatments (0, 3, 6, 9, 12, and 15 mL L^{-1}), six replications, and 1,008 cuttings. Survival, morphometric growth, biomass accumulation, and seedling quality were assessed to determine the optimal propagation treatment. Significant differences ($p \leq 0.05$) were observed among treatments for survival, stem height, stem diameter, root length, and biomass production. The 6 and 9 mL L^{-1} doses produced the most favorable responses, with the highest survival rate reaching 61.42% and the greatest mean stem height of 29.13 cm. These treatments also promoted a more balanced distribution of root and shoot biomass, resulting in greater seedling vigor and improved establishment potential. Overall, the application of 6 mL L^{-1} of ROOT-HOR proved to be the most effective dose, providing a practical and sustainable strategy to enhance the vegetative propagation of *P. racemosa* and strengthen reforestation and ecological restoration efforts in high-Andean ecosystems.

Keywords: *vegetative propagation, hormonal regulation, plant biostimulant, ecological restoration, native species*

Introduction

Ecological restoration is prioritized to address ecosystem degradation. ITTO (2022) recognizes restoration as essential, while CIFOR-ICRAF (2022) emphasizes seedlings. Rooting regulators IBA, NAA, and IAA improve propagation, although effectiveness varies by species and concentration (ITTO, 2024). FAO (2024a) highlights nursery management, whereas FAO (2024b) stresses propagation. In Latin America, FAO (2022a) highlights *Polylepis incana* Kunth, *Alnus acuminata* Kunth, *Cedrela montana* Montz exTurcz, *Escallonia resinosa* Ruiz & Pav, and *Buddleja incana* Ruiz & Pav. FAO (2022b) emphasizes propagation, while IDB (2022) and ECLAC (2022) under-line ecosystem recovery and pressures. In Peru, IIAP (2022) promotes vegetative propagation, SERFOR (2022a) supports native-species recovery, and SERFOR (2022b) emphasizes nursery production. *Polylepis racemosa* has germination below 3% (Flores et al., 1994), making cuttings practical (Ruiz and García, 2019; SERFOR,

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2024) notes standardized protocols are needed. Hormonal propagation has benefited *Retrophyllum rospigliosii* (Pilg.) C.N.Page (More et al., 2021), *Hyptis australis* Epling (Ríos et al., 2022), *Laguncularia racemosa* (L.) C.F.Gaertn. (Castillo et al., 2021), *Pinus hartwegii* Lindl. (Rivera et al., 2021), bamboo (Arancibia and Domínguez, 2019), *Pongamia pinnata* L. Pierre (Cantero et al., 2025), *Solanum lycopersicum* L. (Nkongho et al., 2023), *Vitis davidii* Rom.Caill., Foëx (Zheng et al., 2025), halophytes (Valle-Romero et al., 2025), and *Cyclopia* spp. (Karsen et al., 2025). Restoration urgency is supported by Astudillo et al. (2024), Gómez et al. (2023), Noh et al. (2022), Quenta-Herrera et al. (2022), Zulham et al. (2025), Terefe et al. (2024), Randhir and Klosterman (2024), Endalemaw et al. (2025), Schapheer et al. (2021), Tölgyesi et al. (2023), Silveira et al. (2022), Li et al. (2023), Comer et al. (2022), Christmann et al. (2023), and Monar-Barragán et al. (2023). *Polylepis racemosa* supports water regulation, soil conservation, carbon sequestration, and biodiversity (Servat et al., 2002), but anthropogenic pressures have reduced populations (Mendoza and Cano, 2012). Ramos and Coronel (2021) emphasize rooting substrates. Therefore, this study evaluated ROOT-HOR doses on growth, biomass, survival, and morphophysiological quality of *P. racemosa* in Ayacucho, Peru.

Materials and Methods

Study area and climate

The experiment was conducted under a cold–temperate climate, with an annual mean temperature of 14.64 °C, maximum and minimum averages of 21.71 °C and 7.53 °C, and annual precipitation of 767.5 mm. Rainfall occurs mainly from November to May, while May–September is dry, determining irrigation, shading, and nursery regulation practices (Fig. 1).

Irrigation water quality

Analyses at the LABSAF Canaán–INIA Ayacucho Laboratory showed pH 7.4, electrical conductivity of 379.5 µS/cm, TDS of 242.88 mg/L, and SAR of 0.966. Water was classified as C2S1, indicating medium salinity, low sodicity, and irrigation suitability, although periodic leaching is advisable for salinity-sensitive species.

Substrate properties

Agricultural soil showed pH 7.9, 4.3% organic matter, 0.22% nitrogen, 8.8 mg/kg phosphorus, and 103.22 mg/kg potassium. Its loam texture comprised 42% sand, 39% silt, and 18% clay, favoring aeration and water retention. Electrical conductivity was 10.6 mS/m. Peat and river sand were added at 2:1:1.

Plant material and taxonomic verification

Cuttings of *Polylepis racemosa* (R&P), measuring 8–12 cm and containing 4–8 shoots per segment, were collected from Vilcanchos and Chuschi, Ayacucho. Taxonomic identification was conducted in Dr. Laura Aucasime Medina's laboratory, confirming Division Magnoliophyta, Class Magnoliopsida, Order Rosales, Family Rosaceae, Genus Polylepis, and species *Polylepis racemosa* (R&P) for experimental use.

Materials and equipment

The study employed standardized inputs and tools to ensure experimental precision. **Accordingly**, the following table presents the materials, equipment, and resources used in the vegetative propagation experiment of *Polylepis racemosa* (R&P) (Table 1).

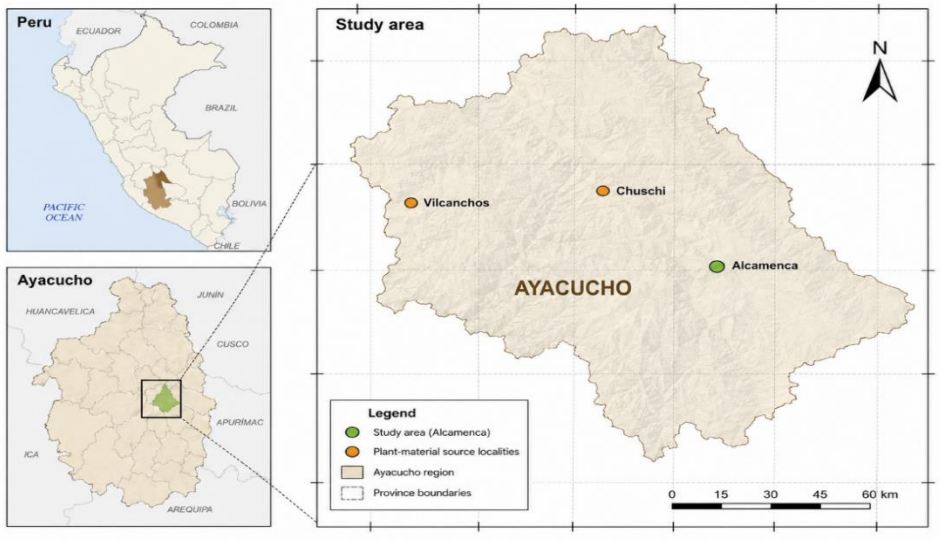


Figure 1
Georeferenced map of the study area in Alcamenca, Ayacucho, Peru.

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Table 1. *Materials, equipment, and resources employed in the vegetative propagation experiment of Polylepis racemosa (R&P)*

Category	Component	Purpose
Biological substrate	Cuttings, black soil (peat), river sand	Vegetative propagation
Desinfection	Formalin (substrate), 90° alcohol (tools)	Sanitary control
Nursery structure	50% Raschel mesh, jute, nylon, posts, 200 L Rotoplas	Protection and irrigation
Tools	Shovel, pickaxe, scissors, sieve, measuring tape, stakes	Land preparation
Measuring equipment	Analytical balance, oven, caliper, GPS, camera, laptop	Measurement and recording
Software	SPSS v26, InfoStat 2023, SAS Planet, Google Earth, Excel, Word	Statistical analysis and writing

Nursery conditions and experimental control

Throughout the experiment, nursery conditions were maintained at 18±2°C, 70–80% relative humidity, and 50% shading using Raschel mesh. Irrigation was applied manually twice daily with chlorine-free water (pH 6.5) to avoid waterlogging. Periodic phytosanitary inspections prevented fungal contamination, while environmental records and calibration controls ensured reproducibility, traceability, and sanitary quality.

Experimental design

The experiment used a Randomized Complete Block Design with six ROOT-HOR doses (0, 3, 6, 9, 12, and 15 ml·L^{-1}), six blocks, and 36 units. Each unit contained 28 Polylepis racemosa cuttings, totaling 1008. Basal portions were immersed for 10 seconds, and treatments were independently randomized within nursery beds. Blocking reduced environmental variability in solar radiation, irrigation, and substrate temperature, improving experimental error control and the reliability of treat-ment comparisons under high-Andean nursery conditions.

Table 2. *Description of the six treatments*

Treatment	ROOT-HOR dose (ml·L^{-1})
T0	0
T1	3
T2	6
T3	9
T4	12
T5	15

Layout of the experimental units and field arrangement

As illustrated below, the following figure presents the randomized distribution scheme of the experimental units within the field (Table 3 and Fig. 2).

Table 3. *Dimensions and characteristics of the experimental field in the* Polylepis racemosa *(R&P) trial*

Description	Unit	Value
Length of the experimental field	m	10
Width of the experimental field	m	7,2
Total field area	m²	72
Number of blocks (experimental beds)	units	6
Length of each experimental bed	m	10
Width of each experimental bed	m	0,5
Area per experimental bed	m²	5
Plot (experimental unit)	m	0,5 × 0,5
Plot area	m²	0,25
Corridors between experimental beds	m	0,6
Distance between cuttings	m	0,8
Cuttings per experimental unit	units	28
Total experimental units	units	36
Total cuttings	unitis	1008

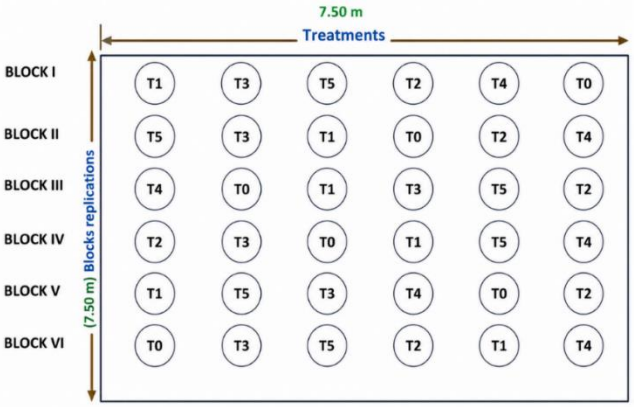


Figure 2. *Randomized Complete Block Design (RCBD) layout of the experimental units in the nursery*

Variables and indicators

Morphological evaluations followed standardized forestry nursery procedures. Stem height and basal diameter were measured using a ruler and digital caliper, respectively. Root length and number were recorded after careful washing. Dry biomass was determined after oven-drying at 70 °C. Dickson Quality and robustness indices were calculated to assess seedling quality.

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Table 4. *Study factors analyzed*

Type of variable	Variable	Indicators and evaluation time
Independent	ROOT-HOR dose	0, 3, 6, 9, 12, 15 ml·L^{-1}
Dependent 1	Vegetative propagation	Sprouting time and %, 30 days; survival %, 120 days
Dependent 2	Morphological growth	Height, stem diameter, roots, shoots, leaves, root length, total/shoot/root dry weight (195 days)
Dependent 3	Seedling quality	Robustness index = Height/Diameter; Dickson Quality Index (DQI) = Total dry weight / [(Height/Diameter) + (Shoot dry weight/Root dry weight)]

Data collection and quality assurance

Measurements were taken between 30 and 195 days after establishment. To ensure impartiality, data were collected by an independent evaluator, double-entered in Excel, and subsequently verified through outlier analysis prior to statistical processing.

Statistical analysis

The statistical analysis was performed according to a Randomized Complete Block Design (RCBD), using the following linear additive model:

$$Yij=\mu+Ti+Bj+\varepsilon ij$$

where: Y_{ij} = observed value of the experimental unit; μ = overall mean; T_i = effect of the i-th ROOT-HOR dose; B_j = effect of the j-th block; ε_{ij} = experimental error associated with each observation.

Normality and variance homogeneity were assessed using Shapiro–Wilk and Levene tests. Treatment effects were evaluated through ANOVA at $p \leq 0.05$, followed by Tukey's HSD test when significant differences occurred. Data transformations and robust criteria were considered when assumptions were violated. Analyses were performed using SPSS v26 and InfoStat 2023.

Seedling quality criteria

Based on SERFOR (2024) and FAO (2022), the following standards were established:

- a well-developed root system free of deformities;
- a lignified, straight, and firm stem;
- shoot-to-root dry weight ratio ≤ 2.5;
- absence of chlorosis or stress symptoms.

Collectively, these criteria ensure vigorous seedlings that are physiologically suitable for high-Andean restoration programs.

Reproducibility considerations

All doses, mixtures, timings, and environmental conditions were thoroughly documented to enable replication. Moreover, meteorological records and instrument calibration logs are available upon institutional request

Results

Polylepis racemosa responses varied with ROOT-HOR dose, mainly in survival, stem growth, rooting, and biomass. Sprouting averaged 20 days and 60.34%, without significant treatment differences ($p > 0.05$), so it was excluded from later comparisons. Low coefficients of variation indicated adequate precision, homogeneous blocks, and reliable statistical analysis (Table 5). ROOT-HOR significantly affected *Polylepis racemosa* survival ($p = 0.0033$), with satisfactory experimental precision (CV = 12.40%). Intermediate doses improved physiological stability, tissue hydration, root differentiation, and stress tolerance. Treatments between 6 and 9 ml·L^{-1} produced the most favorable survival response under high-Andean nursery conditions (Table 6).

Table 5
Analysis of variance for survival percentage of Polylepis racemosa cuttings treated with ROOT-HOR

S.V.	D.F.	SS	MS	F	Pr>F
Treatment	5	1079.56	215.91	4.56	0.0033**
or Error	30	1421.68	47.39		
Total	35	2501.24			
C.V. = 12.40 %					
Mean: 55.52%					

Note. ** = highly significant at $p \leq 0.01$.- C.V. = coefficient of variation
Data recorded 195 days after establishment under nursery conditions in Alcamenca, Ayacucho.

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Table 6. *Effect of ROOT-HOR doses on initial morphometric growth of Polylepis racemosa cuttings*

Variable	S.V.	DF	SS	MS	F	Pr > F	C.V. (%)	Mean (± SE)
Stem height (cm)	Treatments	5	125.58	25.12	3.36	0.0159 *	10.1	27.09 ± 0.85
	Error	30	224.58	7.49				
	Total	35	350.16					
Number of roots units)	Treatments	5	61.38	12.28	1.99	0.1086 ns	15.51	16.00 ± 1.03
	Error	30	184.92	6.16				
	Total	35	246.3					
Stem diameter (mm)	Treatments	5	0.17	0.03	5.13	0.0016 **	11.08	0.73 ± 0.02
	Error	30	0.2	0.01				
	Total	35	0.36					
Root length (cm)	Treatments	5	174.1	34.82	6.71	0.0003 **	9.57	23.81 ± 0.72
	Error	30	155.63	5.19				
	Total	35	329.72					

Note. ns = not significant; * = significant at $p \leq 0.05$; ** = highly significant at $p \leq 0.01$ - SE = standard error.

Significant differences occurred in stem height, diameter, and root length ($p \leq 0.05$), while root number remained unchanged. Intermediate ROOT-HOR doses (6–9 ml·L^{-1}) produced the best morphometric performance, likely by promoting auxin-mediated cell elongation and root differentiation without inhibitory effects. Low variation coefficients confirmed reliable nursery responses (Table 7).

ROOT-HOR significantly influenced total, shoot, and root dry biomass ($p \leq 0.01$). Intermediate doses of 6–9 ml·L^{-1} produced the highest biomass, indicating efficient carbon allocation and physiological balance. Increased root biomass enhanced absorptive capacity, while balanced shoot-to-root ratios reflected greater seedling vigor, structural stability, and post-transplant establishment potential.

Table 7. *Effect of ROOT-HOR doses on biomass accumulation in Polylepis racemosa cuttings*

Variable	S.V.	DF	SS	MS	F	Pr > F	C.V. (%)	Mean (± SE)
Total dry weight (g)	Treatments	5	14.06	2.81	5.55	0.0010 **	15.58	4.90 ± 0.19
	Error	30	15.21	0.51				
	Total	35	29.27					
Shoot dry weight (g)	Treatments	5	10.58	2.12	6.06	0.0005 **	19.15	3.02 ± 0.13
	Error	30	10.47	0.35				
	Total	35	21.05					
Root dry weight (g)	Treatments	5	4.74	0.35	4.75	0.0026 **	18.26	1.88 ± 0.09
	Error	30	2.21	0.07				
	Total	35	3.95					

Note. ** = highly significant at $p \leq 0.01$. - SE = standard error.

Seedling quality

The robustness index exceeded recommended thresholds across treatments, indicating relatively slender seedlings. Intermediate ROOT-HOR doses slightly improved stem proportionality but did not substantially enhance structural balance. Hormonal stimulation favored height growth over radial thickening, producing height-to-diameter imbalances that could reduce resistance to dehydration and mechanical stress after transplantation (Fig. 3).

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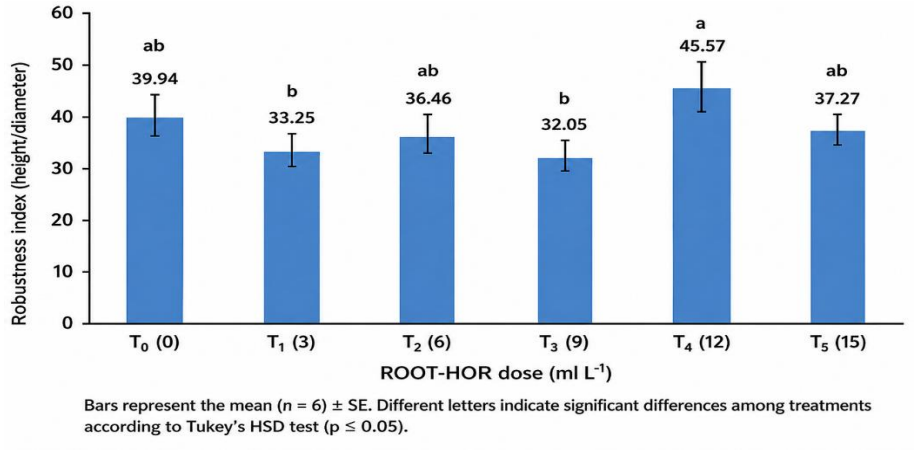


Figure 3
Effect of ROOT-HOR doses on robustness index of Polylepis racemosa seedling

The Dickson Quality Index remained below 0.2 in all treatments, indicating moderate seedling quality despite improved growth and biomass. Intermediate ROOT-HOR doses produced higher DQI values, reflecting better biomass distribution and shoot–root integration. Further optimization of substrate composition and nursery management could improve overall structural and physiological seedling quality (Fig. 4).

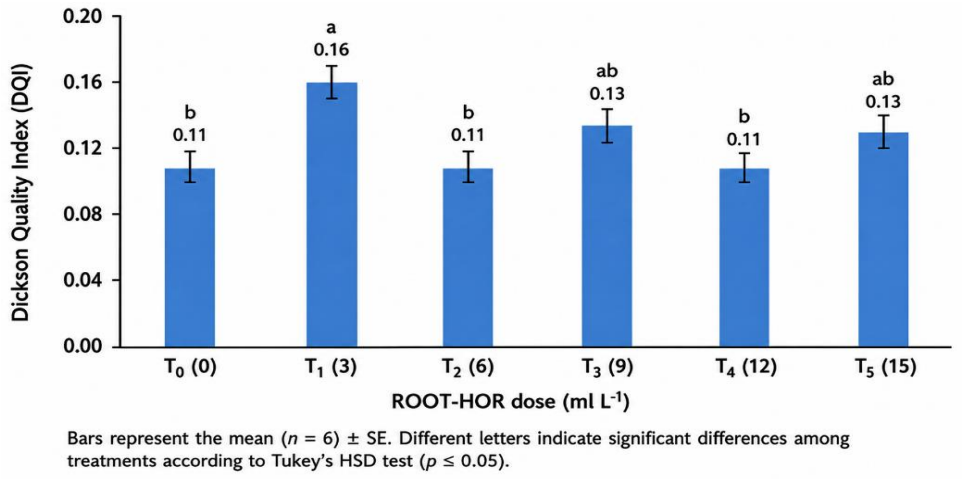


Figure 4
Dickson Quality Index (DQI) of Polylepis racemosa seedlings under different ROOT-HOR doses

Intermediate ROOT-HOR concentrations simultaneously optimized survival percentage and stem growth, particularly between 6 and 9 ml·L^{-1}. This integrated response confirms that moderate hormonal doses promoted a balanced morphophysiological performance during the vegetative propagation process (Fig. 5).

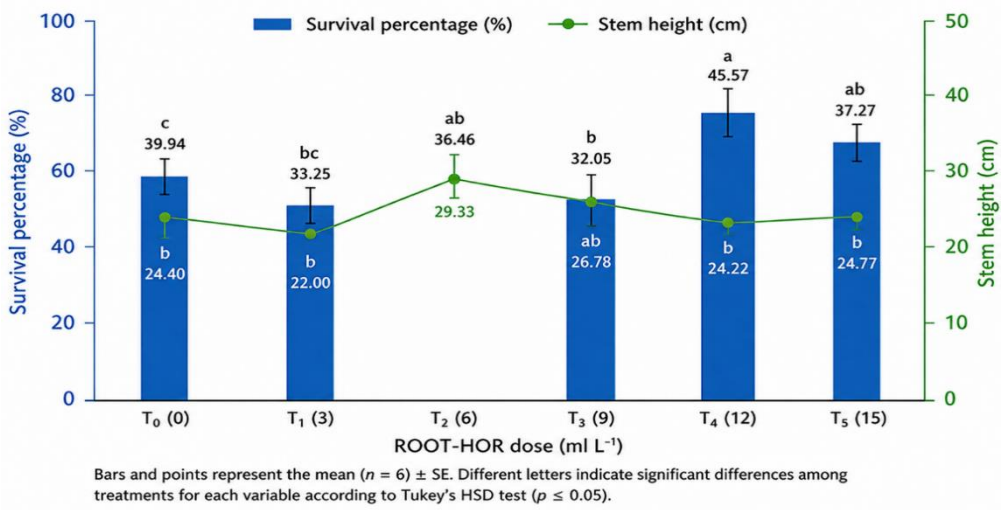


Figure 5
Mean survival percentage and stem height of Polylepis racemosa under different ROOT-HOR doses

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Discussion

The vegetative propagation of Polylepis racemosa showed a clear dose-dependent response to ROOT-HOR, confirming that rhizogenic efficiency depends on the balance between exogenous auxins and endogenous hormonal status. Intermediate doses (6–9 ml L^{-1}) produced the best survival, stem growth, root elongation, and biomass accumulation, whereas higher concentrations reduced physiological efficiency. This bell-shaped response is typical of auxin-regulated processes, where moderate doses stimulate cellular differentiation and excessive levels may become inhibitory or phytotoxic. Ruiz and García (2019) reported a similar pattern in Escallonia resinosa, suggesting narrow hormonal tolerance ranges in woody Andean species. The favorable response at intermediate concentrations may result from enhanced meristematic activity and carbohydrate mobilization toward the basal region. Auxins such as IBA and NAA promote cell dedifferentiation, root primordia formation, and sink strength in rhizogenic tissues, improving hydration, vascular differentiation, and metabolic stability. More et al. (2021) found comparable effects in *Retrophyllum rospigliosii* under controlled nursery conditions. Sprouting showed no significant differences, although low and intermediate doses tended to favor emergence. This suggests that sprouting in P. racemosa depends more on propagule physiological condition and nursery microclimate than auxin concentration alone. Ríos et al. (2022) similarly observed limited shoot variation despite improved rooting in Hyptis australis. Survival at moderate doses may reflect greater membrane stability, osmotic regulation, and conductive tissue development, whereas excessive concentrations may generate hormonal imbalance and ethylene accumulation. Castillo et al. (2021) reported comparable responses in Laguncularia racemosa and emphasized moisture and substrate aeration. Stem height, diameter, and root length improved particularly at intermediate doses, indicating enhanced cambial activity, vascular differentiation, and coordinated aerial and root growth. Cantero et al. (2025) reported similar behavior in Pongamia pinnata. Although root number did not differ significantly, higher averages at 6–9 ml L^{-1} suggest improvement in root functionality rather than quantity. Rivera et al. (2021) likewise found that moderate IBA increased root vigor in *Pinus hartwegii*. Biomass accumulation was one of the clearest responses. Greater total, shoot, and root dry weight indicates improved integration between carbon assimilation, nutrient uptake, and root development, which is particularly important under high-Andean environmental stress. Karsen et al. (2025) demonstrated that hormonal regulation combined with adequate nutrition enhances biomass and rooting in woody species. Increased stem diameter may also reflect improved lignification and vascular strengthening. Zheng et al. (2025) associated auxin regulation with genes involved in vascular differentiation and structural reinforcement in *Vitis davidii*. However, robustness and Dickson Quality Index values revealed structural limitations. High robustness values and DQI below 0.2 indicated excessive height relative to stem thickness and biomass balance, potentially increasing vulnerability after transplantation. Thus, hormonal stimulation alone was insufficient to guarantee optimal seedling quality. Substrate composition, nutrition, moisture, and light regulation must also be optimized. Valle-Romero et al. (2025) and Nkongho et al. (2023) showed that auxins combined with beneficial microorganisms, organic cofactors, or improved substrates enhance physiological resilience, while Arancibia and Domínguez (2019) emphasized that moisture conditions and propagule characteristics strongly modulate hormonal responses. These interactions should therefore be considered jointly when defining propagation protocols for restoration programs.

Conclusions

The application of ROOT-HOR significantly influenced the vegetative propagation of *Polylepis racemosa* under high-Andean nursery conditions, particularly in survival, morphometric growth, root development, and biomass accumulation. Intermediate doses between 6 and 9 ml L^{-1} produced the most favorable physiological responses, with 6 ml L^{-1} identified as the optimal concentration for improving seedling establishment and vigor. These treatments promoted greater survival, stem growth, root elongation, and biomass balance between aerial and below-ground structures, indicating improved rhizogenic efficiency during early development. From an applied perspective, the results demonstrate that ROOT-HOR constitutes a viable technical alternative for the large-scale production of *P. racemosa* seedlings intended for ecological restoration and reforestation programs in degraded high-Andean ecosystems. Nevertheless, although nursery performance was favorable, the structural quality indices suggest that additional improvements in substrate management and environmental control are still required to optimize

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plant quality. One limitation of the present study is that the evaluation was conducted exclusively under nursery conditions; therefore, field validation is necessary to determine long-term survival and adaptation after transplantation. Future research should also examine physiological quality indicators, nutrient dynamics, and the interaction between rooting regulators, substrate composition, and beneficial microorganisms to strengthen propagation protocols for native Andean forest species.

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