Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Efficient *In Vitro* Micropropagation of *Cinchona officinalis* from Nodal Segments

Micropropagación *in vitro* eficiente de *Cinchona officinalis* a partir de segmentos nodales

https://doi.org/10.15446/rfnam.v79.124867

Alex Wilfredo Huatay-Saldaña[1], Jonny Luberli Ramos-Sanchez[1], Delicia Dolores Chuquimango-Álvarez[1], Luis Dávila-Estela[1], Manuel Gregorio Malpica-Rodríguez[1], Nilda Hilario-Román[2], Nadia Masaya Panduro-Tenazoa[3], Elisabeth Viviana Lucero Baldevenites[4], Jorge Arturo Mori-Vásquez[5] and Antony Cristhian Gonzales-Alvarado[5,6,7*]

Keywords:
Conservation
Disinfection
In vitro culture
Rooting
Rubiaceae
Shoot proliferation

CITATION: Huatay-Saldaña AW, Ramos-Sanchez JL, Chuquimango-Álvarez DD, Dávila-Estela L, Malpica-Rodríguez MG, Hilario-Román N, Panduro-Tenazoa NM, Lucero Baldevenites EV, Mori-Vásquez JA and Gonzales-Alvarado AC (2026) Efficient *In Vitro* Micropropagation of *Cinchona officinalis* from Nodal Segments, Revista Facultad Nacional de Agronomía Medellín 79: e124867. doi: https://doi.org/10.15446/rfnam.v79.124867

ABSTRACT

Cinchona officinalis is an emblematic species of the tropical Andes that faces both anthropogenic and environmental pressures, highlighting the urgency of developing strategies for its conservation and propagation. This study aimed to develop and optimize micropropagation using nodal segments through three experiments. The first experiment evaluated disinfection using sodium hypochlorite (NaClO) at different concentrations: 0, 1.5, 2 and 2.5%. The second experiment examined the effect of combining indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) on shoot proliferation, assessing shoot number (SN), shoot length (SL) and survival (%S). The third experiment investigated the influence of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) on root development, measuring root number (RN), root length (RL), and rooting percentage (%E). The best disinfection results were obtained with 2% NaClO, which produced the best outcomes, achieving the lowest contamination rate (5.56%), reduced phenol release (33.33%), and the highest survival rate (61.11%). For shoot proliferation, 2.28 µM IAA combined with 5.33 µM BAP promoted both a higher number (2.5 shoots per plant) and length (23.5 mm per plant) of shoots. In the rooting phase, two treatments were particularly effective: 7.38 µM IBA + 5.37 µM NAA and 9.84 µM IBA + 8.06 µM NAA, yielding more roots (2.93 and 2.47 per plant) and increased root length (5.43 and 5.67 cm per plant, respectively). In conclusion, it was demonstrated that the micropropagation of *C. officinalis* from nodal segments is feasible, offering a practical approach for the conservation and propagation of the species, and providing a solid foundation for large-scale clonal reproduction.

RESUMEN

Palabras clave:
Conservación
Desinfección
Cultivo *in vitro*
Enraizamiento
Rubiaceae
Proliferación de brotes

Cinchona officinalis es una especie emblemática de los Andes tropicales que enfrenta presiones tanto antropogénicas como ambientales, lo que resalta la urgencia de desarrollar estrategias para su conservación y propagación. Este estudio tuvo como objetivo desarrollar y optimizar la micropropagación utilizando segmentos nodales a través de tres experimentos. El primer experimento evaluó la desinfección con hipoclorito de sodio (NaClO) a concentraciones de 0, 1,5, 2 y 2,5%. El segundo examinó el efecto de la combinación de ácido indol-3-acético (AIA) y 6-bencilaminopurina (BAP) en la proliferación de brotes, evaluando el número de brotes (SN), la longitud de brotes (SL) y la supervivencia (%S). El tercero investigó la influencia del ácido indol-3-butírico (AIB) y el ácido naftalenoacético (ANA) en el desarrollo radicular, midiendo el número de raíces (RN), la longitud de raíces (LR) y el porcentaje de enraizamiento (%E). Los mejores resultados de desinfección se obtuvieron con 2% de NaClO, logrando la menor contaminación (5,56%), menor liberación de fenoles (33,33%) y la mayor tasa de supervivencia (61,11%). Para la proliferación de brotes, la combinación de 2,28 µM de AIA con 5,33 µM de BAP promovió un mayor número (2,5 brotes por planta) y longitud (23,5 mm por planta) de brotes. En la fase de enraizamiento, dos tratamientos fueron especialmente efectivos: 7,38 µM de AIB + 5,37 µM de ANA y 9,84 µM de AIB + 8,06 µM de ANA, obteniéndose un mayor número de raíces (2,93 y 2,47 por planta) y un incremento en la longitud radicular (5,43 y 5,67 cm por planta, respectivamente). En conclusión, se demostró que la micropropagación de *C. officinalis* a partir de segmentos nodales es viable, ofreciendo un enfoque práctico para la conservación y propagación de la especie, y proporcionando una base sólida para la reproducción clonal a gran escala.

[1]Facultad de Ciencias Agrarias, Universidad Nacional de Cajamarca, Cajamarca, Perú. ahuatays@unc.edu.pe, jramoss14@unc.edu.pe, ddchuquimangoa@unc.edu.pe, ldavila@unc.edu.pe, mmalpicar@unc.edu.pe
[2]Facultad de Ciencias Agropecuarias, Universidad Nacional Daniel Alcides Carrión, Chanchamayo, Junín, Perú. nhilarior@undac.edu.pe
[3]Facultad de Ingeniería y Ciencias Ambientales, Universidad Nacional Intercultural de la Amazonia, Ucayali, Perú. nmpandurot@unia.edu.pe
[4]Facultad de Ingeniería Industrial y Civil, Universidad de Las Palmas de Gran Canaria, Las Palmas, España. viviana.lucero@ulpgc.es
[5]Facultad de Ciencias Forestales y Ambientales, Universidad Nacional de Ucayali, Pucallpa, Ucayali, Perú. jorge_mori@unu.edu.pe
[6]Centro de Ciências Naturais y Humanas, Universidade Federal do ABC, São Bernardo do Campo, São Paulo, Brazil. antony.gonzales@ufabc.edu.br
[7]Nexus Scientia Project & Consulting I+D+i, Ucayali, Peru.
*Corresponding author

Received: March 14, 2026; Accepted: May 24, 2026
Rev. Fac. Nac. Agron. Medellín 79: e124867. 2026

ISSN 0304-2847 / e-ISSN 2248-7026

CC BY NC SA

The genus *Cinchona* belongs to the family Rubiaceae and is distributed throughout the tropical Andes, particularly in Colombia, Ecuador, and Peru (Coronel-Castro et al. 2024). This genus has attracted great interest from the pharmaceutical industry due to its quinine content, a key antimalarial compound crucial for the treatment of malaria, which still represents a major public health challenge (The Lancet Infectious Diseases 2024). The high demand for this compound has led to the overexploitation of several species within the genus, many of which are now classified as vulnerable or critically endangered. In light of this situation, coordinated efforts are urgently required to support the recovery of these species.

Peru hosts 19 species of the genus Cinchona, distributed across the montane forests of the Andes (Rufasto-Peralta et al. 2023). As part of conservation efforts, the Peruvian government, through the Ministry of Agriculture, approved an action plan for repopulation. This plan prioritized three species (*Cinchona calisaya*, *Cinchona pubescens*, and *Cinchona officinalis*) because of their emblematic status (MINAGRI 2020). Among them, *C. officinalis* is characterized by small and highly fragmented populations as a result of anthropogenic activities, a condition that directly compromises its genetic diversity. Furthermore, natural regeneration is severely restricted due to the high level of seed predation, reaching up to 95%, caused by insects and small mammals. Even under favorable conditions, the seeds that manage to establish exhibit limited development, with low germination rates and reduced regenerative capacity (Sánchez et al. 2021). These constraints directly hinder the species' natural recovery. Therefore, the implementation of effective propagation methods is essential to support conservation efforts and to prevent its inclusion in the IUCN Red List.

Currently, numerous studies on *C. officinalis* have focused on conservation and propagation using conventional methods (Sánchez et al. 2021), as well as advanced biotechnological approaches such as cryopreservation, mycorrhizal bioinoculation, callogenesis, and micropropagation (Armijos-González et al. 2024; Delgado-Paredes et al. 2025). Among these techniques, *in vitro* micropropagation has shown significant potential for plant multiplication. In previous studies, seeds have been used as the initial explant material due to their ease of handling (Delgado-Paredes et al. 2025); however, their use is limited by the difficulty of obtaining viable seeds, particularly under climate change, which alters the phenological and reproductive cycles of species. In such cases, the use of alternative explants, such as nodal segments, has proven to be highly useful (Yücesan et al. 2015). Furthermore, the use of nodal segments as explants can provide greater genetic stability, avoiding the genetic variation that may arise in seed-derived plants (Pandey et al. 2025). This strategy also reduces dependence on seed availability, which is limited by the species' phenology and is not always accessible throughout the year (Gonzales-Alvarado et al. 2022). Moreover, for the species *Cinchona officinalis* there is a notable lack of studies on the use of nodal segments as explant material for its micropropagation, despite their potential relevance for the development of effective methods aimed at the conservation of the species. In this context, the need arises to evaluate their viability as an *in vitro* propagation strategy: will nodal segments be an effective alternative for the micropropagation of this species?

Micropropagation requires methodologies that include plant disinfection, shoot proliferation, rooting, and acclimatization. Disinfection, in particular, is a critical step for the success of subsequent stages. Different concentrations of sodium hypochlorite (NaClO) have been shown to inhibit pathogens, but inappropriate concentrations can induce phytotoxicity and compromise plant material (Mori-Vásquez et al. 2024). Therefore, it is essential to optimize the concentration to avoid damaging the explants. For *C. officinalis*, concentrations of 1, 1.5, and 2.63% NaClO have been reported for seed disinfection (Delgado-Paredes et al. 2025). However, no evidence exists for nodal segments. This knowledge gap motivates the present study, which aims to optimize NaClO concentration for the disinfection of nodal segments, a crucial step to initiate the micropropagation of the species.

Existing micropropagation studies have utilized combinations of auxins and cytokinins, which have been shown to promote both shoot proliferation and increased rooting percentages (Cabahug et al. 2016). Specifically, low concentrations of indole-3-acetic acid (IAA) combined with high concentrations of 6-benzylaminopurine (BAP) have been observed to enhance shoot proliferation in species such as banana (Khan et al. 2021). Conversely,

the combination of low concentrations of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) has proven effective in inducing higher rooting percentages in species such as *Oryza sativa* (Wang et al. 2003). These findings suggest that the use of low concentrations of IAA in combination with high concentrations of BAP may favor shoot proliferation in nodal segments of *C. officinalis*, while the combination of low concentrations of NAA and IBA may enhance rooting efficiency.

In this study, the aim was to micropropagate *C. officinalis* using nodal segments. Specifically, the effect of different concentrations of sodium hypochlorite (NaClO) was evaluated (i), as well the influence of the combination of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) on shoot proliferation (ii), and the effect of the combination of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) on root development (iii).

MATERIALS AND METHODS

Biological material

The experiment was conducted at the Plant Biotechnology Laboratory of the Faculty of Agricultural Sciences, Universidad Nacional de Cajamarca, Peru. For the disinfection experiment, nodal segments (stems) from 8-month-old seedlings, with an average height of 30 cm (Figure 1A), were used. The seedlings were obtained from the nursery of the Instituto Nacional de Agricultura e Innovación (INIA), located in the district of Los Baños del Inca, province and department of Cajamarca, Peru (coordinates 780300.35 m E and 9207919.08 m S). The plants were maintained under constant irrigation conditions in a substrate composed of peat, agricultural soil, and sand in a 3:1:1 (v/v/v) ratio, at ambient temperature and under approximately 30% shade.

Regarding their origin, the seeds were collected from a natural forest in the district of Querocoto, province of Chota, Cajamarca, Peru (coordinates 716119.46 m E and 9296149.93 m S), and subsequently germinated in the nursery to obtain the seedlings used in this study. In order to confirm the taxonomic identity of the species as *Cinchona officinalis* L., a plant sample was sent to the CPUN Herbarium "Isidoro Sánchez Vega" of the National University of Cajamarca, Peru, where the species was confirmed through specialized taxonomic identification.

For the shoot proliferation experiment, all nodal segments that remained aseptic after the disinfection process were used. Finally, for the rooting experiment, nodal segments from all shoot proliferation treatments that produced at least two shoots were selected.

Experimental procedure

Disinfection experiment

For the first disinfection experiment, nodal segments with one apical bud and two axillary buds obtained from *C. officinalis* plants, measuring 1–2 cm in length, were used (Figure 1A). The nodal segments were washed with carbolic soap (6 mg L^{-1}, Danny®) and distilled water for 5 min. The liquid was then removed, and 70% (v/v) ethanol was applied for 60 seconds. Finally, the ethanol was removed and different concentrations of sodium hypochlorite (NaClO) plus three drops of Tween-20 were applied for 10 minutes, followed by three rinses with sterile distilled water. All procedures were carried out in a horizontal laminar flow chamber with high-efficiency particulate air (HEPA) filters (ENVIRCO Corporation®).

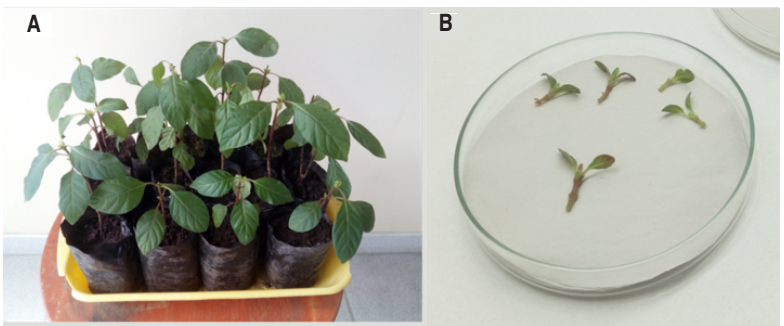


Figure 1. A. *Cinchona officinalis* seedlings grown in a nursery, explant donors, and **B**. Nodal segments.

For the disinfection treatments, a commercial sodium hypochlorite solution (Clorox®, 4%) was diluted to obtain final NaClO concentrations of 1.5, 2, and 2.5%. In the control treatment, NaClO was not applied (Table 1). Murashige and Skoog (MS) medium (1962) supplemented with 3% sucrose, 1 mg L^{-1} thiamine, 0.5 mg L^{-1} pyridoxine, 0.5 mg L^{-1} nicotinic acid, 2 mg L^{-1} glycine, 0.1 g L^{-1} inositol, and adjusted to pH = 5.8 was used as the culture medium. Subsequently, 9 g L^{-1} PhytoTech agar® was added, and 30 mL of the medium was poured into glass jars (7.5 cm high, 4.5 cm base diameter, 100 mL capacity) according to the treatment. The jars were sterilized in an autoclave at 121 °C and 1.1 atm for 20 minutes, after which the nodal segments were inoculated. Each glass jar containing a nodal segment approximately 1–2 cm in length was considered an experimental unit, with 18 biological replicates per treatment.

The inoculated jars were placed in a growth chamber with a 12-hour photoperiod and a light intensity of 400–500 µmol m^{-2} s^{-1}, using 100 W fluorescent lamps, at a temperature of 23±1 °C and a constant relative humidity of 75% throughout the entire experiment. The variables evaluated for disinfection were the percentage of contamination (%C per plant), percentage of phenolization (%Ph per plant), and percentage of survival (%S). All these variables were assessed 15 days after *in vitro* inoculation.

Shoot proliferation experiment

For shoot proliferation, nodal segments that remained aseptic for 30 days after inoculation of the explant in the disinfection experiment were used (Figure 1B). Additionally, segments that showed bud development were subsequently excised and used as new explants for the experiment. The same culture medium protocol employed in the disinfection experiment was used, with the addition of different combinations of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP), as well as a control treatment without growth regulators. The treatments were as follows: T1 (1.14 µM IAA + 1.33 µM BAP), T2 (1.14 µM IAA + 2.66 µM BAP), T3 (1.14 µM IAA + 5.33 µM BAP), T4 (2.28 µM IAA + 1.33 µM BAP), T5 (2.28 µM IAA + 2.66 µM BAP), T6 (2.28 µM IAA + 5.33 µM BAP), T7 (4.57 µM IAA + 1.33 µM BAP), T8 (4.57 µM IAA + 2.66 µM BAP), T9 (4.57 µM IAA + 5.33 µM BAP), and T10 (control, without phytohormones) (Table 1). Nodal segments were subsequently inoculated into the culture media. Each experimental unit consisted of a glass jar containing five nodal segments measuring 1–2 cm in length, with 30 explants per treatment (six jars per treatment). The jars were maintained in a growth room under the same conditions as the disinfection experiment.

Table 1. Distribution of treatments in shoot proliferation and rooting.

T	EPB: IAA + BAP (µM)	Number of biological replicates	EE: IBA + NAA (µM)	Number of biological replicates
T1	1.14+1.33	30	4.92+5.37	30
T2	1.14+2.66	30	4.92+8.06	30
T3	1.14+5.33	30	4.92+10.74	30
T4	2.28+1.33	30	7.38+5.37	30
T5	2.28+2.66	30	7.38+8.06	30
T6	2.28+5.33	30	7.38+10.74	30
T7	4.57+1.33	30	9.84+5.37	30
T8	4.57+2.66	30	9.84+8.06	30
T9	4.57+5.33	30	9.84+10.74	30
T10	0 (control)	30	0 (control)	30
	Total	300		300

T: Treatment, EPB: Shoot proliferation experiment, and EE: Rooting experiment.

The variables evaluated for shoot proliferation were the number of shoots per plant (SN per plant), shoot length per plant (SL per plant), and survival rate (%S). SL was measured using a graduated ruler. Evaluations were conducted over 60 days at four observation intervals.

Rooting experiment

For rooting, nodal segments from the shoot proliferation experiment that produced at least two shoots were used. The same culture medium protocol employed in previous experiments was used, with the addition

of different combinations of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA), as well as a control treatment without growth regulators. The treatments were as follows: T1 (4.92 µM IBA + 5.37 µM NAA), T2 (4.92 µM IBA + 8.06 µM NAA), T3 (4.92 µM IBA + 10.74 µM NAA), T4 (7.38 µM IBA + 5.37 µM NAA), T5 (7.38 µM IBA + 8.06 µM NAA), T6 (7.38 µM IBA + 10.74 µM NAA), T7 (9.84 µM IBA + 5.37 µM NAA), T8 (9.84 µM IBA + 8.06 µM NAA), T9 (9.84 µM IBA + 10.74 µM NAA), and T10 (control, without phytohormones) (Table 2). The nodal segments were subsequently inoculated into the culture media. Each experimental unit consisted of a glass jar containing five nodal segments measuring 1–2 cm in length, with 30 explants per treatment (six jars per treatment). The jars were maintained in a growth room under the same conditions as those used in the disinfection and shoot proliferation experiments.

The variables evaluated for rooting were the number of roots per plant (RN per plant), root length per plant (RL per plant), survival rate (%S), and rooting rate (%E). RL was measured using a graduated ruler; in the case of coiled roots, the measurement was performed by following their complete trajectory along the ruler. Evaluations were conducted over 60 days at four observation intervals.

Data analysis

All experiments were conducted using a completely randomized design (CRD). For each evaluated variable, the assumptions of normality (Shapiro–Wilk test, $P>0.05$) and homogeneity of variances (Levene's test, $P>0.05$) were verified. As all variables met these assumptions, an analysis of variance (ANOVA) was performed ($P\leq0.05$), followed by mean comparisons using Duncan's test $P\leq0.05$. For the disinfection experiment, linear or nonlinear regressions were applied depending on the goodness of fit, as indicated by the coefficient of determination (R^2) (Gonzales-Alvarado et al. 2025).

Additionally, principal component analysis (PCA) was performed for each experiment, considering all evaluated variables. In the disinfection experiment, PCA was conducted using the ggplot2 package (Wickham 2016), incorporating 95% confidence ellipses via biplot. For the shoot proliferation and rooting experiments, PCA was carried out using the Multivariate Analysis package (Lima 2021), allowing the identification of clustering patterns among treatments and the assessment of variable contributions to overall system variability. Furthermore, correlation matrices were determined using Spearman's coefficient with the *corrplot* package (Wei et al. 2024), considering correlations with $-0.6 \leq P \geq 0.6$ as strong. All statistical analyses were performed in R Studio, version 4.5.3. (2026.01.1+403).

RESULTS AND DISCUSSION

Effect of NaClO concentration on the disinfection of nodal segments of *Cinchona officinalis*

Contamination is a major factor that interferes with plant development *in vitro* (Mori-Vásquez et al. 2024). In this study, the effect of NaClO concentration was significant ($P\leq0.05$) on contamination (%C), phenolization (%Ph), and survival (%S) (Figure 2). The 2% NaClO was found to reduce contamination in the nodal segments of *Cinchona officinalis* by over 90%, while maintaining low levels of phenolization (33.33%) and a higher survival rate (61.11%) (Figure 2A-F). Similar results were reported by Dongli et al. (2023) in *Hedera helix*, where the application of 2% NaClO for 10 minutes yielded optimal outcomes.

This effect can be attributable to NaClO acting as an oxidizing agent; at higher concentrations, it can induce the production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). These ROS may generate oxidative stress in plant tissues, leading to the degradation of cellular components and the activation of defense mechanisms (Khare et al. 2015). This study represents the first report of sterilization for nodal segments of *C. officinalis*, constituting a crucial advance for subsequent experimental phases, including shoot proliferation and *in vitro* rooting.

Moreover, all disinfection variables showed differentiated trends (Figure 2G). %C progressively decreased with increasing NaClO concentration, fitting a second-order polynomial regression with a high coefficient of determination (R^2=0.9968). %Ph followed a linear model, showing a progressive increase with NaClO concentration (R^2=0.8494). For %S, the most suitable model was a second-order polynomial regression (R^2=0.7567), showing an increase up to its maximum at T3, followed by a decrease at T4. The principal component analysis of the disinfection variables indicated that treatments T2 and T3 were mainly associated with survival (%S). In

contrast, contamination (%C) was strongly associated with treatment T1, while T4 was closely linked to phenolization (%Ph). Treatment T2 also showed a slight association with phenolization (Figure 2H). The PCA explained 65.5 and 22.04% of the total variability for components 1 and 2, respectively. These findings indicate that NaClO concentrations higher than 2% promote the release of phenolic compounds, which negatively affect the viability of nodal segments. Therefore, we recommend the use of 2% NaClO to achieve optimal results.

Effect of Indole-3-acetic Acid (IAA) and 6-Benzylaminopurine (BAP) on shoot proliferation of *Cinchona officinalis*

The balance between auxins and cytokinins is considered crucial for plant development, particularly for shoot formation. In general, all treatments produced shoots originating from both pre-existing and adventitious buds, and no callus formation was observed. This trend has also been reported by Armijos-González et al. (2024), who described the proliferation of shoots from both pre-existing and adventitious buds as a result of the action of growth regulators. The results showed that treatment T6 (2.28 µM IAA and 5.33 µM BAP) produced the highest number of shoots (2.5±0.19 SN per plant) and shoot length (23.5±1.1 mm per plant), with a strong correlation between these variables (Table 2). The temporal behavior of these variables evaluated between 15- and 60-days post-inoculation *in vitro*, was modeled using second-degree polynomials (Figure 3). In general, a progressive increase in shoot length was identified, whereas shoot number

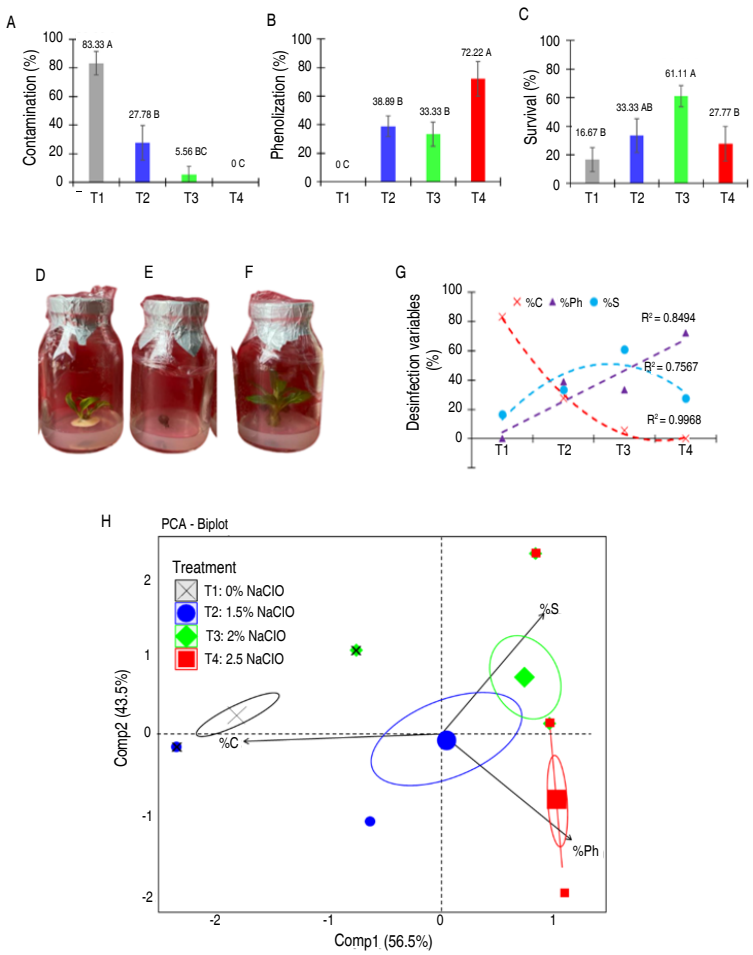


Figure 2. A. Percentage of contamination, **B**. Percentage of phenolization, **C**. Percentage of survival, NaClO response. **D**: contamination, **E**. Phenolization, and **F**. Survival (explant without contamination). **G**. Regression analysis of the variables evaluated, and **H**. Principal component analysis. T1: 0% NaClO. T2: 1.5% NaClO. T3: 2% NaClO. T4: 2.5%. %C: Percentage of contamination ($y = 12.498x^2 – 89.709x + 159.71$. R^2=0.9968). %Ph: Percentage of phenolization ($y = 21.x – 16.665$. R^2=0.8494). %S: Percentage of survival ($y = -12.5x^2 + 68.608x - 43.05$. R^2=0.7567).

did not show a progressive pattern, fitting a polynomial model in all cases. Particularly, treatment 6 presented a differential model compared to the other treatments (Figure 3F). For shoot number (SN), the model was described as $y = -0.2557x^2 + 2.1043x - 1.738$, with a coefficient of determination $R^2=0.9718$. For shoot length (SL), the model was $y = -1.0714x^2 + 12.249x - 10.946$, with $R^2=0.9968$, indicating excellent statistical accuracy for describing the kinetics of shoot proliferation. Such models are essential for understanding the dynamic response of treatments under controlled conditions (Gonzales-Alvarado et al. 2025).

Table 2. Effect of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) on shoot proliferation of *Cinchona officinalis* (mean ± standard error). Different letters represent significant differences according to Duncan's test, $P \leq 0.05$.

DPI	T	IAA + BAP (µM)	SN	SL (mm)	S (%)
15	T1	1.14+1.33	1.3 ± 0.14^{AB}	6.87 ± 0.76^{CD}	83.33 ± 6.92^{A}
	T2	1.14+2.66	1.43 ± 0.13^{AB}	9.3 ± 0.89^{ABC}	86.67 ± 6.31^{A}
	T3	1.14+5.33	1.43 ± 0.16^{AB}	8.1 ± 0.85^{ABC}	86.67 ± 6.31^{A}
	T4	2.28+1.33	1.53 ± 0.15^{A}	9.2 ± 1.01^{ABC}	86.67 ± 6.31^{A}
	T5	2.28+2.66	1.7 ± 0.15^{A}	8.93 ± 0.86^{ABC}	90 ± 5.57^{A}
	T6	2.28+5.33	1.67 ± 0.11^{A}	9.97 ± 0.61^{AB}	100 ± 0^{A}
	T7	4.57+1.33	1.37 ± 0.12^{AB}	8.93 ± 0.82^{ABC}	90 ± 5.57^{A}
	T8	4.57+2.66	1.4 ± 0.1^{AB}	10.7 ± 1.01^{A}	96.67 ± 3.33^{A}
	T9	4.57+5.33	1.37 ± 0.13^{AB}	7.6 ± 0.82^{BC}	86.67 ± 6.31^{A}
	T10	0 (Control)	1.03 ± 0.09^{B}	4.83 ± 0.39^{D}	90 ± 5.57^{A}
30	T1	1.14+1.33	1.57 ± 0.17^{BCD}	11.57 ± 1.28^{A}	83.33 ± 6.92^{A}
	T2	1.14+2.66	1.8 ± 0.17^{ABC}	14.7 ± 1.38^{A}	86.67 ± 6.31^{A}
	T3	1.14+5.33	1.53 ± 0.16^{BCD}	12.33 ± 1.32^{A}	83.33 ± 6.92^{A}
	T4	2.28+1.33	1.97 ± 0.21^{ABC}	14.2 ± 1.56^{A}	86.67 ± 6.31^{A}
	T5	2.28+2.66	2.03 ± 0.18^{AB}	13.73 ± 1.31^{A}	90 ± 5.57^{A}
	T6	2.28+5.33	2.27 ± 0.16^{A}	15.43 ± 0.85^{A}	100 ± 0^{A}
	T7	4.57+1.33	1.5 ± 0.14^{CD}	14.03 ± 1.19^{A}	90 ± 5.57^{A}
	T8	4.57+2.66	1.57 ± 0.13^{BCD}	14.87 ± 1.24^{A}	96.67 ± 3.33^{A}
	T9	4.57+5.33	1.5 ± 0.15^{CD}	12.2 ± 1.4^{A}	86.67 ± 6.31^{A}
	T10	0 (Control)	1.27 ± 0.12^{D}	7.63 ± 0.6^{B}	90 ± 5.57^{A}
45	T1	1.14+1.33	1.67 ± 0.19^{BC}	16.13 ± 1.71^{B}	83.33 ± 6.92^{A}
	T2	1.14+2.66	1.9 ± 0.18^{AB}	19.2 ± 1.58^{AB}	90 ± 5.57^{A}
	T3	1.14+5.33	1.77 ± 0.16^{BC}	16.33 ± 1.63^{B}	86.67 ± 6.31^{A}
	T4	2.28+1.33	2.03 ± 0.22^{AB}	18.3 ± 1.69^{AB}	86.67 ± 6.31^{A}
	T5	2.28+2.66	2.17 ± 0.2^{AB}	17.37 ± 1.4^{AB}	90 ± 5.57^{A}
	T6	2.28+5.33	2.37 ± 0.16^{A}	21.17 ± 0.93^{A}	100 ± 0^{A}
	T7	4.57+1.33	1.7 ± 0.15^{BC}	18.83 ± 1.55^{AB}	93.33 ± 4.63^{A}
	T8	4.57+2.66	1.77 ± 0.14^{BC}	19.17 ± 1.23^{AB}	96.67 ± 3.33^{A}
	T9	4.57+5.33	1.63 ± 0.16^{BC}	16.53 ± 1.75^{AB}	90 ± 5.57^{A}
	T10	0 (Control)	1.27 ± 0.12^{C}	9.77 ± 0.72^{C}	90 ± 5.57^{A}
60	T1	1.14+1.33	1.73 ± 0.19^{BC}	20.17 ± 1.96^{AB}	83.33 ± 6.92^{A}
	T2	1.14+2.66	1.93 ± 0.17^{B}	21.77 ± 1.65^{AB}	90 ± 5.57^{A}
	T3	1.14+5.33	2 ± 0.19^{AB}	18.2 ± 1.79^{B}	86.67 ± 6.31^{A}
	T4	2.28+1.33	2.07 ± 0.22^{AB}	20.23 ± 1.73^{AB}	86.67 ± 6.31^{A}
	T5	2.28+2.66	2.2 ± 0.21^{AB}	18.83 ± 1.39^{AB}	90 ± 5.57^{A}
	T6	2.28+5.33	2.5 ± 0.19^{A}	23.5 ± 1.1^{A}	100 ± 0^{A}
	T7	4.57+1.33	1.8 ± 0.15^{BC}	21.43 ± 1.63^{AB}	93.33 ± 4.63^{A}
	T8	4.57+2.66	1.8 ± 0.15^{BC}	22.8 ± 1.37^{AB}	96.67 ± 3.33^{A}
	T9	4.57+5.33	1.67 ± 0.17^{BC}	20.57 ± 1.75^{AB}	90 ± 5.57^{A}
	T10	0 (Control)	1.3 ± 0.12^{C}	12.6 ± 0.91^{C}	90 ± 5.57^{A}

DPI: days post-inoculation. T: treatment, 3-IA: indole-3-acetic acid, BAP: 6-benzylaminopurine, NS: Number of shoots, SL: Shoot length, and %S: Survival percentage.

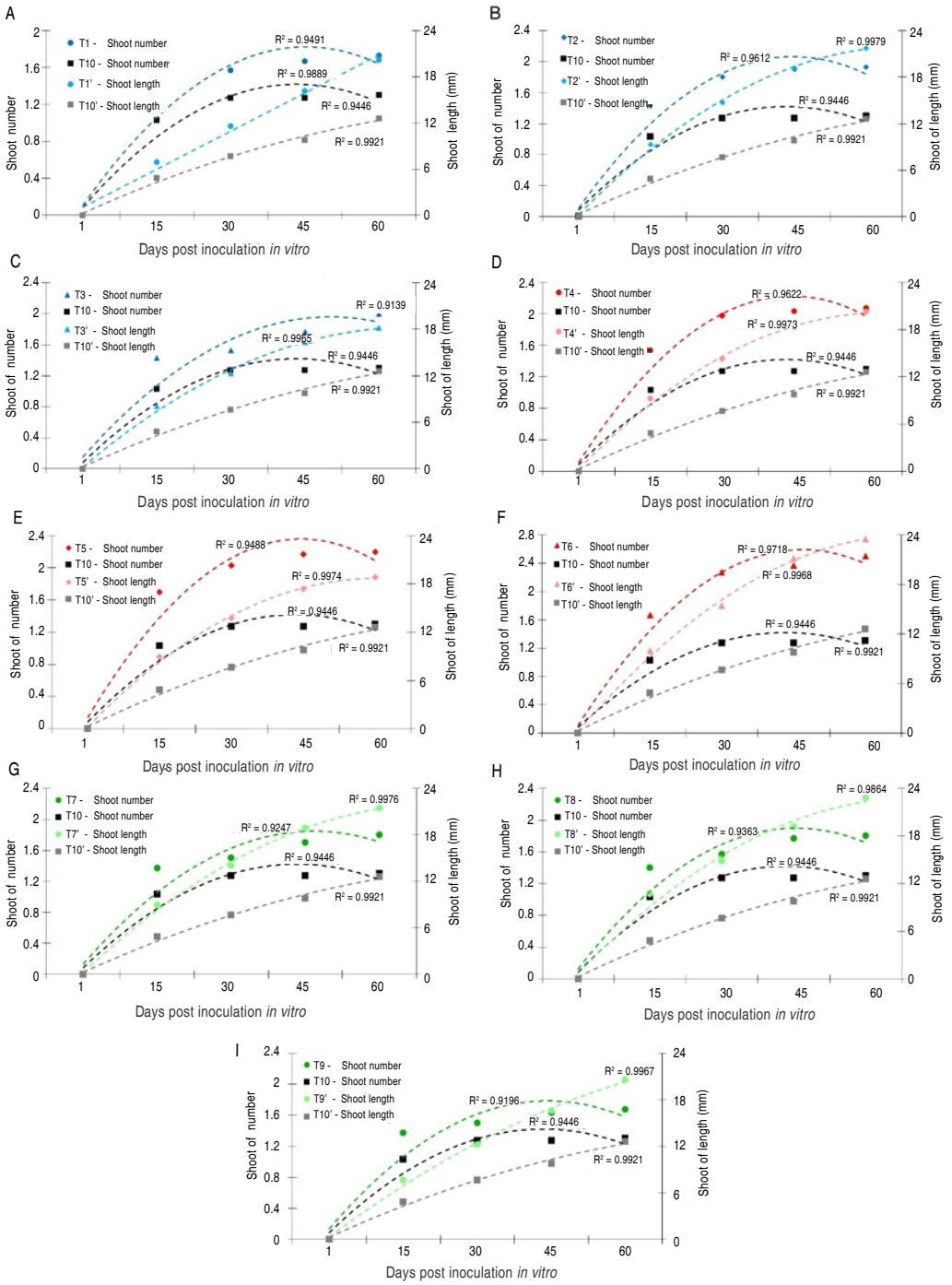


Figure 3. A-I. Second-degree polynomial models of shoot number and shoot length per treatment. T10' corresponds to the control treatment. T1: $y = -0.1893x^2 + 1.5187x - 1.22$; $R^2 = 0.9491$. T1': $y = 4.96x - 3.932$; $R^2 = 0.9889$. T2: $y = -0.2193x^2 + 1.7487x - 1.422$; $R^2 = 0.9612$. T2': $y = -1.0257x^2 + 11.498x - 10.218$; $R^2 = 0.9979$. T3: $y = -0.1614x^2 + 1.4026x - 1.086$; $R^2 = 0.9139$. T3': $y = -0.9064x^2 + 9.9016x - 8.742$; $R^2 = 0.9965$. T4: $y = -0.24x^2 + 1.904x - 1.552$; $R^2 = 0.9622$. T4': $y = -1.1029x^2 + 11.573x - 10.202$; $R^2 = 0.9973$. T5: $y = -0.2521x^2 + 1.9999x - 1.606$; $R^2 = 0.9488$. T5': $y = -1.15x^2 + 11.51x - 10.108$; $R^2 = 0.9974$. T6: $y = -0.2557x^2 + 2.1043x - 1.738$; $R^2 = 0.9718$. T6': $y = -1.0714x^2 + 12.249x - 10.946$; $R^2 = 0.9968$. T7: $y = -0.1764x^2 + 1.4516x - 1.14$; $R^2 = 0.9247$. T7': $y = -0.9257x^2 + 10.83x - 9.664$; $R^2 = 0.9976$. T8: $y = -0.1936x^2 + 1.5584x - 1.238$; $R^2 = 0.9363$. T8': $y = -1.0007x^2 + 11.411x - 9.718$; $R^2 = 0.9864$. T9: $y = -0.19x^2 + 1.5x - 1.176$; $R^2 = 0.9196$. T9': $y = -0.5279x^2 + 8.1741x - 7.336$; $R^2 = 0.9967$. T10: $y = -0.16x^2 + 1.244x - 0.998$; $R^2 = 0.9446$. T10': $y = -0.3329x^2 + 5.0111x - 4.406$; $R^2 = 0.9921$.

It was observed that higher BAP levels (5.33 µM) tend to enhance shoot proliferation (Figure 4A); however, this effect is modulated by IAA concentration, as levels above 2.28 µM reduce proliferation (Table 2). Similar results were reported by Khan et al. (2021) in banana explants, where 5.71 µM IAA combined with 11.1 µM BAP promoted higher shoot numbers. For shoot length, T6 was superior, whereas other treatments ranged from 18.83 to 21.77 mm per plant, and the control exhibited the shortest shoots (12.6±0.91 mm per plant). These findings support the proposed hypothesis, suggesting that in *Cinchona officinalis*, the optimal balance for shoot proliferation and elongation requires a lower proportion of IAA relative to BAP.

The IAA concentration should not exceed 2.28 µM, as higher levels may inhibit shoot production. This effect is likely attributable to the antagonistic interaction between auxins and cytokinins; lower IAA concentrations reduce lateral shoot inhibition, allowing more axillary buds to develop into shoots (Pei-gen X 2008). Furthermore, Xu et al. (2017) reported that IAA can activate genes involved in cell expansion and elongation, inducing the expression of small auxin RNA (SAUR) genes, which promote growth by activating plasma membrane H^+-ATPases, ases, leading to cell wall loosening and solve uptake.

Principal component analysis revealed that T6 was associated with SN, SL, and %S (Figure 4C). Correlation analysis among the variables (%S, SN and SL) indicated coefficients below r=0.6 (Figure 4D), consistent with findings by Lebedev et al. (2018) in raspberry. This low correlation may result from including all treatment data, including the control, which can influence inter-variable relationships (Gonzales-Alvarado and Cardoso 2024).

Although a limited number of variables were considered in this study, the comprehensive analysis provided valuable insights into shoot production dynamics. It is recommended that future research includes biochemical analyses, such as chlorophyll composition and nutrient content, as well as physicochemical studies, including pH, electrical conductivity and biomass accumulation. Molecular approaches should also be explored to elucidate the mechanisms underlying IAA- and BAP-induced cell proliferation. Finally, these results may contribute to the design of conservation and ecological restoration programs for *C. officinalis*.

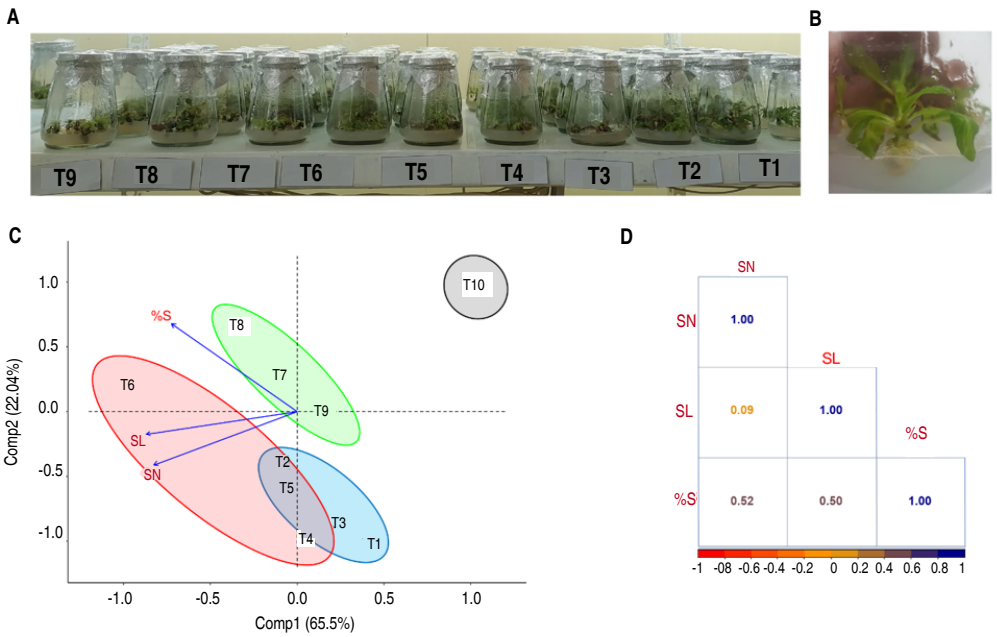


Figure 4. A. Proliferation treatments at 60 days. **B**. Control treatment. **C**. Principal component analysis (PCA) of the shoot proliferation experiment, and **D**. Correlation analysis.

Effect of Indole-3-butyric Acid (IBA) and Naphthaleneacetic Acid (NAA) on rooting of *Cinchona officinalis*

Rooting is considered a critical process for *in vitro* plant propagation because it ensures survival and successful development during *ex vitro* acclimatization (Lebedev et al. 2018). In this study, the highest rooting rate of 76.67±7.85% was achieved in treatment T8, consisting of 9.84 µM IBA + 8.06 µM NAA, evaluated 60 days post-inoculation *in vitro* (Table 3; Figure 5A). This result was significantly higher than previous reports for *C. officinalis* by Delgado-Paredes et al. (2025), which documented rooting percentages below 70%.

Regarding root length, treatments T4, T7 and T8 were identified as superior, reaching 5.43±1.36, 5.63±1.09 and 5.67±0.99 cm per plant, respectively (Figure 5B). Notably, treatments T4 and T7, despite not having the highest rooting percentages, were statistically similar to T8 and also exhibited a greater number and length of roots. These characteristics are known to enhance nutrient absorption and improve adaptation during the acclimatization phase (Kurtz et al. 2022).

The best results were obtained with low concentrations of NAA combined with moderate (7.38 µM) to high (9.84 µM)

Table 3. Effect of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) on the rooting of *Cinchona officinalis* (mean ± standard error). Different letters represent significant differences according to Duncan's test, $P \leq 0.05$.

DPI	T	IBA + NAA (µM)	RN	RL (cm)	S (%)	E (%)
30	T1	4.92+5.37	0.07±0.07^{BC}	0.03±0.03^{B}	100±0^{A}	3.33±3.33^{CD}
	T2	4.92+8.06	0.1±0.06^{BC}	0.23±0.13^{B}	96.67±3.33^{A}	10±5.57^{BCD}
	T3	4.92+10.74	0±0^{C}	0±0^{B}	96.67±3.33^{A}	0±0^{D}
	T4	7.38+5.37	0.93±0.38^{A}	1.2±0.46^{A}	100±0^{A}	33.33±8.75^{A}
	T5	7.38+8.06	0.5±0.18^{B}	0.4±0.13^{B}	100±0^{A}	26.67±8.21^{AB}
	T6	7.38+10.74	0.07±0.07^{BC}	0.03±0.03^{B}	100±0^{A}	3.33±3.33^{CD}
	T7	9.84+5.37	0.4±0.16^{BC}	0.5±0.21^{B}	100±0^{A}	20±7.43 ^{ABC}
	T8	9.84+8.06	0.23±0.11^{BC}	0.23±0.11^{B}	100±0^{A}	13.33±6.31^{BCD}
	T9	9.84+10.74	0.13±0.08^{BC}	0.17±0.1^{B}	100±0^{A}	10±5.57^{BCD}
	T10	0 (Control)	0±0^{C}	0±0^{B}	100±0^{A}	0±0^{D}
45	T1	4.92+5.37	0.2±0.11^{CD}	0.3±0.15^{CD}	100±0^{A}	13.33±6.31^{CD}
	T2	4.92+8.06	1.4±0.64^{AB}	1.47±0.63^{BCD}	96.67±3.33^{A}	26.67±8.21^{BC}
	T3	4.92+10.74	0.27±0.13^{CD}	0.57±0.26^{CD}	96.67±3.33^{A}	16.67±6.92^{BCD}
	T4	7.38+5.37	2.1±0.45^{A}	3.43±0.9^{A}	100±0^{A}	53.33±9.26^{A}
	T5	7.38+8.06	1±0.29^{BC}	1.77±0.54^{BC}	100±0^{A}	40±9.1^{AB}
	T6	7.38+10.74	0.2±0.11^{CD}	0.63±0.39^{CD}	100±0^{A}	13.33±6.31^{CD}
	T7	9.84+5.37	1.1±0.33^{BC}	2.5±0.7^{AB}	100±0^{A}	36.67±8.95^{ABC}
	T8	9.84+8.06	0.67±0.21^{BCD}	1.63±0.54^{BCD}	100±0^{A}	33.33±8.75^{ABC}
	T9	9.84+10.74	1.07±0.28^{BC}	1.63±0.63^{BCD}	100±0^{A}	40±9.1^{AB}
	T10	0 (Control)	0±0^{D}	0±0^{D}	100±0^{A}	0±0^{D}
60	T1	4.92+5.37	1.13±0.31^{BCD}	2.83±0.81^{AB}	100±0^{A}	40±9.1^{BC}
	T2	4.92+8.06	2.73±0.92^{A}	3.77±1.1^{AB}	96.67±3.33^{A}	40±9.1^{BC}
	T3	4.92+10.74	0.73±0.28^{CD}	1.5±0.49^{BC}	96.67±3.33^{A}	26.67±8.21^{C}
	T4	7.38+5.37	2.93±0.63^{A}	5.43±1.3^{6}A	100±0^{A}	56.67±9.2^{AB}
	T5	7.38+8.06	1.6±0.35^{ABC}	3.03±0.67^{AB}	100±0^{A}	50±9.28^{BC}
	T6	7.38+10.74	0.77±0.21^{CD}	2.27±0.68^{BC}	100±0^{A}	36.67±8.95^{BC}
	T7	9.84+5.37	2.6±0.52^{A}	5.63±1.0^{9}A	100±0^{A}	63.33±8.95^{AB}
	T8	9.84+8.06	2.47±0.45^{AB}	5.67±0.9^{9}A	100±0^{A}	76.67±7.85^{A}
	T9	9.84+10.74	2.63±0.51^{A}	4.23±1.1^{AB}	100±0^{A}	60±9.1^{AB}
	T10	0 (Control)	0±0^{D}	0±0^{C}	100±0^{A}	0±0^{D}

DPI: days post-inoculation. T: Treatment. IBA: Indole-3-butyric acid. NAA: Naphthaleneacetic acid. RN: Number of roots. RL: Root length. S (%): Survival percentage. E (%): Rooting percentage.

concentrations of IBA. However, these findings differed from initial hypothesis, which predicted that low concentrations of both IBA and NAA would produce higher rooting percentages. Fifteen days post-inoculation, callus formation was observed at the base of the nodal segments, which subsequently induced the development of root primordia, leading to the formation of adventitious roots of indirect origin. This behavior is consistent with that reported by Gonzales-Alvarado et al. (2022), who described callus formation followed by the development of root primordia and their subsequent differentiation into roots. In cases where callus formation was absent, roots did not develop, as observed in the control treatment (Figure 5C). These results contrast with those reported by Ătefančič et al. (2015), who found that callus formation negatively affected rooting percentage in cherry trees treated with IBA and NAA. On the other hand, the presence of callus does not necessarily imply somaclonal variation, as reported in Hibiscus sabdariffa by Konar et al. (2018).

Principal component analysis associated treatments T4 and T7 with the variables RN, RL and E (%), consistent with findings by Rebetzke et al. (2022). Correlation analysis revealed strong relationships among RN, RL and E (%) (Figure 5D), with coefficients exceeding r=0.94. Similar correlations were reported by Huanyu et al. (2023), with values above r=0.91. In contrast, no correlation was observed between survival and the other variables (RN, RL and E (%)). This lack of correlation may be explained by the inclusion of all treatment data, including the control, which can influence inter-variable relationships (Gonzales-Alvarado and Cardoso 2024). It is recommended that future studies focus on the acclimatization phase to further support the conservation and propagation of *C. officinalis*.

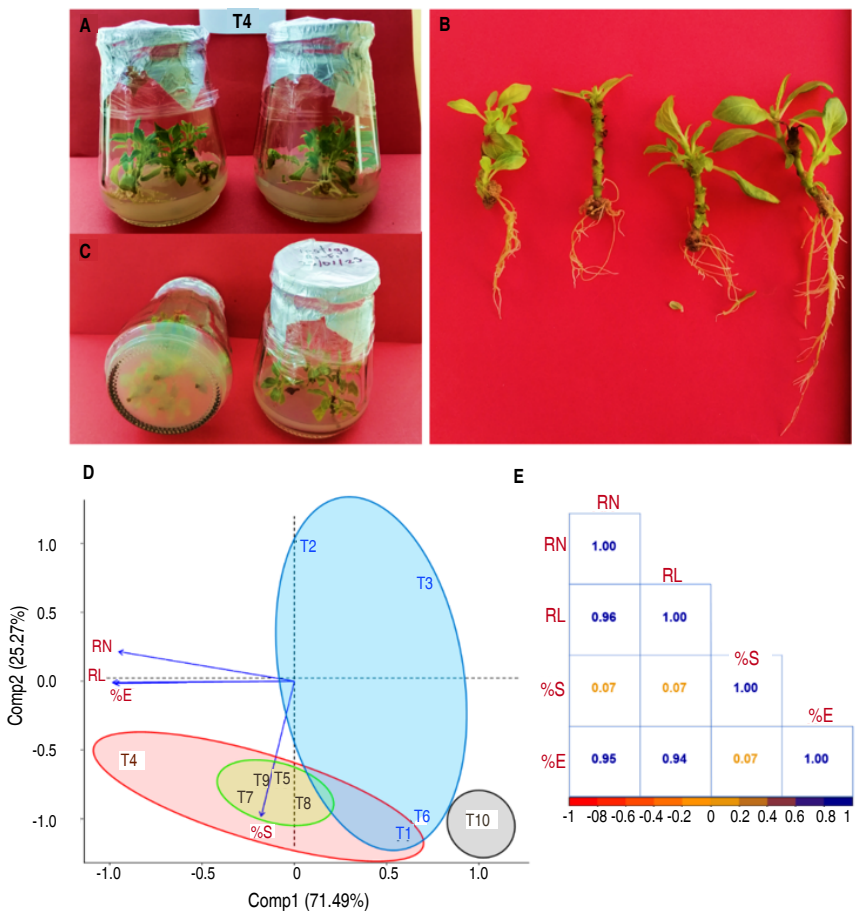


Figure 5. Rooting response. **A**. Treatment 8 to 60 days post-*in vitro* involution. **B**. Root length to 60 days. **C**. Control treatment. **D**. Principal Component Analysis (PCA) of the rooting experiment, and **E**. Correlation analysis. RN: Number of roots. RL: Root length. E (%): Rooting percentage. S (%): Survival percentage.

CONCLUSION

This study demonstrates the feasibility of nodal segments for the micropropagating *Cinchona officinalis*, providing a strategic approach for the conservation of this endangered species. In particular, a 2% sodium hypochlorite concentration yielded the best results, achieving the lowest contamination rate, reducing phenol release and increasing explant survival. For shoot proliferation, treatment with 2.28 µM IAA in combination with 5.33 µM BAP promoted a higher number and greater length of shoots. During the rooting phase, two treatments proved especially effective: 7.38 µM IBA + 5.37 µM NAA (T4) and 9.84 µM IBA + 8.06 µM NAA (T7). Both consistently enhanced the number of roots per nodal segment and root length. These findings directly contribute to species conservation efforts and provide a solid foundation for large-scale clonal propagation; furthermore, future studies should focus on the acclimatization phase and on optimizing the nutritional composition of culture media.

ACKNOWLEDGMENTS

We express our sincere gratitude to all the individuals and institutions that contributed to the development of this research. In particular, we would like to acknowledge the invaluable support of Teresita del Niño Jesús Moreno Huamán and Juan Seminario Cunya, head of the Plant Biotechnology Laboratory at the National University of Cajamarca, for providing the technical and logistical resources essential for carrying out the experimental work.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest of any kind in the preparation and publication of the manuscript.

REFERENCES

Armijos-González R, Ramón P and Cueva-Agila A (2024) *Cinchona officinalis* L. *ex situ* conservation by *in vitro* slow growth and cryopreservation techniques. Plant Cell Tissue Organ Culture 158:6. https://doi.org/10.1007/s11240-024-02784-8

Ăţefančič M, Ăţampar F and Osterc G (2015) Influence of endogenous IAA levels and exogenous IBA on rooting and quality of leafy cuttings of *Prunus* 'GiSelA 5'. The Journal of Horticultural Science and Biotechnology 81(3):508–512. https://doi.org/10.1080/14620316.2006.11512095

Cabahug RAM, Soh SY and Nam SY (2016) Effects of Auxin and Cytokinin Application on Leaf Cutting Propagation in *Echeveria* Species. Flower Research Journal 24(4): 264–273. https://doi.org/10.11623/FRJ.2016.24.4.04

Coronel-Castro E, Meza-Mori G, Torres JMC et al (2024) Potential distribution and identification of critical areas for the preservation and recovery of three species of *Cinchona* L. (Rubiaceae) in Northeastern Peru. Forests 15(2):321. https://doi.org/10.3390/f15020321

Delgado-Paredes GE, Rojas-Idrogo C, Vásquez-Díaz C et al (2025) *In vitro* propagation and germplasm conservation of three emblematic forest species of Peru. Notulae Scientia Biologicae 17(1):12005. https://doi.org/10.55779/nsb17112005

Dongli P, Anwar M and Yingying S (2023) The establishment of a rapid propagation system for *Hedera helix* petioles as explants. Rendiconti Lincei Scienze Fisiche e Naturali 34:275–281. https://doi.org/10.1007/s12210-022-01113-5

Gonzales-Alvarado AC and Cardoso JC (2024) Development, Chlorophyll Content, and Nutrient Accumulation in *in vitro* Shoots of *Melaleuca alternifolia* under light wavelengths and 6-BAP. Plants 13(20):2842. https://doi.org/10.3390/plants13202842

Gonzales-Alvarado AC, Hilario-Román N, Revilla-Chávez JM et al (2025) Woody plant medium optimizes *in vitro* germination and development of *Calycophyllum spruceanum.* Revista Facultad Nacional de Agronomía Medellín 78(3):11353–11363. https://doi.org/10.15446/rfnam.v78n3.119370

Gonzales-Alvarado AC, Mori-Vasquez JM, Tuisima LL and Revilla JM (2022) Influencia de la desinfección, medios de cultivo y fitohormonas en el desarrollo morfogénico *in vitro* de germoplasma de *Guazuma crinita* Mart. Folia Amazónica 31(1):57–70. https://doi.org/10.24841/fa.v31i1.572

Huanyu C, Lizhou H, Angyan R et al (2023) Growth regulators on the shooting and rooting of *Tamarix chinensis* stem cuttings. Rhizosphere 25:100679. https://doi.org/10.1016/j.rhisph.2023.100679

Khan A, Bashir A, Erum S et al (2021) Effects of 6-Benzylaminopurine and Indole-3-acetic acid on growth and root development of banana explants in micropropagation. Sarhad Journal of Agriculture 37(1):9–13. http://doi.org/10.17582/journal.sja/2021/37.1.9.13

Khare T, Kumar V and Kishor PBK (2015) Na^+ and Cl^- ions show additive effects under NaCl stress on induction of oxidative stress and the responsive antioxidative defense in rice. Protoplasma 252:1149–1165. https://doi.org/10.1007/s00709-014-0749-2

Konar S, Karmakar J, Ray A, Adhikari S and Bandyopadhyay TK (2018) Regeneration of plantlets through somatic embryogenesis from root derived calli of *Hibiscus sabdariffa* L. (Roselle) and assessment of genetic stability by flow cytometry and ISSR analysis. PlosOne 13(8):e0202324. https://doi.org/10.1371/journal.pone.0202324

Kurtz LE, Borbas LN, Brand MH and Lubell-Brand JD (2022) *Ex vitro* rooting of *Cannabis sativa* microcuttings and their performance compared to retip and stem cuttings. American Society for Horticulture Science 57(12):1576–1579. https://doi.org/10.21273/hortsci16890-22

Lebedev V, Arkaev M, Dremova M et al (2018) Effects of growth regulators and gelling agents on *ex vitro* rooting of raspberry. Plants 8(1):3. https://doi.org/10.3390/plants8010003

Lima M (2021) Multivariate analysis: Multivariate statistical analysis. R package version 1.0.2.

MINAGRI – Ministerio de Agricultura y Riego (2020) Resolución directoral ejecutiva N° 067-2020-MINAGRI-DVDIAR-AGRO RURAL-

DE. 1–5. Perú. https://www.actualidadambiental.pe/wp-content/uploads/2020/06/RDE-067-2020-AG-AGRORURAL-DE-1.pdf

Mori-Vásquez JA, Panduro Tenazoa NM, Muñoz Fernandez VJ et al (2024) Effect of disinfection, leaf development, and phytohormones on callus induction of *Simarouba amara* Aubl. Ciencia y Tecnología Agropecuaria 25(3):e3662. https://doi.org/10.21930/rcta.vol25_num3_art:3662

Pandey H, Misra V, Sharma A et al (2025) Nodal culture for efficient regeneration and CRISPR/Cas-based genome editing in recalcitrant horticultural crops. Horticulture Advances 3:28. https://doi.org/10.1007/s44281-025-00080-9

Pei-gen X (2008) Effect of plant growth regulator on proliferation of axillary buds of *Anoectochilus roxburghii*. The Chinese Pharmaceutical Journal 43(23):1777–1780. https://api.semanticscholar.org/CorpusID:88099710

Rebetzke GJ, Zhang H, Ingvordsen CH et al (2022) Genotypic variation and covariation in wheat seedling seminal root architecture and grain yield under field conditions. Theoretical and Applied Genetics 135:3247–3264. https://doi.org/10.1007/s00122-022-04183-z

Rufasto-Peralta YL, Baselly-Villanueva JR, Alva-Mendoza DM et al (2023) Estimación de la calidad de sitio de *Cinchona pubescens* (Rubiaceae), en el bosque montano La Palma, Chota, Perú. Lilloa 60(2):259–279. https://doi.org/10.30550/j.lil/1826

Sánchez Santillán T, Meléndez Mori J, Morales Rojas E et al (2021) Clonal propagation of the cinchona tree (*Cinchona officinalis* L.): an alternative for conserving Peru's national tree. Bioagro 33(3):215–222. https://doi.org/10.51372/bioagro333.7

The Lancet Infectious Diseases (2024) A new dawn for malaria prevention. The Lancet Infectious Diseases 24(2):107. https://doi.org/10.1016/S1473-3099(24)00012-4

Wang S, Taketa S, Ichii M et al (2003) Lateral root formation in rice (*Oryza sativa* L.): Differential effects of indole-3-acetic acid and indole-3-butyric acid. Plant Growth Regulation 41 (1):41–47. https://doi.org/10.1023/A:1027384528993

Wei T, Simko V, Levy M et al (2024) *Corrplot: Visualization of a Correlation Matrix* (V.0.9). https://cran.r-project.org/web/packages/corrplot/index.html

Wickham H (2016) Programming with ggplot2. In H. Wickham (Ed.), Ggplot2: Elegant graphics for data analysis. Springer International Publishing 241-253. https://doi.org/10.1007/978-3-319-24277-4_12

Xu YX, Xiao MZ, Liu Y et al (2017) The small auxin-up RNA OsSAUR45 affects auxin synthesis and transport in rice. Plant Molecular Biology 94:97–107. https://doi.org/10.1007/s11103-017-0595-7

Yücesan BB, Mohammed A, Arslan M and Gürel E (2015) Clonal propagation and synthetic seed production from nodal segments of cape gooseberry (*Physalis peruviana* L.), a tropical fruit plant. Turkish Journal Agriculture and Forestry 39(5):797–806. https://doi.org/10.3906/tar-1412-86