ARTÍCULO DE INVESTIGACIÓN

Optimizing *ex vitro* Acclimatization of *Cinchona spp.*: Towards the establishment of an industrial crop in Colombia

Optimización de la aclimatación *ex vitro* de *Cinchona spp.*: Hacia el establecimiento de un cultivo industrial en Colombia

Juan David Saavedra Correa*, Silvia Lizette Bustamante Rodríguez**, Robert Theiler***

DOI: 10.15446/rev.colomb.biote.v27n1.118377

ABSTRACT

Efforts to optimize *ex vitro* transfer techniques of un-rooted i*n vitro* shoots of *Cinchona spp.* (Rubiaceae) a native tree of the andean forests, are crucial for promoting sustainable cultivation practices of this medicinal plant. Renowned for its historical significance and effectiveness in treating malaria and other ailments, the tree holds a prominent position in botanical research, food industry, and pharmaceutical applications due to its production of alkaloids such as quinine and quinidine. This study aimed to improve the acclimatization process of un-rooted *in vitro* shoots of Cinchona, facilitating their transplantation to field conditions for establishing a plantation in Colombia. The results revealed that compared to commercial available substrates (pH higher than 5.5), the utilization of peat moss substrate (pH lower than 4 and electric conductivity lower than 100 µS / cm^{-1}) led to significantly higher survival rates (>87%) and improved growth outcomes. This underscores the efficacy of peat moss in facilitating the acclimatization process of *ex vitro* plants over a 12-week period, ensuring robust development and survival of the plants, which guarantees vigorous specimens for field plantation.

Key words: Cinchona*, Ex vitro* transfer, Acclimatization, Unrooted shoots.

RESUMEN

Los esfuerzos para optimizar las técnicas de transferencia *ex vitro* de brotes no enraizados *in vitro* de *Cinchona spp.* (Rubiaceae), un árbol nativo de los bosques andinos, son cruciales para promover prácticas de cultivo sostenible de esta planta medicinal. Reconocida por su importancia histórica y su eficacia en el tratamiento de la malaria y otras dolencias, la planta ocupa una posición destacada en la investigación botánica, la industria alimentaria y las aplicaciones farmacéuticas debido a su producción de alcaloides como la quinina y la quinidina. Este estudio tuvo como objetivo mejorar el proceso de aclimatación de los brotes de Cinchona propagados *in vitro* no enraizados, facilitando su trasplante a condiciones de campo y sentando las bases para establecer una plantación en Colombia. Los resultados revelaron que, en comparación con los sustratos comerciales disponibles (pH superior a 5.5), la utilización de sustrato

* MSc - Instituto de Biotecnología Universidad Nacional de Colombia, Bogotá, Colombia. Mail: jusaavedrac@unal.edu.co - https://orcid.org/0000-0003-1527-0428

** MSc -Instituto de Biotecnología Universidad Nacional de Colombia, Bogotá, Colombia. Mail: slbustamanter@unal.edu.co - https://orcid.org/0000-0002-2595-9758

*** PhD - Formerly Horticultural Department, Agroscope Changins-Wädenswil, Wädenswil, Switzerland. Mail: theiler.robert.colombia@gmail.com - https://orcid.org/0009-0003-3705-6542

de turba (pH inferior a 4 y conductividad eléctrica inferior a 100 µS / cm^{-1}) condujo a tasas de supervivencia significativamente más altas (>87%) y mejores resultados de crecimiento. Esto destaca la eficacia de la turba en la facilitación del proceso de aclimatación de plantas *ex vitro* durante un período de 12 semanas, asegurando un desarrollo y supervivencia robustos de las plantas, lo que garantiza especímenes vigorosos para la plantación en campo.

Palabras clave: Cinchona, Transferencia *ex vitro*, Aclimatación, Brotes no enraizados.

Recibido: enero 17 de 2025 **Aprobado:** 10 de abril de 2025

INTRODUCTION

Originating in the Andean regions of Bolivia, Peru, Ecuador, and Colombia, *Cinchona spp.* holds a profound historical significance as a traditional remedy known as "quina-quina" for treating symptoms of fever (Taylor, 1943). The bark of the Cinchona tree is highly valued for its bioactive alkaloids —quinine, quinidine, cinchonine, and cinchonidine— which confer its medicinal properties (Schaepmeester, 2021). In addition, due to the bitter taste, quinine is actively used in the beverage industry for producing tonic water (Nikolaeva *et al.*, 2019; Pain, 2019). Global production of Cinchona alkaloids is estimated at approximately 600 tons per year, with 40% destined to the food and beverage industry and 60% for pharmaceutical applications (Schaepmeester, 2021; Yip *et al.*, 2023). This high global demand for Cinchona bark and its derivatives underscores the critical need for sustainable cultivation practices, to ensure the availability of the resource.

The therapeutic use of cinchona trees dates back to the early 16th century, when Spanish colonizers documented that indigenous Andean civilizations used their bark to treat fevers (Díaz-Piedrahita, 2003; Sandoval & Echandía, 1986). By the 17th century, Jesuit missionaries in Peru recognized its therapeutic effectiveness in combating malaria (Jaramillo-Arango, 1949; Achan *et al.*, 2011), in the following decades, the cinchona bark trade to Europe became popular; however, concerns about overexploitation increased in the 18th century, prompting scientific expeditions to the Andean mountains by Dutch, English, and French scientists in the mid-19th century, to obtain seeds and seedlings and start plantations in their colonies (Taylor, 1943; Crawford, 2014, 2016). Subsequently, the establishment of Cinchona plantations in Java (Indonesia), India, and Africa, during the late 19th century transformed the global Cinchona bark trade and malaria treatment (Roersch van der Hoogte & Pieters, 2014).

In the 20th century, efforts to improve Cinchona plantations productivity were established in central Africa by the Dutch, Belgians, and Germans at Bukavu, Democratic Republic of Congo (Pharmakina SA, 2016). However, the spread of Cinchona root disease caused by the soil-borne pathogen *Phytophthora cinnamomi* led to high losses of trees in certain plantations (Theiler, 2014). Pharmakina S.A. initiated a project with ACW (Agroscope Changins-Wädenswil, Switzerland) to identify and multiply *P. cinnamomi* tolerant plants (mainly obtained by seed-grown field plants) through *in vitro* techniques. Some of these *P. cinnamomi* tolerant Cinchona genotypes were kept *in vitro* at ACW and eventually transferred to the Tissue Culture laboratory at the Instituto de Biotecnología (IBUN) at the Universidad Nacional de Colombia for *in vitro* micropropagation and *ex vitro* acclimatization (Theiler, 2014). This attempt is part of a broader initiative to establish a small plantation in Colombia to produce cinchona bark. These undertakings are indispensable for initiating a sustainable cultivation and preservation of the Cinchona tree in Cundinamarca, Colombia.

In vitro culture techniques provide a disease-free controlled environment for the massive propagation of Cinchona plants in short periods (Armijos-González & Pérez-Ruiz, 2016; Moraes *et al.*, 2021; Vivanco-Galván *et al.*, 2022; Duta-Cornescu *et al.*, 2023), setting the foundation for subsequent transplantation to field conditions. However, the transition from *in vitro* to *ex vitro* conditions poses significant challenges. *In vitro* plants are characterized by underdeveloped cuticles and low stomatal functionality, making them highly susceptible to desiccation, transplant shock, and nutrient deficiencies when transferred to *ex vitro* environments (Perea Dallos *et al.*, 2009; Mahendra *et al.*, 2020). Consequently, this step impacts plant survival and growth rates; thus, micropropagated plants often lack the physiological adaptations necessary to survive *ex vitro*, leading to reduced survival rates and hindered growth (Oakes *et al.*, 2020; Shiwani *et al.*, 2022).

Therefore, optimizing *ex vitro* acclimatization techniques becomes essential for starting sustainable cultivation and conservation practices for *Cinchona spp.,* improving plant health and overall productivity. Several studies have focused on the *in vitro* conservation of Cinchona species (Hunter, 1972; Armijos-González & Pérez-Ruiz, 2016; Lima-Jimenez, 2018; Serrano *et al.*, 2019; Vivanco-

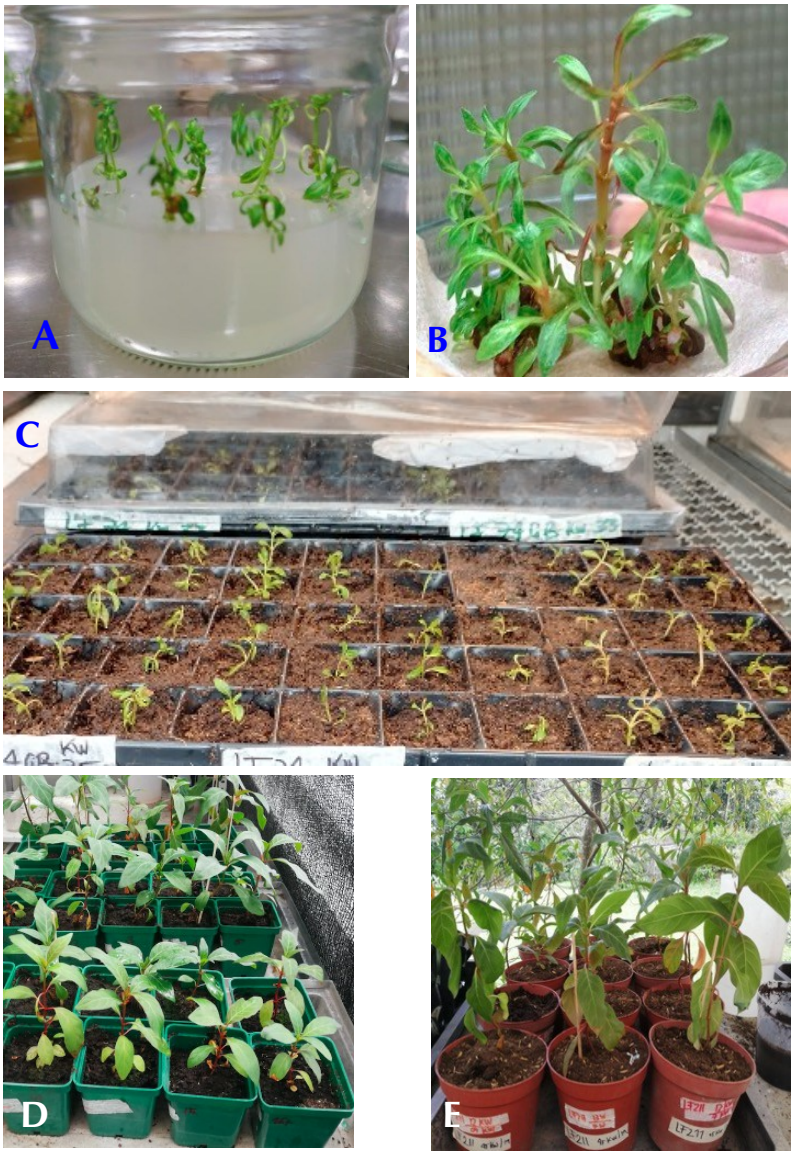

Figure 1. Acclimatization *ex vitro* of *Cinchona spp.* genotypes. (a) Freshly subculture shoots *in vitro*. (b) Clusters of micropropagated *in vitro* shoots after 12-week culture period. (c) Un-rooted *in vitro* shoots were transferred to seedling trays for the initial stage of the acclimatization phase. (d) After 12 weeks of acclimatization with substrate S5. (e) Cinchona plants are ready for transfer to soil after 6 months under shade conditions.

Galván *et al.*, 2022), nevertheless, there is a lack of research related to the *ex vitro* acclimatization process in cinchona. To address this knowledge gap, this study aims to refine *ex vitro* acclimatization techniques for *Cinchona spp.* by evaluating various substrates, facilitating the transition of *in vitro* plants to field conditions, as well as the long-term preservation of this economically significant plant species and establishing a future sustainable production system in Colombia.

MATERIALS AND METHODS

Selection of Plant Material

Four different genotypes of *Cinchona spp.* hybrids of *Cinchona calisaya* (formerly *C. ledgeriana*) and *Cinchona pubescens* (formerly *C. succirubra)* (Andersson, 1998; Schaepmeester, 2021), were selected based on their potential tolerance to the pathogen *Phytophthora cinnamomi*. These selections were part of a research project between Pharmakina (DRC) and ACW

Table 1. pH and Conductivity average values of each substrate evaluated during the acclimatization stage.

Substrate	pH	Electric Conductivity (EC)
		µS / cm $^{-1}$
(S1) Compost / Garden soil (1:1)	7.00	90
(S2) Sandy Soil	5.10	<50
(S3) Commercial garden soil (Anasac®)	5.25	320
(S4) Peat substrate / Commercial garden Soil (Anasac®) (1:1)	5.38	910
(S5) Peat moss substrate (Pindstrub®)	3.73	86

(Switzerland), aimed at ensuring a sustainable quinine bark supply in central Africa nearly four decades ago. These genotypes, identified as LC29, LF211, LF40, LF74 and LF74GB (an *in vitro* selection of LF74 with larger leaf blades), were transferred to the laboratory of Plant Tissue Culture at the Instituto de Biotecnología (IBUN) of the Universidad Nacional de Colombia, in Bogotá, approximately ten years ago (Theiler, 2014).

Before acclimatization, the Cinchona plants were maintained and propagated *in vitro* by shoots in a modified Murashige and Skoog (M&S) medium supplemented with 1 mg/L of benzylaminopurine (BAP) and 0.1 mg/L of indole-3-butyric acid (IBA) (Hunter, 1979), in glass jars of 300 and 450 ml (Figure 1a), *in vitro* subcultures were performed every 12 weeks to prepare the plants for transfer to *ex vitro* conditions (Figure 1b). Maintaining a temperature range of 24°C ± 3°C, with a photoperiod consisting of 14 hours of light and 10 hours of darkness under white light, at an intensity of 25–35 µmol m-2 s-1 photon flux density (PFD).

Acclimatization and Substrate Selection

In vitro shoots from each Cinchona genotype, with a minimum of three pairs of leaves, without roots and a length of ≥2 cm, were chosen for the acclimatization and hardening phase. This selection process aligns with established protocols recognized for enhancing plant adaptability to external conditions (Jagiełło-Kubiec *et al*., 2021; Shiwani *et al*., 2022). The rootless shoots were placed in seedling trays (Figure 1c) equipped with transparent plastic covers , which created a microenvironment conducive to maintaining humidity levels for four weeks, facilitating a gradual transition from *in vitro* to *ex vitro* conditions.

Initially, a preliminary experiment was conducted to assess the survival rates of the selected shoots across five substrate variations: compost and garden soil mix (1:1) (S1), Sandy soil (S2), Commercial garden soil (Anasac®) (S3), peat substrate mixed with commercial garden soil (1:1) (S4), and peat moss substrate (Pindstrub®) (S5) (Table 1).

The trays were placed in a greenhouse at the IBUN, to maintain optimal controlled environmental conditions, with a temperature range of 25-28°C during the day and 17-22°C during the night, and an exposure to 12 hours of daily light with a light intensity between 16 to 24 µmol m^{-2} s^{-1}, and a relative humidity (RH%) level above 70%. After four weeks the survival rate was calculated according to the equation: Survival Rate (%) = (Number of surviving plants / Total number of plants) × 100.

Second stage of acclimatization

Upon completion of the initial acclimatization phase, the plant shoots were exposed to ambient conditions by gradually removing the covers, allowing them to adapt incrementally to environmental conditions. Additionally, plants were watered two times per week and received a sprayed foliar fertilization with a diluted M&S medium salts solution at pH 5.8 every two weeks. During twelve weeks' period, continuous monitoring of plant growth parameters, including shoot length, nodes, and leaf count, was conducted to evaluate the effectiveness of the acclimatization technique employed.

Data collection, and statistics analysis

During the initial stage, 100 un-rooted *in vitro* shoots (20 per genotype) were planted in each of the five substrates under evaluation to assess viability based on survival rates. After identifying the substrate with the highest survival rate in a period of four weeks, the main experiment proceeded with 15 *in vitro* rootless shoots per Cinchona genotype chosen as replicates, which was repeated three times over the course of a year. Observations on shoot length, leaf pairs, and node number, were recorded during 12th week after transfer to *ex vitro* conditions. The final survival rate and growth parameters

data were analyzed statistically using the nonparametric Kruskal-Wallis Test (Kruskal & Wallis, 1952), as the data did not meet the assumptions of normality and homogeneity of variances. The differences amongst the mean values ($P < 0.05$) were calculated by Wilcoxon signed-rank test using the using the R program (v.4.0.5. R Development Core Team, 2021). All the results are presented in the form of mean ± SD.

RESULTS AND DISCUSSION

In vitro multiplication

Five *in vitro* genotypes of *Cinchona spp*. originating from established Congolese plantations (Theiler, 2014), were micropropagated using a modified MS medium (Hunter, 1979). The micropropagation resulted in plant clusters with shoots ranging from 2 to 3 cm in length within a three-month subculture cycle (Figure 1b). Before the acclimatization phase, it was determined to exclude the *in vitro* rooting phase as part of protocol optimization efforts, recognizing the inherent complexities associated with *in vitro* rooting, including plant recalcitrance and slow root initiation particularly in woody plants species including Cinchona tree (Abdalla *et al*., 2022).

Survival rates in the different substrates

Rootless shoots were transplanted into the five soil substrates under evaluation (Table 1), throughout the initial 4-week acclimatization period in the greenhouse. Shoots of *Cinchona spp*. were covered during this phase to facilitate the hardening process while maintaining high humidity levels. After this period, the average survival rate was assessed (Figure 2). The peat moss substrate (S5) yielded an 87.3% survival rate, which was significantly higher ($P < 0.05$) compared to the survival rates in the other remaining substrates (S1, S2, S3, and S4). where the survival rate dropped to below 20%.

Survival rates following the initial stage of acclimatization exhibited a pronounced inclination towards acidic soils, the experiment indicated the limitation to successfully establish *in vitro* shoots in soils where the pH concentration is above 5.5. Consequently, peat moss substrate (Pindstrub®) (S5) was selected as the optimal choice with an average pH of 3.65 and electrical conductivity (EC) of 86 $\mu S/cm^{-1}$. S5 is characterized by having a low pH, high water retention capacity, and high porosity (Shin *et al*., 2012; Pandey *et al*., 2019). These properties are advantageous for rootless shoots as they ensure consistent moisture around the developing basal area where root initiation typically occurs, accompanied by the promotion of soil aeration ensuring sufficient oxygen supply necessary for gas exchange and nutrient absorption (Clapa *et al*., 2013; Ben & Friedman, 2018). Additionally, the combination of low EC and acidic nature could probably have minimized salt stress in the micropropagated plants and could have provided a more balanced and readily available nutrient source compared to higher EC substrates (Prasad, 2022). Furthermore, in the peat moss substrate (S5), survival rates were consistent across all genotypes, with no significant differences in survival rates between them ($P > 0.05$). This commercial substrate stood out for its performance compared to others evaluated, emphasizing its uniform

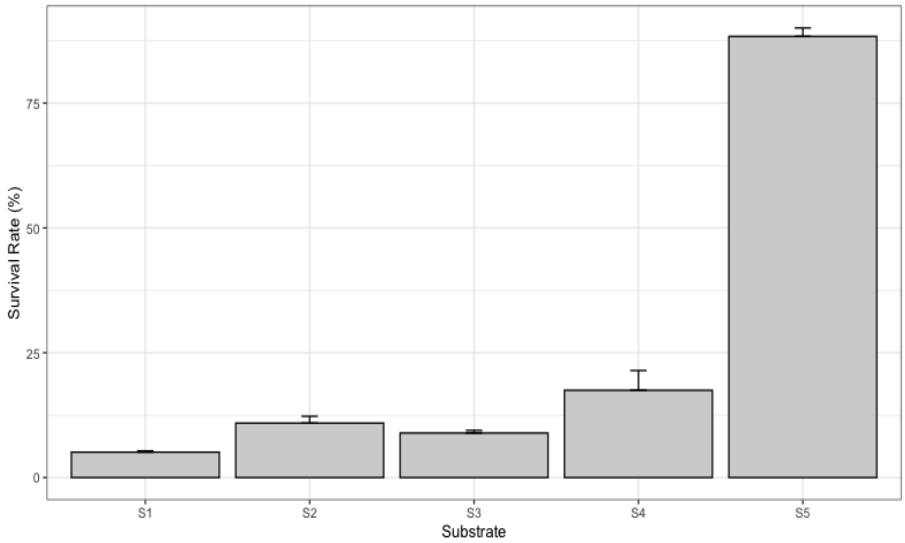

Figure 2. Effect of different substrates on *ex vitro* survival percentage of Cinchona rootless shoots during 4 weeks, S1 - Compost and garden soil mix (1:1), S2 - Sandy soil, S3 - Commercial garden soil (Anasac®), S4 - Peat substrate mixed with commercial garden soil (1:1), and S5 - Peat moss substrate (Pindstrub®).

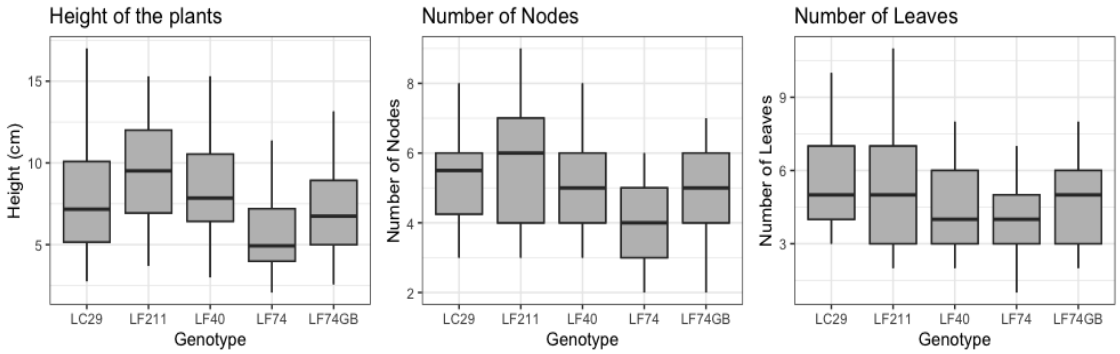

Figure 3. Growth variations among the *Cinchona spp.* genotypes after an acclimatization period of 12-weeks in the substrate S5.

Table 2. *Cinchona spp.* growth by genotype over a period of 12 weeks of acclimatization. Means in each column followed by the same letters are not significantly different according to Wilcoxon's rank sum tests at $P < 0.05$. Values are mean ± standard error.

Genotype	Shoots Height (cm)	Number of Nodes	Number of leaf pairs
	Mean ±SE	Mean ±SE	Mean ±SE
LC29	7.91 ±0.52 a	5.35 ±0.18 a	5.36±0.26 a
LF211	9.42 ±0.46 b	5.67 ±0.20 a	5.40 ±0.33 a
LF40	8.51 ±0.46 ab	5.12 ±0.16 ac	4.45 ±0.25 b
LF74	5.61 ±0.35 c	4.03 ±0.15 b	4.37 ±0.21 b
LF74GB	7.06 ±0.38 ab	4.86 ±0.16 c	4.72 ±0.24 ab

efficacy in supporting survival across various genetic backgrounds within the Cinchona genotypes.

Conversely, the low survival rates observed in substrates S1, S2, S3, and S4 compared to peat moss (S5) are probably due to a combination of factors related to their pH and electrical conductivity (EC). Most of the substrates have a neutral to slightly acidic pH (around 7.0 and 5.25. respectively) which might not be optimal for Cinchona shoots development. This observation aligns with existing reports highlighting the plant's preference for acidic soils in its natural habitats (Gómez Silvera *et al.*, 2016; Villar Cabeza *et al.*, 2018; Rufasto Peralta, 2021). Additionally, Substrates such as S3 and S4, which have a high EC value (320 and 910 µS/cm^{-1} respectively) could have induced salt stress in the rootless shoots, hindering their ability to absorb water and nutrients (Zsolt *et al.*, 2020). While S2 has a more favorable pH (5.10), its very low EC (<50 µS/cm^{-1}) might indicate a lack of readily available nutrients, likely contributing to the poor survival rates seen in these substrates.

Second stage of acclimatization

Due to these results, the shoots were maintained in peat moss substrate for an additional eight weeks, after this period the growth of *Cinchona spp.* genotypes were evaluated (Table 2). Wilcoxon's rank sum tests indicated there were significant differences ($P < 0.05$) between genotypes in terms of shoot height, the number of nodes and leaf pairs. Genotype LF211 stood out with the tallest shoots (9.4 cm ±3.0), the highest average number of nodes (5.6 ±1.3), and the most leaf pairs (7.0 ±2.5). Conversely, LF74 exhibited the lowest values in all three categories: shoot height (5.6 cm ±2.4), number of nodes (4.0 ±1.0), and number of leaf pairs (4.9 ±1.0). LC29, LF40 and LF74GB displayed intermediate values for all parameters.

The variations in growth parameters among the different Cinchona genotypes (Figure 3) underline the significance of their distinct physiological responses and the interplay between genetics and environmental factors during *ex vitro* acclimatization. This diversity in growth

Figure 4. Acclimatized Cinchona plants after transplantation to field conditions in Sasaima, Cundinamarca, Colombia.

attributes reflects the inherent variations dictating the plants' growth patterns and resource allocation strategies (Espinosa-Leal *et al.*, 2018). LF211's taller shoot height and greater number of nodes and leaves suggest a robust growth phenotype, potentially indicating an adaptation to the peat moss substrate. In contrast, LF74's comparatively shorter shoot height and fewer nodes and leaf pairs may imply a less vigorous growth phenotype, emphasizing the importance of genotype selection for optimal cultural practices. However, the sustained high survival rates exhibited in all genotypes, despite differences in growth parameters, demonstrate the promising potential of Cinchona genotypes to thrive under the controlled *ex vitro* environmental conditions proposed in this research.

Furthermore, a key aspect of successful *ex vitro* acclimatization involves gradually exposing the plants to fluctuating humidity conditions (Hazarika *et al.*, 2006; Perea Dallos *et al.*, 2009; Chandra *et al.*, 2010; Deng *et al.*, 2015; Mahendra *et al.*, 2020). By systematically removing the transparent covers for limited periods throughout the last 8 weeks of the experiment, the plants progressively acclimated to fluctuations in humidity and temperature, mimicking the *ex vitro* environment. This approach culminated in the complete removal of covers at week 10, signifying a milestone indicating sufficient adjustment for field transplantation, and promoting enhanced plant resilience and vitality. After twelve weeks the *Cinchona spp.* plants from the greenhouse at IBUN were transplanted to plastic pots (Figure 1d) and relocated to a farm situated in the coffee production area of Sasaima, Cundinamarca, where the acclimatized Cinchona plants were carefully maintained under shaded and protected conditions, in a substrate comprised of a (1:1) mixture of peat moss substrate (S5) and field soil with a pH level averaging 6.5 and EC concentrations ranging between 380-425 $\mu S/cm^{-1}$. Over the course of six months in this environment, the plants grew to a height of ≥30 cm (Figure 1e), after which they were transplanted into field conditions (Figure 4).

CONCLUSION

The optimization of *ex vitro* acclimatization techniques for un-rooted *in vitro* shoots of *Cinchona spp.* genotypes, particularly through substrate selection and systematic management of environmental factors, underscore the role of strategies in promoting survival rates on *in vitro* plant shoots, maintaining high levels of relative humidity (>90%) during this stage is essential to encourage plant survival and facilitate the transition from rootless *in vitro* shoots to *ex vitro* conditions. Likewise, the research highlighted the effectiveness of the peat substrate (pH 3.73 and EC of 86 $\mu S/cm^{-1}$) with a high survival rate compared to other substrates. Furthermore, the analysis of growth parameters showed variations between the genotypes, indicating their different physiological responses and their adaptability to the peat substrate. These conditions during the acclimatization phase were essential since it improved the resistance and vitality of the plants for a successful transplantation to field conditions. These

results can contribute to the establishment of a commercial plantation in Colombia, generating a robust stock material from hybrid plants. Likewise, our findings could have a connotation for conservation efforts, in the reintroduction processes of threatened Cinchona species through *in vitro* culture techniques.

ACKNOWLEDGEMENTS

We thank the Instituto de Biotecnología de la Universidad Nacional de Colombia (IBUN), particularly the Laboratory of Plant Tissue Culture for the support provided in the development of this research.

REFERENCES

Abdalla. N., El-Ramady. H., Seliem. M. K., El-Mahrouk. M. E., Taha. N., Bayoumi. Y., Shalaby. T. A., & Dobránszki. J. (2022). An Academic and Technical Overview on Plant Micropropagation Challenges. *Horticulturae*. 8(8). Article 8. https://doi.org/10.3390/horticulturae8080677

Achan. J., Talisuna. A. O., Erhart. A., Yeka. A., Tibenderana. J. K., Baliraine. F. N., Rosenthal. P. J., & D'Alessandro. U. (2011). Quinine. an old antimalarial drug in a modern world: Role in the treatment of malaria. *Malaria Journal*. 10(1). 144. https://doi.org/10.1186/1475-2875-10-144

Andersson. L. (1998). A revision of the genus Cinchona (Rubiaceae—Cinchoneae). Memoirs of The New York Botanical Garden. 80: 1–75.

Armijos-González. R., & Pérez-Ruiz. C. (2016). *In vitro* germination and shoot proliferation of the threatened species *Cinchona officinalis L* (Rubiaceae). *Journal of Forestry Research*. 27(6). 1229-1236. https://doi.org/10.1007/s11676-016-0272-8

Ben. N., & Friedman. S. (2018). Review and Evaluation of Root Respiration and of Natural and Agricultural Processes of Soil Aeration. *Vadose Zone Journal - Wiley Online Library*. 17(1:47). https://doi.org/10.2136/vzj2017.06.0119

Chandra. S., Bandopadhyay. R., Kumar. V., & Chandra. R. (2010). Acclimatization of tissue cultured plantlets: From laboratory to land. *Biotechnology letters*. 32. 1199-1205.

Clapa. D., Fira. A., & Joshee. N. (2013). An efficient *ex vitro* rooting and acclimatization method for horticultural plants using float hydroculture. *HortScience*. 48 (9). 1159-1167. https://doi.org/10.21273/HORTSCI.48.9.1159

Crawford. M. J. (2014). An empire's extract: chemical manipulations of cinchona bark in the eighteenth-century spanish atlantic world. *Osiris*. 29(1). 215-229. https://doi.org/10.1086/678104

Crawford. M. J. (2016). The andean wonder drug: cinchona bark and ımperial science in the spanish atlantic. 1630-1800. University of Pittsburgh Press.

Deng. Z. C., Jin. H., & He. H. (2015). An efficient micropropagation system for *Morinda officinalis* How. (Rubiaceae). an endangered medicinal plant. *Journal of Agricultural Science and Technology*. 17(6). 1609-1618.

Díaz-Piedrahita. S. (2003). Las quinas en el mundo y en Colombia. *Revista Medicina Colombia*. Vol. 25(2 (62)). 128-132 (in Spanish).

Duta-Cornescu. G., Constantin. N., Pojoga. D.-M., Nicuta. D., & Simon-Gruita. A. (2023). Somaclonal variation—advantage or disadvantage in micropropagation of the medicinal plants. *International Journal of Molecular Sciences*. 24(1). 838. https://doi.org/10.3390/ijms24010838

Espinosa-Leal. C. A., Puente-Garza. C. A., & García-Lara. S. (2018). *In vitro* plant tissue culture: Means for production of biological active compounds. *Planta*. 248(1). 1-18. https://doi.org/10.1007/s00425-018-2910-1

Gómez Silvera. A., Beraun Macedo. L. A., Gómez Rengifo. O. J., & Llatas Ducep. E. (2016). Procesos de regeneración natural de la quina o cascarilla *(Cinchona spp.):* En los bosques de neblina del distrito de Kañaris. región Lambayeque. Instituto Nacional de Innovación Agraria. (in Spanish) https://bit.ly/4eFu6Dk

Greenwood. D. (1992). The quinine connection. *Journal of Antimicrobial Chemotherapy*. 30(4). 417-427. https://doi.org/10.1093/jac/30.4.417

Hazarika. B. N., Teixeira da Silva. J., & Talukdar. A. (2006). Effective acclimatization of *in vitro* cultured plants: Methods. physiology and genetics. *Floriculture*. ornamental and plant biotechnology. 2. 427-438.

Hunter. C. S. (1979). *In vitro* Culture of *Cinchona Ledgeriana* L. *Journal of Horticultural Science*. 54(2). 111-114. https://doi.org/10.1080/00221589.1979.11514857

Jagiełło-Kubiec. K., Nowakowska. K., Łukaszewska. A. J., & Pacholczak. A. (2021). Acclimation to *ex vitro* conditions in ninebark. *Agronomy*. 11(4). 612. https://doi.org/10.3390/agronomy11040612

Jaramillo-Arango. J. (1949). A critical review of the basic facts in the history of Cinchona. *Botanical Journal of the Linnean Society*. 53(352). 272-311. https://doi.org/10.1111/j.1095-8339.1949.tb00419.x

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association*, *47*(260), 583–621.

Lima Jiménez, N. R., Moreno Serrano, J. A., Eras Guamán, V. H., Minchala Patiño, J., González Zaruma, D., Yaguana Arévalo, M., & Valarezo Ortega, C. (2018). Propagación *in vitro* de Cinchona officinalis L a partir de semillas. *Revista de Investigaciones Altoandinas*, 20(2), 169–178.

Mahendra. R., Chauhan. N., Sharma. J., Rana. K., & Bakshi. M. (2020). *Ex-vitro* establishment of tissue cultured plants in fruit crops-a review. *International Journal of Current Microbiology and Applied Sciences*. 9. 3321-3329. https://doi.org/10.20546/ijcmas.2020.911.397

Moraes. R. M., Cerdeira. A. L., & Lourenço. M. V. (2021). Using micropropagation to develop medicinal plants into crops. *Molecules*. 26(6). 1752. https://doi.org/10.3390/molecules26061752

Nikolaeva. A. A., Korotkova. E. I., Tomsk Polytechnic University. Lipskikh. O. I., & Tomsk Polytechnic University. (2019). Determination of quinine in drugs and beverages by fluorimetric method. Bulletin of the Karaganda University. «Chemistry» Series. 94(2). 56-61. https://doi.org/10.31489/2019Ch2/56-61

Oakes. A. D., Pilkey. H. C., & Powell. W. A. (2020). Improving *ex vitro* rooting and acclimatization techniques for micropropagated american chestnut. *Journal of Environmental Horticulture*. 38(4). 149-157. https://doi.org/10.24266/0738-2898-38.4.149

Pain. S. (2019). A toast to tonic. New Scientist. 244(3261). 62-64. https://doi.org/10.1016/S0262-4079(19)32439-X

Pandey. K., Kuldeep. P., & Kumar. P. (2019*). In vitro* and *ex vitro* approaches for hardening of tissue culture plants (1.a ed.). New Delhi Publishers. https://www.researchgate.net/profile/Nilanjaya-Dr/publication/372629569_Plant_stress_Biology/links/64c2a0b76f28555d86d7fef2/Plant-stress-Biology.pdf#page=231

Perea Dallos. P., Teresa. G., A. C. M. H., Gabriel. G. M., & E. C. S. J. (2009). Cultivo de tejidos vegetales *in vitro*: Manual de prácticas de laboratorio. (in Spanish) https://bit.ly/4cdyQhX

Pharmakina SA. (2016). Quinine sulfate manufacturers. exporters & wholesalers: about pharmakina. *Pharmakina*. https://www.pharmakina.com/about-pharmakina/quinine-manufacturers-company-background/

Prasad. M. (2022). Review of the use of Peat Moss in Horticulture. https://bit.ly/3VDCHhl

Roersch van der Hoogte. A., & Pieters. T. (2014). Science in the service of colonial agro-industrialism: The case of cinchona cultivation in the Dutch and British East Indies. 1852–1900. Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences. 47. 12-22. https://doi.org/10.1016/j.shpsc.2014.05.019

Rufasto Peralta. Y. (2021). Calýdad de sýtýo de *cinchona sp.,* en relacýón a varýables edafoclýmátýcas en el bosque montano la palma. Provincia de chota [Universidad Nacional Autónoma de Chota]. https://bit.ly/4ccGYPO

Sandoval. Y., & Echandía. C. (1986). La historia de la quina desde una perspectiva regional: Colombia.1850-1882. Anuario Colombiano de Historia Social y de la Cultura. 0(13-14). 153-187 (in Spanish).

Schaepmeester. D. H. (2021). Trees agaınst malarıa : alkaloıd concentratıons and management of cınchona trees ın peru and the DR Congo [Ghent University]. https://bit.ly/3xovr0S.

Serrano, J. A. M., Ruíz, C. P., Fierro, I. M., & Fierro, J. M. (2019). Effect of culture medium on morphogenic processes *in vitro* in Cinchona officinalis L. *Revista de La Facultad de Ciencias Agrarias UNCuyo*, 51(1), 55–68.

Shin. B. K., Son. J. E., & Choi. J. M. (2012). Physico.chemical properties of peatmoss and coir dust currently used as root medium components for crop production in korean plant factories. *Journal of Bio-Environment Control*. 21(4). 362-371.

Shiwani. K., Sharma. D., & Kumar. A. (2022). Improvement of plant survival and expediting acclimatization process. en S. Gupta & P. Chaturvedi (Eds.). Commercial scale tissue culture for horticulture and plantation crops (pp. 277-291). Springer Nature. https://doi.org/10.1007/978-981-19-0055-6_12

Taylor. N. (1943). Quinine: the story of cinchona. The Scientific Monthly. 57(1). 17-32.

Theiler, R. (2014, year). Cinchona: A journey around the world [Seminar]. Personal communication, Seminario de Investigación del Instituto de Biotecnología, Universidad Nacional de Colombia, Bogotá.

Topcuoğlu. B., & Turan. M. (2018). Peat. BoD – Books on Demand.

Villar Cabeza. M. Á., Marcelo Bazán. F. E., & Baselly Villanueva. J. R. (2018). Estudio silvicultural de la quina: *Cinchona officinalis* L. Instituto Nacional de Innovación Agraria. https://bit.ly/45Cj0Lv

Vivanco-Galván. O., Jiménez. Y., Castillo Malla. D. P., & Lucero. H. (2022). Blue LED light enhances the growth of *Cinchona officinalis* L. cultured *in vitro*. En R. Shinar. I. Kymissis. & E. J. List-Kratochvil (Eds.). *Organic and hybrid Sensors and Bioelectronics*. XV (p. 35). SPIE. https://doi.org/10.1117/12.2633167

Yip. A., Sivarajah. S., & Natkunarajah. J. (2023). H22 Barking up the right tree: History of quinine. *British Journal of Dermatology*. 188(Supplement_4). ljad113.304. https://doi.org/10.1093/bjd/ljad113.304

Zsolt. S.-V., González Orenga. S., Cantor. M., Denisa Andreea. J., Boscaiu. M., & Vicente. O. (2020). Effects of drought and salinity on two commercial varieties of *Lavandula angustifolia* Mill. *Plants*. 9. 637. https://doi.org/10.3390/plants9050637.