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Influence of *Cinchona officinalis* (Rubiaceae) seedling size on survival and stem deformation after replanting

Influencia del tamaño de plántula de *Cinchona officinalis* (Rubiaceae) en la supervivencia y deformación del tallo posterior al repique

Efeito do tamanho das mudas de *Cinchona officinalis* (Rubiaceae) na sobrevivência pós-transplantio

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ABSTRACT

Cinchona officinalis, known as cinchona tree, is a species of high medicinal value that became popular for its antimalarial and febrifuge properties and is listed as an endangered species. The objective of this study was to determine the influence of *C. officinalis* seedling size on survival and steam deformation after pricking out. A

completely randomized design was applied with three treatments according to seedling height; 1) from 2.0 to 5.0 cm, 2) from 5.1 to 8.0 cm and 3) greater than 8.1 cm. For each treatment, three replicates and ten seedlings (experimental unit) were used for each replicate. The lowest survival rate was recorded in group 3 (30.0 ± 10 %), while the highest survival rate was recorded in group 1 (86.7 ± 5.8 %). Regarding the effect of *C. officinalis* seedling size on the presence of steam deformation, group 1 had the lowest deformation (20.0 ± 10 %), while group 3 had the highest number of individuals with deformed steam (83.3 ± 15.3 %). It is concluded that the survival and morphological characteristics of *C. officinalis* at 60 days in the nursery are directly proportional to the size of the seedlings used during the replanting process.

Keywords: *Cinchona officinalis*; Replanting; Nursery forest.

RESUMEN

Cinchona officinalis L.*,* conocida como árbol de la quina, es una especie de alto valor medicinal que se popularizó por su uso antimalárico, propiedades febrífugas y se encuentra catalogada como especie en peligro de extinción. El objetivo de este estudio fue determinar el efecto del tamaño de plántula de *C. officinalis* L. en la supervivencia y deformación del tallo posterior al trasplante. Se aplicó un diseño completamente al azar con tres tratamientos según la altura de la plántula; 1) de 2,0 a 5,0 cm, 2) de 5,1 a 8,0 cm y 3) mayor a 8,1 cm. Por cada tratamiento se empleó tres repeticiones y diez plántulas (unidad experimental) por cada repetición. Se registró la menor tasa de supervivencia en el grupo 3 (30,0 ± 10 %), mientras que la mayor tasa de supervivencia se registró en el grupo 1 (86,7 ± 5,8 %), en cuanto al efecto del tamaño de la plántula de *C. officinalis* L.sobre la presencia de deformaciones en el tallo el grupo 1 fue el que presentó la menor deformación (20,0 ± 10 %), mientras que el grupo 3 fue el que presentó el mayor número de individuos con el tallo deformado (83,3 ± 15,3 %). Se concluye que la supervivencia y características morfológicas de *C. officinalis* L. guardan una relación directamente proporcional al tamaño de las plántulas utilizadas en el trasplante.

Palabras clave: *Cinchona officinalis*; Trasplante; Vivero forestal.

RESUMO

Cinchona officinalis L., conhecida como árvore cinchona, é uma espécie de alto valor medicinal que se tornou popular por suas propriedades antipalúdicas, febrífugas e está listada como uma espécie ameaçada de extinção. O objetivo deste estudo era determinar o efeito do tamanho da muda de *C. officinalis* L. na sobrevivência e deformação do caule após o transplante. Um projeto completamente aleatório foi aplicado com três tratamentos de acordo com a altura das mudas; 1) 2,0 a 5,0 cm, 2) 5,1 a 8,0 cm e 3) maior que 8,1 cm. Para cada tratamento, foram utilizadas três réplicas e dez mudas (unidade experimental) por réplica. A menor taxa de sobrevivência foi registrada no grupo 3 (30,0 ± 10 %), enquanto a maior taxa de sobrevivência foi registrada no grupo 1 (86,7 ± 5,8 %). Com relação ao efeito do tamanho da muda *de C. officinalis* L. na presença de deformação do caule, o grupo 1 teve a menor deformação (20,0 ± 10 %), enquanto o grupo 3 teve o maior número de indivíduos com caules deformados (83,3 ± 15,3 %). Conclui-se que as características de sobrevivência e

morfológicas de *C. officinalis* L. são diretamente proporcionais ao tamanho das plântulas utilizadas no transplante.
Palavras-chave: *Cinchona officinalis*; Transplante; Viveiro florestal.

INTRODUCTION

Peru is a mega diverse country due to the variety of flora and fauna species, ecosystems and its genetic and cultural resources (Fajardo *et al*., 2014). In the country, the presence of plants of medicinal and food importance stands out (De-la-Cruz *et al*., 2007). Such is the case of the genus *Cinchona* (*C. officinalis* L., *C. pubescens* Vahl, *C. micrantha* Ruiz and Pav.) whose bark contains quinine alkaloids (especially: quinine, quinidine, cinchonine and cinchonidine), which were used for more than three centuries as the only treatment against malaria (Loayza *et al*., 2010). *Cinchona officinalis* was exploited for centuries and its bark was exported to many parts of the world, the most conservative figures state that between the XVII and XVIII century approximately half a million kilograms of bark were exported to Europe per year (Roersch van der Hoogte and Pieters 2015), according to the last study conducted more than three decades ago, populations were stable, however, there is considerable pressure due to the expansion of the agricultural and logging frontier (Zevallos 1989).

Andean forests with *Cinchona* species presence have been subject to anthropogenic pressure over the last 350 years (López, 2016). The remaining trees of *C. officinalis* are restricted to scattered individuals located in fragmented habitats as a result of burning and urban expansion (Huamán *et al*., 2019). Therefore, it is important to understand aspects related to its propagation in order to generate management and conservation plans.

Among the methods used to reverse the depredation of an area is the installation of forest plantations (Ferez et *al*., 2015); this mechanism promotes a rapid recovery of the forest structure, generating a suitable habitat for the reestablishment of ecological succession (Holl and Aide 2011).

Various factors influence the development of seedlings during the nursery stage; within which are the availability of water, shade, nutrients, substrate, weeds, transplant age, pests and diseases (Poorter *et al.,* 2012a). The effect of management practices in nurseries on the quality of seedlings has been studied in different forest species including Pinus palustris Mill (South et al., 2005), *Acacia koa* (Dumroese *et al.,* 2011), these studies they focused on the growth of the seedlings, which were carried out in research stations.

One of the stages that ensures success in the production of forest seedlings and subsequent installation in the final field is the replstting, so it is necessary to know factors such as the height of the seedling, morphological and physiological characteristics of the species and damage to the root system to ensure the survival of seedlings (Calegari *et al*., 2011). The highest seedling mortality is usually observed in the first two months after replanting (Viani and Rodrigues 2007; Turchetto *et al*., 2016) this is associated with water stress, when the root system of the seedlings has not yet been restored which causes a partial closure of the stomata for a prolonged period (Taiz

and Zeiger, 2008). Therefore, once individual seedlings overcome this stage, the chances of survival increase (Turchetto *et al*., 2016).

There are no studies describing the influence of *C. officinalis* seedling size on post-repipe survival, however, other types of botanical and ecological studies of this species have been developed (Zevallos 1989; Aymard 2019) this fact makes the research pioneering in the area.

Therefore, the present work seeks to determine the influence of *C. officinalis* seedling size on survival and trunk deformation after pricking out.

MATERIALS AND METHODS

Study area

The test was conducted from February 8 to April 8, 2021 in the community of La Cascarilla (UTM coordinates 732697.45 E, 9372588.42 S), province of Jaén, Peru, the area has an average elevation of 1 810 m. a.s.l., and corresponds to premontane rainforest (bhP) (Holdridge 1987). Annual precipitation is 1,730 mm, with a minimum temperature of 13.0 °C and a maximum of 20.5 °C (Figure 1).

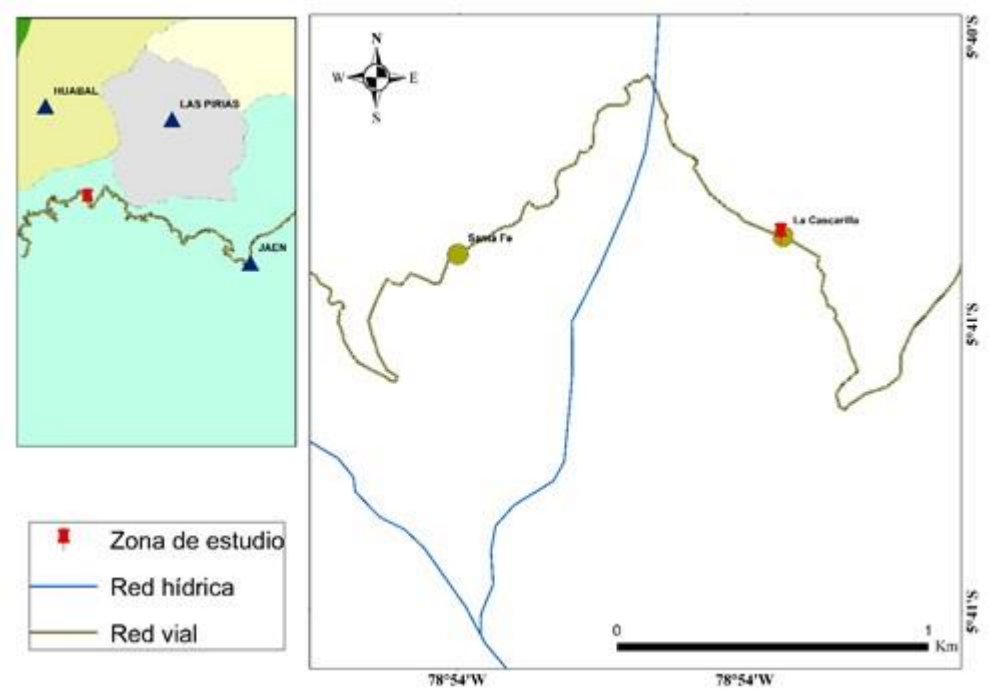

Figure 1. - Location of the study area in the community of La Cascarilla, province of Jaén, Peru

Study species

Cinchona officinalis is a species with seed dispersal generally anemochorous; in the arboreal state it can reach a height of 11-15 m, with a cylindrical trunk, 30 to 40 cm in diameter; the branching is sympodial; with an irregular globular crown, its leaves are simple, opposite and decussate, measuring 8 to 26.8 cm long (not including the petiole) and 7 to 18 cm wide, flowers in the form of terminal panicles 20 to 25 cm long, slightly pubescent. Flowers hermaphrodite, actinomorphic; calyx gamosepalous, about 4 mm long, cylindrical, with 5 small lobes; corolla white-red, with fused petals, 1.5 cm long, fruit in the form of a dark brown capsule, ellipsoid in shape, 0.8 to 2.5 cm long and 0.4 to 0.8 cm wide, dehiscent (Zevallos 1989).

Plant material

Ninety seedlings of *C. officinalis* were selected from the almacigueras beds, with good vigor and phytosanitary quality, with an average age of five months; they were classified into three groups according to their size: 1) from 2.0 to 5.0 cm, 2) from 5.1 to 8.0 cm and 3) larger than 8.1 cm. The seedlings were carefully removed, avoiding damage to the root system; they were then placed in containers with clean water, where they remained for 30 minutes until planting.

In the nursery the seedlings were placed in polyethylene bags with a volume of 684.4 cm^3 (7.5 cm in diameter and 15.5 cm in height); containing a substrate collected from the forest, whose physical-chemical characteristics were; texture: sandy loam, pH: 4.2 ± 0.1, EC: 0.5 ± 0.0 dS/m, P: 8.5 ± 2.5 mg/kg, total N: 0.3 ± 0.0 %, Ca: 1.2 ± 0.0 meq/100 g, K: 82.7 ± 1.7 mg/kg, Na: 0.4 ± 0.0 meq/100 g, Mg: 0.3 ± 0.0 meq/100 g.

During pruning, it was taken into account that solar radiation and temperature should be relatively medium or low, therefore, this activity was carried out in the afternoon to avoid dehydration and wilting; additionally, the leaves of the seedlings were cut at 50 % of the total area to regulate their transpiration.

The seedlings remained in environments protected by a green raschel mesh (65 % shade), 2 irrigations per day were applied with a nebulized system to maintain the relative humidity of the environment and avoid wilting of the seedlings. Cultural work in the nursery was constant during the entire study phase.

Experimental design

The experiment was set up under a complete randomized design with 3 treatments (groups), 3 replicates and 10 subsamples per replicate. The duration was 60 days (Table 1).

Table 1. - Classification of treatments according to *C. officinalis* seedling size

Group	Seedling size
1	2.0 - 5.0 cm
2	5.1 - 8.0 cm
3	> 8.1 cm

Data recording

Data recording had two phases, the first was daily (first week after pricking out), recording the number of live seedlings and deformed stems. The second phase consisted of a weekly evaluation, recording the same parameters described above, until the end of the research. The data were recorded in a field notebook and then transferred to the Excel digital template, in which survival was expressed as a percentage.

Data analysis

C. officinalis seedling survival data were subjected to analysis of variance (ANOVA) in order to determine the existence or not of significant statistical differences between treatments, then comparison of means was performed with Tukey's HSD post hoc test (P = 0.05). StatGraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, VA, USA) was used.

RESULTS

Figure 2A shows the cumulative survival rate expressed as a percentage of *C. officinalis* seedlings after replanting. The lowest survival rate was observed in group C (30.0 ± 10 %) whose stem height was greater than 8.1 cm, while the highest survival rate was recorded in group A (86.7 ± 5.8 %) which was comprised of seedlings between 2 and 5 cm in height. There were no significant differences between groups 1 and 2, but there were significant differences between groups 1 and 2 and test group 3. The mortality of *C. officinalis* seedlings is concentrated in the first six days after replanting; thereafter, no mortality of *C. officinalis* seedlings was recorded. The highest mortality rate was observed on the next day after replanting for all study groups, being higher in group 3 (4.3 ± 0.5 %) (Figure 2B).

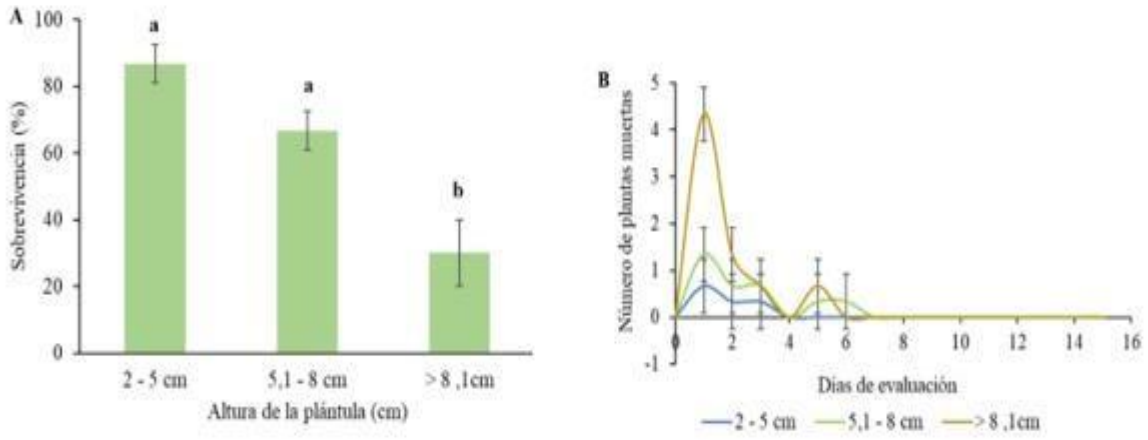

Figure 2. - Effect of the size of different groups on the survival of *C. officinalis* seedlings (A) and daily mortality after repotting of dead *C. officinalis* seedlings (B). Averages followed by different lowercase letters indicate significant differences between means according to a Tukey's post hoc test (P = 0.05)

Figure 3 shows the effect of *C. officinalis* seedling size on the presence of stem deformation in the seedling after replanting. Group 1 presented a lower incidence of stem deformation (20.0 ± 10 %), while group 3 seedlings presented a higher number of individuals with stem deformation (83.3 ± 15.3 %) (Figure 3).

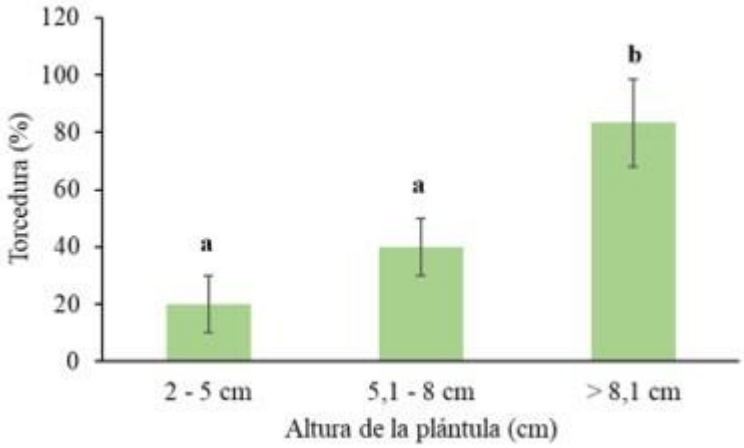

Figure 3. - Effect of the size of the different groups on the stem deformation of *C. officinalis* seedlings. Averages followed by different lowercase letters indicate significant differences between means according to a Tukey's post hoc test (P = 0.05).

DISCUSSION

The results of the study indicate a close relationship between seedling size and post repropagation survival and the presence of stem deformations. The mortality of *C. officinalis* seedlings was concentrated in the first seven days after replatting, this result is consistent with the results obtained by Viani and Rodrigues (2007). Seedling mortality is associated with the water stress that seedlings suffer after repotting when the root

system has not yet recovered, generating a partial closure of the stomata, for a prolonged period causing chronic photoinhibition (Taiz and Zeiger 2008); consequently, when seedlings overcome this stage, the probability of survival increases (Turchetto *et al*., 2016).

When the seedlings are taken to the nursery, there are alterations in the microclimate, such as an increase in temperature, humidity and luminosity; such changes reduce the photosynthetic capacity of the plant by photoinhibition and degradation of photosynthetic pigments (Kitao*et al*., 2000), which causes a decrease in the efficiency of carboxylation and reduces the efficiency of photosystem II (Gilmore and Govindjee 1999). In this context, it is hypothesized that the percentage of seedling survival would increase if the replanting were carried out in periods with temperatures between 18 and 22 °C, and that in the following months there are no extreme temperatures.

Factors such as seedling size, climatic conditions, physiological and morphological characteristics of the species, the time elapsed from the extraction of seedlings from the nursery to the pricking out and the damage caused to the root system, have a significant influence on the survival of seedlings after pricking out (Calegari*et al*., 2011).

The highest mortality and manifestation of stem deformation in *C. officinalis* seedlings was observed in the third group of trials (height > 1.5 m). *officinalis seedlings* was observed in the third test group (height > 8.1 cm). 8.1 cm), this would be related to the damage caused to the root system which generates a water imbalance in the seedling, losing more water than the roots can absorb (Lüttge 2008), in addition, the aerial biomass is greater in seedlings taller than 8. This generates a water imbalance since larger seedlings tend to be more demanding in water, so that mortality rates are higher when there is water stress (Turchetto *et al*., 2016).

CONCLUSION

C. officinalis seedlings at 60 days in the nursery survive and show their morphological characteristics in direct proportion to their size during pruning. Resulting, the smaller the size of the seedlings, the lesser the ditrimental effect caused by the edaphoclimatic conditions and the physiological behavior of the species. It is necessary to do more studies related to this subject, in order to find efficient methodologies that allow the mass production in nursery of this species with high medicinal value.

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Conflict of interests:
The authors declare not to have any interest conflicts.

Authors' contribution:
The authors have participated in the writing of the work and analysis of the documents.