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Assessment of Seed Quality and Germination Response in the Species of the Genus *Polylepis*

Cecilia Vega Krstulovic, Jorge Quezada Portugal, Paola Rocabado Koya, Gabriela Villegas Alvarado and Juan Carlos Bermejo Franco

Abstract

To contribute to the knowledge of sexual reproduction of endangered native species of the genus *Polylepis* (*P. neglecta*, *P. incarum* and *P. pacensis*) in Bolivia, different germination studies were performed to obtain saplings for reforestation as a very important measure recommended for the recovery and conservation of this genus. Initially, seed trees were identified in natural populations (*P. pacensis* and *P. incarum*) and also in green areas of the Municipality of La Paz (*P. neglecta*). The characterization of physical and physiological quality (laboratory and greenhouse tests) of the collected seeds was carried out. The physical quality test, showed a high degree of impurities (47%), so it was required to collect a greater amount of plant material. *P. incarum*, *P. pacensis* and *P. neglecta* showed 10, 8 and 6% humidity respectively. A low germination percentage was obtained: 10% (*P. neglecta*), 8% (*P. pacensis*) and 2% (*P. incarum*), issue reported for several *Polylepis* species. Under greenhouse conditions the highest germination percentage (19%) was displayed in the substrate sand + peat 1:1. On the other hand, *P. neglecta* showed a higher germination percentage (between 10 and 15%), *P. pacensis* and *P. incarum*: 8 and 2%, respectively. Our data suggest feasible alternatives to improve the massive propagation of these species under greenhouse conditions.

Keywords: germination, greenhouse, *Polylepis*, reforestation, seeds, viability

1. Introduction

The arborescent genus *Polylepis* (Rosaceae) is distributed along the Andes mountain range from Venezuela, Colombia, Ecuador, Perú, Bolivia, Argentina (Córdoba), to northern Chile [1].

In Bolivia, it is in an altitudinal range from 3200 to 5200 m, forming true forests in the Eastern Cordillera associated with other species; while in the Western Cordillera it forms monospecific patches [2, 3]. The genus *Polylepis* includes between 15 and 28 recognized species, being Bolivia one of the main centers of wealth with 14 species [3–6].

These forests represent important islands for the conservation of biodiversity in the Andes [7]. They also play a central role in high Andean ecology as a habitat for many species of plants, animals and as an important source of resources for local...
inhabitants [8]. Nevertheless, because of their high fragmentation, Polylepis forests are considered one of the most threatened ecosystems in Bolivia [2, 9].

The degradation of Polylepis forests began millennia ago, mainly due to anthropogenic activities such as felling for firewood and charcoal production and human ignited fires to stimulate pastures regrowth in order to enabling agricultural fields and reforestation with exotic species (eucalyptus and pines), factors that have led to the destruction and reduction of ~90% of the area occupied by these forests, restricting them to specialized microhabitats and modifying their floristic and faunal composition [8, 10]. To this must be added the low germination rates (2–50%) reported for different Polylepis species, which can be related to the high percentages of empty or non-viable seeds due to the anthropogenic action on their habitat. [1, 11–13].

Consequently, within the framework of the strategy for the conservation of Polylepis (Quewiña) forests and biodiversity associated in Bolivia, it was determined that these forested formations are priorities for conservation through the development and implementation of integral projects to restore and recover these ecosystems and their biodiversity, which include the transplantation of juvenile plants and reforestation with native species [14].

In this regard, scientific publications about propagation and reforestation in Polylepis are insufficient in Bolivia. So it is essential to get more information about reproductive biology and establishment for this genus.

Nevertheless, there are outstanding experiences in other countries that have generated basic information on different aspects for germination and saplings production for the natural forests recovery. Such as the research carried out in Chile that generated information about P. tarapacana seeds collection through the identification of seed trees; seeds germination in a suitable substrate for saplings obtaining; and their transplantation to a definitive place [15]. On the other hand, in Argentina a methodology for the production of Polylepis australis seedlings for reforestation has been standardized due to several years of research [12].

In this sense, the study aims to generate information on the sexual reproduction of Polylepis species: P. neglecta, P. incarum and P. pacensis, to achieve the understanding of the germinative processes focused on the attributes of physical and physiological quality that can contribute to the generation of standardized treatments to obtain plant material ready to be planted and useful as a tool to implement an integrated management of afforestation and/or reforestation.

2. Methodology

2.1 Selection of species

The species were selected based on the availability of seed trees, that is, Polylepis species located in areas near the Department of La Paz-Bolivia were considered, mainly due to the restriction in the geographical area established by the funder to carry out the present investigation. In that sense, the species P. neglecta, P. incarum and P. pacensis were selected.

On the other hand, the state of conservation was considered, of each species that in the case of P. neglecta is classified as vulnerable (VU) and P. pacensis is within the category endangered (EN) [16]. P. incarum qualifies within the category EN [17].

Finally, the growth rate of the species was taken into account, a key aspect for the subsequent production processes of the species of interest. There are reports that P. neglecta seedlings show rapid growth (up to 50 cm per year), while P. incarum is recognized as a fast-growing tree. Therefore, both species are considered as potential for reforestation [2].
2.1.1 Location and selection of seed trees of Polylepis

In order to verify the zones with forested fragments of *Polylepis*, the sampling points were georeferenced and the selection of potential seed trees was carried out according to specimens that visually presented the highest relative density of fruits in the canopy (presence of branches with fertile structures distributed in the different expositions of the glass). Likewise, basic allometric parameters such as height, trunk diameter DAP$_{50}$ (measured at 50 cm from the base) were recorded, marked and measured and verified by specialists from the National Herbarium of Bolivia (LPB) of the Universidad Mayor de Bolivia. San Andrés (UMSA).

Field trips were made to areas near the Municipality of La Paz, where sectors with *P. pacensis* forests were located, such as the town of Cohoni, at ~75 km from the city of La Paz, between coordinates 16°42′42.9″ S and 67°50′18.4″ W and at an altitude of 3539 m. In it were located different points with presence of fragments of these forests (Figure 1a). These populations were settled on slopes and ravines of steep slope; depending on its accessibility, 8 trees were selected and recorded in fruiting state (i.e., presence of immature, mature and remaining fruits), with an average height of 3.7 m and an average DAP$_{50}$ of 17.5 cm.

In the Municipality of Puerto Carabuco, located in the third section of the Camacho Province of the Department of La Paz, at an average altitude of 3860 m, between the coordinates 15°32′17″ S and 69°25′06″ W, located two main points with presence of fragments of *P. incarum* forests (Figure 1b), settled on slopes with steep slopes, where 33 specimens were marked, in which floral and fruit structures were differentiated (mature, immature and old). The average height and DAP$_{50}$ recorded were 3.5 m and 27.8 cm, respectively.

In the case of *P. neglecta*, a number of previously identified seedbeds were found in the La Paz Botanical Garden (Figure 1c), of the University Campus of the Faculty of Pure and Natural Sciences of the UMSA, with the following coordinates 16°32′18″ S and 68°04′7.99″ O, at 3411 m; where 6 trees were marked in flowering and fruiting state. Based on the measurements made, an average height of 3.9 and 26.5 cm of DAP$_{50}$ was calculated.

2.1.2 Seed collection

To collect the seeds of the species under study, trees with mature clusters with a minimum of 25% flowering with respect to the total crown were visually selected, and based on criteria of coloration (fruits of light brown color) and degree of adhesion to the cluster (easily removable). However, in the process, immature (green) seeds were observed that were quite attached to the bunch and old seeds (dark brown) very loose on contact with the hand (Figure 2). The collection of seeds was done manually to loosen the fruits. Even so, it was inevitable to obtain samples with different degrees of maturity. The amount of seed collected per tree per species was variable. In the laboratory of the Unit of Plant Biotechnology (U.B.V.-U.M.S.A.) Was homogenized (mixture of primary samples) and reduced to ~100 g in the species *P. neglecta* and *P. incarum*, and only 30 g in the case of *P. pacensis*. From these samples, the distribution of the seeds was carried out according to the quantity required for each of the developed analyzes.

2.2 Analysis of seed quality

Based on the methodology standardized by the International Seed Testing Association [18], for each species under study, the physical and physiological seed quality tests were carried out at the facilities of the U.B.V. of the U.M.S.A.
2.2.1 Physical quality

The physical quality analysis included the determination of parameters such as physical purity, moisture content and the weight of 1000 seeds. The physical purity analysis consisted in examining a sample of 100 g of seeds per species (with the exception of *P. pacensis* for not obtaining the sample size) and separating the pure seed from the impurities (seed of other species and inert matter) with the aid of microscopy.
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of magnifying glasses, to then weigh them and calculate the percentage of purity according to the following formula [19]: purity percentage = (pure seed/total weight of the original sample) × 100.

Subsequently, we proceeded to determine the moisture content of the seed through the method recommended by the ISTA [18]. Eight samples of 100 seeds were weighed, dried in an oven at a temperature of 103 ± 2°C for 17 hours and then weighed again. The humidity percentage was calculated from the following formula: humidity percentage = (original weight − weight after stove drying/original weight) × 100.

For the evaluation of the weight of 1000 seeds, these were separated manually, with 8 repetitions randomly per species and each of them was weighed in an analytical balance.

2.2.2 Physiological quality

An exploratory study was previously carried out using the tetrazolium viability test based on staining patterns that allowed the reliable evaluation of the viability of
the seeds of the species under study. In accordance with the procedure established by
the ISTA [18], the seeds were pre-conditioned, placed for 24 h on moistened paper
towel. This triggered the activation of the enzymatic system, besides facilitating the
cutting of the seed and the development of a sharper and uniform coloration. Next,
a cross section of the seeds was made to expose the embryo directly to the action
of the tetrazolium and facilitate its penetration. Five repetitions of 20 seeds were
made per Petri dish, making a total of 100 seeds per species. Subsequently, the seeds
were stained by immersing them in the tetrazolium solution at a concentration of
0.5% for a period of 24 h in the dark and at room temperature. To proceed with the
interpretation of the test, a stereoscope was used. In this way, the seeds were clas-
sified, according to the colored zones, in different staining categories established
according to the ISTA standards: Category 1: living tissue (TV1, intense red tissue
staining, TV2, bright or intense pink tissue staining, TV3, light pink tissue staining);
Category 2: deteriorated tissue (TD1, garnet red tissue coloration. TD2, milky pink
tissue coloration); Category 3: dead tissue (TM1, discoloration of discolored/whit-
ish or hyaline tissue TM2, necrotic tissue with brown coloration). Given that empty
seeds were found (without an embryo), Category 4 (SSTE, seed without embryonic
tissues) was added to describe this condition.

The main attribute considered in the physiological quality of seeds refers to their
germination capacity. In this sense, a germination test was carried out under controlled
environmental conditions of humidity, temperature and light in the growth room of
the U.B.V., which has shelves with artificial light, programmed with a photoperiod of
16 h light/8 dark and an average temperature of 25°C. Six seeds were planted per Petri
dish, with a total of 240 seeds for each species. The number of repetitions (Petri dish)
per species was 40. As a substrate paper, towel was used, which provided adequate
porosity for moisture retention. This trial lasted 30 days, time that was waited for the
maximum germination to occur. During this period, the count of (1) germinated seeds
(those that developed a seedling with essential structures, such as roots, stems and
cotyledons) and (2) ungerminated seeds.

2.3 Seed germination in greenhouse

Considering the information available in previous works on Polylepis seeds,
two trials were carried out in the greenhouse of the La Paz Botanical Garden-
U.M.S.A. In a first (preliminary) trial, four combinations of substrates were tested:
S1, sand + peat (1:1) (control substrate); S2, sand + peat + black earth (1:1:1); S3,
sand + peat + humus (2:1:1); and S4, sand + black earth (1:1). Prior to planting
the seeds, pregerminative soaking in water was carried out for 24 h. Two hundred
and forty seeds were sown per species; half was subjected to this procedure. The
study was conducted in July, August and September 2015. The data obtained were
analyzed statistically through a completely randomized design with trifactorial
arrangement; the factors were: Species (A), soaking (B) and substrates (C), with
10 repetitions per treatment. Then a second (final) test was carried out, in which
the two best substrates from the preliminary test (S1 [sand + peat 1:1] and S4
[sand + black earth 1:1]) were used to verify the observed response in the previous
study and establish treatments for subsequent production processes. In both cases,
the pregerminative treatment (seed soaking) was applied. Two hundred and forty
seeds per species were planted, of which 120 were soaked in water to standardize a
 technique for the propagation of keñua seedlings. This study was carried out in the
months of September and October 2015. The results were analyzed through a com-
pletely randomized design with a bifactorial arrangement; the factors were species
(A) and treatments of substrates with and without soaking (B), with 40 repetitions
per treatment.
3. Results

3.1 Analysis of seed quality

3.1.1 Physical quality

The results regarding the parameter of physical quality purity of seeds showed that in 100 g of seed of *P. neglecta*, 57% corresponded to pure seed and, in the case of *P. incarum*, 50%. As regards *P. pacensis*, this information was not generated since only a maximum of 30 g of the 100 g required for this analysis was reached. With respect to the moisture content of the seeds, in the case of *P. neglecta* species 6%, 8% for *P. pacensis* and 10% for *P. incarum* were recorded. The average values obtained for the parameter weight of 1000 seeds for each species showed that 1000 seeds of *P. neglecta* weigh 4.78 g, in the case of *P. incarum* 9.65 g and in the species *P. pacensis* 5.31 g.

3.1.2 Physiological quality

Depending on the results obtained in the tetrazolium test, different coloring patterns could be differentiated in the tissues of the seeds. In the species *P. neglecta* 40% of seeds were observed with intense red tissue staining (TV1), 22% with dead tissue (TM2) and 17% of empty seeds (without embryo). In *P. incarum* 48% of seeds with brown coloration (TM2) and 20% with discoloration of colorless/whitish or hyaline tissue (TM1) was verified, condition that indicates tissue death in both cases. In the species *P. pacensis* 58% of seeds with dead tissue (TM2) and 21% of empty seeds were recorded, without the formation of embryonic tissues (SSTE). The rest of the percentages presented in smaller proportion in the three species correspond to other categories that are shown in Table 1.

In turn, the different staining categories established to determine the viability of the seeds can be observed in Figure 3.

In general, this test allowed us to obtain an approximation to determine the viability of *Polylepis* seeds through the differentiation of colorations of the observed tissues. Giving as final results a high percentage of non-viable seeds in the species *P. incarum* and *P. pacensis* (76 and 85% respectively), while in *P. neglecta* a lower percentage of non-viable seeds was recorded (42%), results that to some extent support the low germination rates observed in both species described below (Figure 4).

### Table 1

Percentage of seeds according to the categories of tetrazolium staining (TZ).

<table>
<thead>
<tr>
<th>Species</th>
<th>Category 1: Living tissue</th>
<th>Category 2: Degenerated tissue</th>
<th>Category 3: Dead tissue</th>
<th>Category 4: Empty seed</th>
<th>Total seeds evaluated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TV1</td>
<td>TV2</td>
<td>TV3</td>
<td>TD1</td>
<td>TD2</td>
</tr>
<tr>
<td><em>P. neglecta</em></td>
<td>40</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. incarum</em></td>
<td>6</td>
<td>15</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. pacensis</em></td>
<td>9</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TV1: intense red tissue coloration, TV2: bright or intense pink tissue coloration, TV3: coloration of right pink tissue, TD1: coloration of germinal red tissue, TD2: milky pink tissue coloration, TM1: coloration of discoloration/hyaline or hyaline tissue, TM2: brown tissue coloration, SSTE: empty seed without embryonic tissues.
The results of the germination test showed a germination rate of 42% for *P. neglecta* and 10% for *P. incarum* and *P. pacensis*, after 30 days of evaluation, defined as the observation period in which the maximum occurred accumulated germination (Figure 5a–c).

### 3.1.3 Seed germination in greenhouse

The results of the preliminary test, at 45 days of evaluation (maximum germination time), showed only significant statistical differences with respect to the factors species (A) and substrates (C), not so for the soaking factor (B), nor for any of the interactions between factors. Accordingly, both factors were analyzed separately through Duncan’s multiple range test ($\alpha = 0.05$), which he identified as the highest germination percentage to that registered for *P. neglecta* (15%), since it is statistically different from those obtained for *P. incarum* and *P. pacensis* (Figure 6a).

Regarding the differences identified for the substrate factor, through the Duncan test ($\alpha = 0.05$) it was verified that the substrate S1 (sand + peat 1:1) had the highest germination rate (19%) in relation to the other substrates (Figure 6b). In this sense, when

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**Figure 3.**
Colored seeds with tetrazolium, distributed among living, deteriorated, dead tissues and empty seeds.

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dealing with evaluation factors it can be affirmed that the substrate S1 is the one that presents the best germination rate for all the species under study and the species with the best germinative behavior regardless of the substrate or the soaking is *P. neglecta*.

In the final test the results showed significant statistical differences with respect to the species under study (A) and the treatments (B), and not so for the interaction (AB).
In this way, the Duncan test ($\alpha = 0.05$) again identified \textit{P. neglecta} as the species that reached the highest percentage of germination (10%), followed by \textit{P. pacensis} (8%) and finally \textit{P. incarum} (2%) (Figure 7a). Likewise, this test revealed that the treatments with the highest percentage of germination correspond to the substrate S1 (sand + peat 1:1), independently of the effect of the pregerminative soaking treatment (Figure 7b). In summary, it can be confirmed that the S1 substrate, independently of the soaking, was the one that presented the best germination rate for the three species. The best germinative behavior, regardless of the substrate or soaking, was the species \textit{P. neglecta}.

4. Discussion

4.1 Analysis of seed quality

With respect to physical quality parameters, in the case of physical purity, the seeds presented a high degree of impurities (47% on average), which makes it difficult to obtain pure seeds for future planting. Its importance lies in the fact that a lot of seeds with a lot of inert material is of lower quality and implies the need to use more of this material in order to make the process more effective [20].

The moisture content is one of the most important factors that affects the maintenance of the quality of the seeds. Thus, dry and healthy seeds can be kept under appropriate storage for many more years, while wet seeds can deteriorate in a few days. The
storage time of seeds decreases as the moisture content increases, since it has a domi-
nant effect on the predominance and activity of insects and fungi during storage [21]. The moisture content affects the metabolic activity of the seeds in the germination and the deterioration of these; therefore, knowledge of this attribute allows choosing the most appropriate procedure for the harvest or collection of seeds, their conditioning, the preservation of the physical, physiological and sanitary quality of the seed [20]. In the case of the species studied, there are no recommended humidity parameters for storage. However, according to the ISTA [18] it is considered as a norm that the humidity of the seeds to be commercialized should not exceed 13% [20]. The percentage of humidity of all the species under study was low, which could be considered orthodox seeds, which are those whose moisture content can be reduced to values between 5 and 10% and store them at temperatures below zero without damaging them; its conservation for long periods without losing its germinative power [22]. According to the document published by the Mining Company Doña Inés de Collahuasi SCM [15], seeds of *P. tarapacana* stored for a year in a dry cellar at room temperature did not lose their viability, so the percentage of germination was not seen affected.

The weight of 1000 seeds allows to establish the necessary amount of seeds to achieve a certain number of plants. If the value of this parameter is known, it is easy to determine the amount of seeds to be used per reforestation area [21]. According to what was observed in the present study, it can be inferred that to have 1000 seeds of *P. neglecta* and *P. pacensis*, between 4 and 6 g is required, while in *P. incarum* about 9 g would be required.

In relation to the physiological quality analyzes, through the viability test of tetrazolium in *Polylepis* seeds, high percentages of non-viable seeds and empty seeds were verified, which would be related to the low percentage of germination registered in different species of the *Polylepis* genus. In this regard, it is mentioned that in the collection of seeds corresponding to *P. incana*, many trees came to have almost all their seeds empty [11]. Another very interesting result is the one reported for *P. australis* (Córdoba-Argentina), in which they analyzed the effect of the seed mass, the geographic region, the forest fragments and the individual trees on the germination of the seeds, and reported that seeds that did not germinate were almost always empty [23]. Likewise, these authors refer to Hensen (unpublished data), who found a high percentage of empty and non-viable seeds in several Bolivian *Polylepis* species (*P. hieronymi*, 80%, *P. tomentella*, 84%, *P. besseri*, from 90 to 100%, *P. racemosa*, 100%, *P. tarapacana*, 100%). The presence of high percentages of empty seeds seems to be a characteristic in *Polylepis* species, which represents a problem when making afforestation or reforestation plans. Likewise, the existence of a positive correlation between the mass and seed germination of *P. australis* was determined, that is, the weight of the seed could be a quick way to evaluate the viability of these and, therefore, it would be a practical alternative for the selection of seeds and ensure higher germination rates [23].

Based on the germination test carried out, available reports recorded low germination rates in the species studied, which was verified through the data obtained for this study. Experiences of germination of *P. incana* in Petri dishes from samples taken at different altitudes showed germination percentages between 8 and 34% in a period of 15–49 days (collection of seeds at 3100 m). Likewise, samples collected at an altitude of 3600 m, with pregerminative treatment consisting of soaking in water for 42 h prior to sowing, showed germination percentages between 12 and 44% [24].

### 4.2 Seed germination in greenhouse

Different experiences in germination for the production of seedlings in other *Polylepis* species under greenhouse conditions, report results similar to those
presented in this chapter. Regarding the type of substrate, they recommend a mixture of sand + peat in a 1:1 ratio, which in the tests carried out in the present study turned out to be the substrate in which the greatest number of seeds of the three species analyzed [2] germinated. Although some researches recommend to subject the seeds to a soaking in water for 24 h as a pregerminative treatment [25, 26], the results in this work did not show a marked effect on the germination of the species under study, so their use resulted indistinct depending on the data obtained.

With relationship the time of germination, the first seedlings emerged between 20 and 25 days in \textit{(P. neglecta, P. incarum and P. pacensis), registering this event until 45 days in the different tests performed}. Studies carried out on \textit{P. tarapacana} showed that the appearance of seedlings was concentrated between 30 and 40 days after sowing [15], whereas in the case of \textit{P. australis}, the first seedlings were observed at 20 days after sowing and were increasing until 60 days [12].

The percentages of germination obtained were variable, in \textit{P. neglecta} 10% was registered, in the case of \textit{P. pacensis} 8% and for the species \textit{P. incarum} 2%. There are several reports about the low germinative power of \textit{Polylepis} species. For example, for \textit{P. incana} (Peru) there is information that the percentage of germination varies between 2 and 15% [27]. In \textit{P. tomentella} there is a 3.3% [28], and in \textit{P. australis} there is a variation of 10–50% of germination percentage under experimental conditions [12]. In the study carried out by the Mining Company Doña Inés de Collahuasi SCM [15] it is mentioned that the percentage of germination in massive propagation in \textit{P. tarapacana} fluctuated remarkably according to the origin of the seeds, in a range of 2–10%, with an average close to 4%. In \textit{P. besseri}, the conclusion of the germinative stage occurred between 50 and 65 days, with a germination rate of 3–10% in experimental plots [1], whereas, in \textit{P. racemosa}, the germinative success was of 2–15%, in a period of 60–70 days [25]. Therefore, the success rate of propagation will depend mainly on the amount of viable seeds and the adequate environmental conditions (soil, humidity and temperature) that are provided for germination [11].

On the other hand, it is important to take into account the distribution of the seed trees from which the samples were obtained, either in the city or in the field, an aspect that can respond to the low germination rate presented in the species under study. The crossing between neighboring plants can lead to a biparental depression of inbreeding, that is, a loss of biological effectiveness (vigor, viability, fecundity) due to the reduction of genetic variability due to homozygosity, which reduces the chances of survival of the species [23]. For this reason, it should be taken into account that the collection of seeds takes place in places where a broad dispersion flow can be evidenced that, in a certain way, guarantees greater genetic variability within that population.

5. Conclusions

Through this study, it was possible to generate basic reference information, providing information on the location, description and selection of seedlings of \textit{Polylepis} in areas near or belonging to the Municipality of La Paz, to determine parameters related to the physical and physiological quality of seeds, and also the development of basic procedures for their initial cultivation in controlled conditions (greenhouse).

In this way, it is intended to contribute to the biological knowledge of the species examined, filling some gaps of information to understand their current status and plan the development of appropriate \textit{in situ} and \textit{ex situ} conservation strategies for the species under study through restoration or reforestation programs with seedlings obtained by seed.
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