Morphology, dormancy and pre-germination treatments for three native forest species in southern Ecuador

Morfología, dormancia y tratamientos pre-germinativos para tres especies forestales en el sur del Ecuador

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ABSTRACT

The high Andean forests have high rates of biodiversity, however, currently the loss of native forest is alarming, especially in countries where deforestation is increasing. However, generating restoration plans entails the production of plants, which must be propagated through seed, but the limited information about acquiescence, dormancy and germination techniques in native species is a barrier. We hypothesized that dormancy and acquiescence can be break through the application of chemical or physical techniques, and is specific to each species. The seeds of the species *Podocarpus sprucei*, *Clusia* sp. and *Viburnum triphyllum* were collected within the Irquis-Yanuncay Protective Forest in Azuay, Ecuador. Morphological descriptions, imbibition and viability tests, pre-germinative treatments of scarification (GA_3 and H_2SO_4) and stratification (hot) were applied. After analyzing the tests carried out, it was determined that *Clusia* sp. does not present acquiescence or dormancy, while *P. sprucei* presents endogenous acquiescence and morphological dormancy, meanwhile, *V. triphyllum* presents exogenous/endogenous dormancy and morphophysiological dormancy. Furthermore, the best pre-germinative treatment to stimulate germination in *P. sprucei* (62%) and *V. triphyllum* (80%) was acid scarification of H_2SO_4; likewise, hot stratification at 30°C applied in *Clusia* sp. generated greater germination (89%) and at 40°C (82%) in *V. triphyllum*. This information allowed us to understand and complement information on morphology, acquiescence, dormancy, and pre-germination treatments of these native species and in the future, promote their use in restoration projects.

Keywords: Podocarpus, Clusia, Viburnum, warm stratification, chemical scarification, restoration, Andes.

RESUMEN

Los bosques alto andino cuentan con altas tasas de biodiversidad, sin embargo, en la actualidad la pérdida de bosque nativo es alarmante, sobre todo en países en donde la deforestación está en aumento. No obstante, el generar planes de reforestación conlleva la producción de plantas, las cuales deben propagarse a través de semilla, pero la escasa información acerca de latencia, dormancia y técnicas de germinación en especies nativas es una barrera. Nuestra hipótesis fue que la dormancia y latencia puede ser eliminada al aplicar técnicas químicas o físicas y es dependiente de cada especie. Las semillas de las especies *Podocarpus sprucei, Clusia* sp. y *Viburnum triphyllum* fueron colectadas dentro del Bosque Protector Irquis-Yanuncay en Azuay, Ecuador. Se realizaron descripciones morfológicas, pruebas de imbibición, y viabilidad, donde se aplicaron tratamientos pregerminativos de escarificación (GA_3 y H_2SO_4) y estratificación (caliente). Luego de analizar las pruebas realizadas, se pudo evidenciar que *Clusia* sp. no cuenta con latencia ni dormancia, mientras que, *P. sprucei* presenta latencia endógena y dormancia morfológica, entre tanto, *V. triphyllum* presenta latencia exógena/endógena y dormancia morfofisiológica. Además, el mejor tratamiento pregerminativos para estimular la germinación en *P. sprucei* (62%) y *V. triphyllum* (80%) fue la escarificación ácida de H_2SO_4; asimismo, la estratificación caliente a 30°C aplicada en *Clusia* sp. generó mayor germinación (89%) y a 40°C (82%) en *V. triphyllum.* Esta información permitió comprender y complementar información sobre morfología, latencia, dormancia, y tratamientos pregerminativos de estas especies nativas y en el futuro, fomentar su uso en reforestación.

Palabras clave: Podocarpus, Clusia, Viburnum, estratificación caliente, escarificación química, restauración, Andes.

INTRODUCTION

The Andean forests are characterized by having a wide floristic diversity; however, they are threatened with the constant increase in the agricultural frontier, which allows thousands of hectares to be deforested annually. In Ecuador, the deforestation rate is above 11% superior to a theoretical stable system (Sierra et al. 2021), which leads to negative effects such as the loss of biodiversity and therefore of its ecosystem services (Lawrence & Vandecar, 2015). To combat this bad practice, Ecuador developed a National Forest Restoration Plan for 2019-2030, whose goal includes the restoration of 30,000 hectares of degraded ecosystems in the period 2019-2021 (MAE, 2019). According to Moreno-Mateos et al. (2020), a valid approach in long-term restoration is to reestablish interaction networks at the community level, therefore, the species selected for restoration purposes should include ecological attributes as the dispersion of its seed. There are native Andean Forest species that have potential for use in ecological restoration such as *Clusia* sp., *Podocarpus sprucei* Parl. and *Viburnum triphyllum* Benth.

Clusia genera with over 300 species, is one of the largest of the Clusiaceae family (Gustafsson et al. 2007). The flowers are morphologically highly diverse and offer either nectar, pollen or resin as a reward for pollinators (Gustafsson & Bittrich, 2002). For example, the new species *Clusia nubium* (Clusiaceae) from cloud-forests of southwestern Ecuador, is characterized by male flowers with a resin-secreting synandrium (Gustafsson & Borchsenius, 2016). *Clusia alata* also from the southern region of Ecuador presents an important antioxidant and medicinal capacity (Armijos et al. 2018) or *Clusia flaviflora* that is frequent in native inter-Andean forests in the Ecuadorian provinces such as Azuay (Minga et al. 2019) and Carchi (Olivera & Cleef, 2009); this species predominates at altitudes around 3,500 meters above sea level.

The family of Podocarpaceae is the second largest family within the conifers consisting of 19 genera, 194 species and nine varieties (Knopf et al. 2012). DNA sequence and morphological data determined similarity between *Podocarpus sprucei* Parl.—in western Ecuador and northern Peru (Knopf et al. 2012). Sarmiento et al. (2018) identified *Podocarpus oleifolius* "romerillos" and *Podocarpus rospigliosii* "mogollón" in a protected forest in the south-east of Ecuador, but at a lower frequency than the abundant "guabisay" *P. sprucei.*

The genus *Viburnum* belongs to the family Adoxacea although it was previously assigned to the family Caprifoliacea (Lopez, 2003). Worldwide, it has 175 species of shrubs and small trees that have been described by using DNA sequencing (Winkworth & Donoghue, 2004). In Ecuador, eight species of *Viburnum* have been identified: *V. obtectum*, *V. stipitatum*, *V. hallii*, *V. jamesonii*, *V. pichinchense*, *V. toronis*, *V. triphyllum* and the endemic *V. divaricatum* (Lopez, 2003). These species have been genetically grouped into the Oreinodontotinus clade by using barcoding (Clement & Donoghue, 2012). In Ecuador this genus is distributed in humid forests from 750 to 3,800 meters above sea level (Lopez, 2003). For example, in southern Ecuador, *V. triphyllum* and *V. pichinchense* grow in secondary forest communities (Jadán et al. 2020); and in northern Ecuador *V. triphyllum* is part of the Andean cloud forest tree lines (Sarmiento and Frolich, 2002).

Seeds as the reproductive organ of trees and plants resort to dormancy when necessary to proceed with germination under favorable conditions; this is a key process for the establishment of plant communities in an ecosystem (Li et al. 2010; Youssef et al. 2012). Dormancy is the absence of germination in a period of time under any combination of environmental and physical factors that are favorable for germination (Soppe & Bentsink, 2016), which is determined by genetics mediated in part by hormones such as abscisic acid and gibberellins (Klupczyńska & Pawłowski, 2021). According to Baskin and Baskin, (2004), Pausas and Lamont (2022), five classes of dormancy are distinguished in seeds (physiological, physical, morphological, morpho-physiological and combined), therefore, seeds of different species require specific treatments to break it and allow germination; however, these processes may vary in different populations of the same species (Née et al. 2017; Commander et al. 2013).

For the Andean region, there is a lack of information about the morphology, germination and dormancy of important forest species, therefore, from the perspective of propagation of forest mountain species with ecological and socioeconomic importance. We hypothesized that dormancy and acquiescence can be break through the application of chemical or physical techniques, and is specific to each species. In relation the previously mentioned, the following objectives were proposed: 1) Describe the seed morphological characteristics of three native high Andean species (*Clusia* sp., *Podocarpus sprucei* Parl. and *Viburnum triphyllum* Benth), and 2) Evaluate scarification and stratification treatments that can disrupted morphological and morphophysiological dormancy of Andean species. These processes described above in context with restoration and reforestation could affect the propagation rates and reproduction of native plants utilized for these purposes.

METHODS

Study area. Seeds collections of *Clusia* sp. (duco), *Podocarpus sprucei* Parl (romerillo) and *Viburnum triphyllm* Benth (rañas) was carried out within the Yanuncay-Irquis Protective Forest, Azuay, Ecuador (3° 03' 48.2'' S, 79° 03' 59.94'' W), whose altitude ranges between 2,640 and 3,800 m a.s.l., in Cuenca canton, Azuay province (Figure 1). Seed evaluations were carried out in the Seed laboratory located in the Faculty of Agricultural Sciences of the University of Cuenca.

Seed selection and collection. The selected species have ecological importance mainly as food for birds and at a socioeconomic level due to the quality of wood specially in the case of *P. sprucei*. The seeds came from at least 10 individuals per species spatially distributed in the forest. The best phenotypic characteristics of the trees by spe-

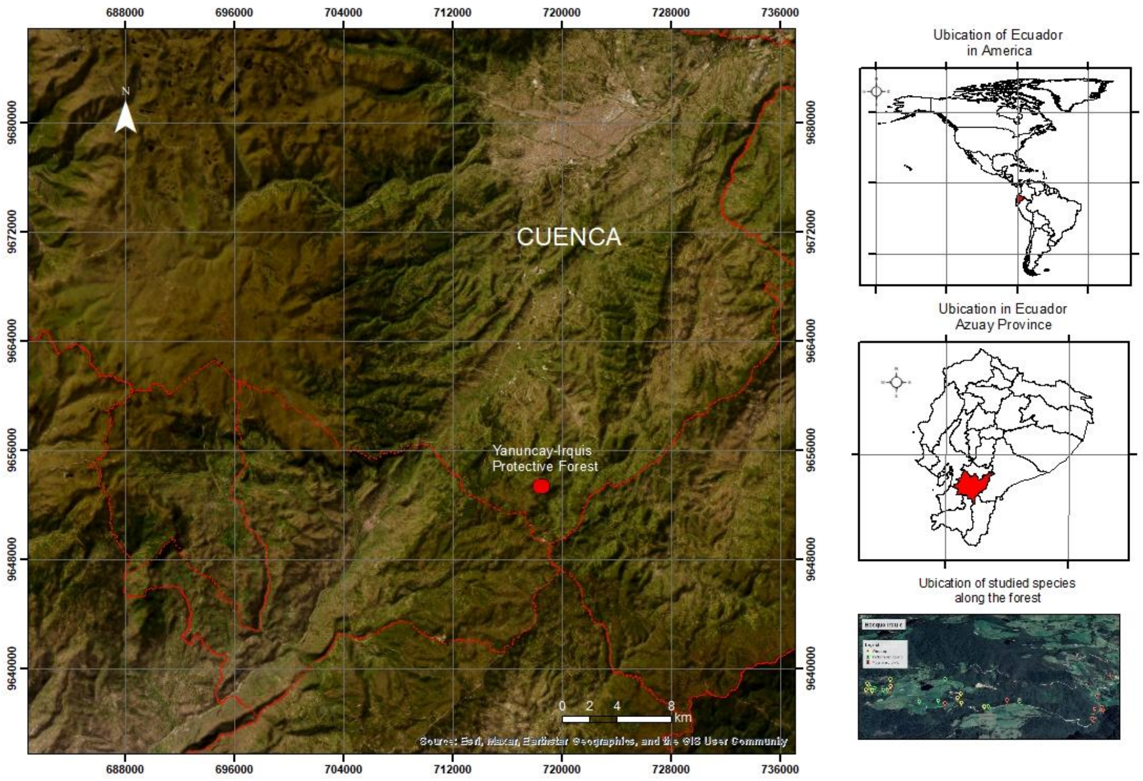

Figure 1. Location map of the Yanuncay-Irquis Protective Forest in the province of Azuay, Ecuador.

Mapa de ubicación del bosque protector Yanuncay-Irquis en la provincia del Azuay, Ecuador.

cies were considered, and fruit collection was carried out according to the fruiting period of each species, *Clusia* sp. between September and October 2018, *P. sprucei* between September and December 2018 and *V. triphyllum* between January and February 2019. The ripe fruits were taken to the laboratory for seed processing.

Morphological and physical characterization of seeds. For the morphological description of the seeds, a total of 100 seeds randomly selected from the total batch were used for each species. The parameters measured were the length and width of the seed in mm with a digital caliper. Additionally, 25 embryos were measured using a stereomicroscope with camera (Infinity analyze). To determine the position and type of the embryo in the seed, a cross section was made, for this purpose a stereomicroscope equipped with a camera (MShot Image Analysis System) was used. The classification of the embryo was based on that proposed by Baskin and Baskin (2007) and previously Martin (1946).

Additionally, with another batch of seeds distributed in 10 repetitions with 15 seeds each, an imbibition test was carried out (150 seeds per species), which consisted of submerging the seeds in distilled water and then weighing (g) them during the first 8, 12, 24, 36, 48 and 72 hours. Finally, for viability tests, 100 embryos of each species were extracted distributed in four repetitions (25 per repetition), the embryos were immersed in 1% tetrazolium chloride for 24 hours, subsequently those embryos that were stained and the results were counted. They were expressed in percentages.

Pre-germinative treatments. Prior to the application of the treatments, a disinfection protocol was applied to the entire batch of seeds, which consisted of rinsing them for 3 to 5 minutes with running water, followed by applying three drops of liquid soap and a final rinse with plenty of distilled water. The pre-germinative treatments applied to the seeds of the three species were: 1) Hot stratification, which consisted of immersing the seeds in hot water at temperatures of 30°C, 40°C and 70°C for 12 hours; 2) Immersion in hormonal solution: seeds were soaked in gibberellic acid at 300 ppm for 12, 24, 36 and 48 hours; 3) Immersion of seeds in 10% sulfuric acid for 3, 5 and 10 minutes, and 4) Control treatment. Once the treatments were applied, the seeds were placed in Petri dishes with previously sterilized paper towels. The experimental units were placed in the growth room with the following environmental conditions: maximum temperature (25°C), minimum temperature (16°C), mean temperature (19°C), relative humidity (64%), 12 hours of light and 12 hours of darkness.

Experimental design. A completely randomized design was applied. At the species level, 11 treatments were made up of four repetitions with 25 seeds each (100 seeds treatment^{-1} and 1,100 seeds species^{-1}). Germination control was carried out every three days for 18 weeks for the species *Clusia* sp. and 40 weeks for the species *P. sprucei* and *V. triphyllum*. It should be noted that a seed was considered germinated when it had its elongated radicle and the first embryonic leaves (ISTA, 2016). To calculate the germination percentage, the equation [1] was used (Islam et al. 2012).

$$G\ (\%) = \text{number germinated seeds}/\text{number sown seeds}*100 \quad [1]$$

Statistical analysis. The analysis was carried out for each species for the imbibition and viability test; and, for the treatment factor the response variable analyzed was germination. The Kruskal-Wallis test was applied ($P < 0.05$). Likewise, to detect significant differences between treatments, a Nemenyi post hoc test was applied ($P < 0.05$). Also, an accumulative germination plot was elaborated using GraphPad Prism. Finally, metrics of germination (rate, speed and energy) were calculated through the package “germinationsmetricts”, likewise, statistical analyses and the rest of plots were performed in R Studio IDE (R Core Team, 2024).

RESULTS

Seed and embryo size. The seeds of *V. triphyllum* presented larger sizes in relation to the other species analyzed; while the smallest seeds corresponded to *P. sprucei*. Regarding the size of the embryos, the largest corresponded to *Clusia* sp. and those of smaller size to *V. triphyllum* (Table 1 and Figure 2).

Position and type of embryo. It was determined that the position of the embryos of *P. sprucei* and *Clusia* sp. corresponds to axillary division, since they are located in the center of the seed (Figure 3). The position of *V. triphyllum* embryos is classified as lateral division, because it is located at the lower end of the seed (Figure 3C). Regarding the

Table 1. Mean size of seeds and embryos of the species *P. sprucei*, *Clusia* sp. and *V. triphyllum* SD: Standard deviation, n: number of evaluated seeds.

Media del tamaño de semillas y embriones de las especies *P. sprucei*, *Clusia* sp. and *V. triphyllum. SD: desviación estándar, n: número de semillas evaluadas.*

Species		Seed size (n = 100)		Embryo size (n = 25)		
		Length (mm)	Width (mm)	Lenght (mm)	Width (mm)	SD
P. sprucei	Mean	6.4	5.5	3.4	1.0	0.53
	SD	0.3	0.3	0.3	0.1	
Clusia sp.	Mean	8.4	3.8	8.2	3.1	0.98
	SD	0.5	0.3	0.9	0.3	
V. triphyllum	Mean	8.9	6.8	2.2	1.0	0.25
	SD	0.8	0.7	0.2	0.1	

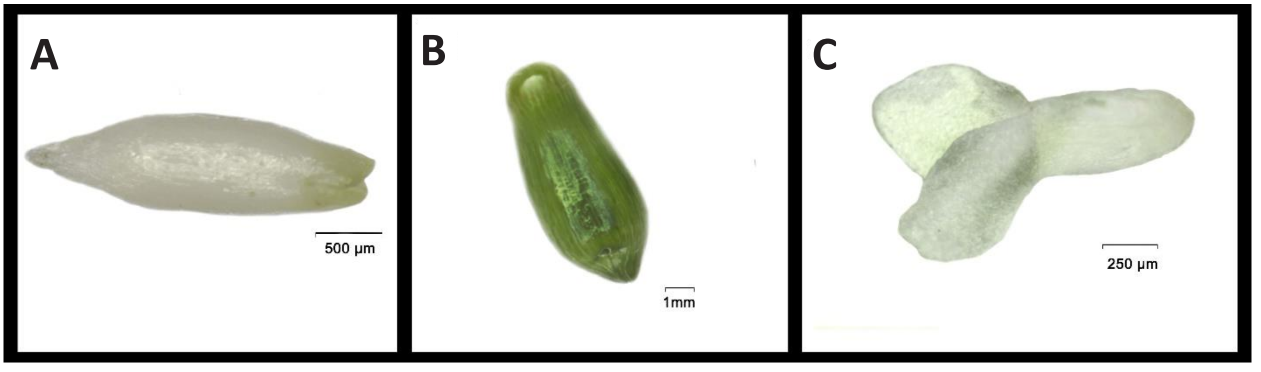

Figure 2. Pictures from seed embryo of the next species: *P. sprucei* (A)*, Clusia* sp. (B) y *V. triphyllum* (C).

Fotografías del embrión de las semillas de las siguientes especies: *P. sprucei* (A)*, Clusia* sp. (B) y *V. triphyllum* (C).

type, in the case of *P. sprucei*, it was classified as underdeveloped linear and for *Clusia* sp., it was classified as fully developed linear; the embryos were longer than wide in relation to the size of the seed (Figure 3A and 3B). The type of embryo of *V. triphyllum* is classified as rudimentary, due to its small size in relation to the endosperm and represents around a quarter of the total size of the seed.

Imbibition test. The results showed that the imbibition tests (after 72 h) for *P. sprucei* and *Clusia* sp. had an increase in their weight as the hours passed, and it was statistically significant, indicating that the seed coat is permeable and allows water to enter the endosperm. *V. triphyllum* had an increase, however, this was not statistically significant, therefore, the seeds of this species present exogenous dormancy (Figure 4).

Viability test. Podocarpus sprucei, *Clusia* sp. and *Viburnum triphyllum* had high viability with 50%, 88% and 79% respectively. Only in *P. sprucei* 5% were reported as empty seeds (Figure 5). The results of viability tests indicated that *Clusia* sp. mean is higher than that of other species (Supplementary material 1).

Effect of pre-germinative treatments. P. sprucei seeds presented higher percentages of germination in the acid scarification treatment (sulfuric acid) for 3 minutes with 62%, while the treatment with the lowest germination was hot

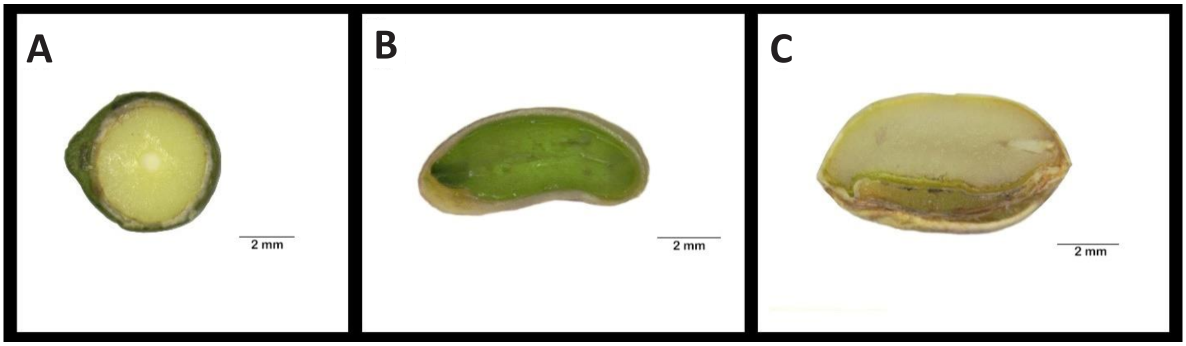

Figure 3. Pictures from embryo position of the next species: *P. sprucei* (A), *Clusia* sp. (B) y *V. triphyllum* (C).

Fotografías de la posición del embrión de las siguientes especies: *P. sprucei* (A), *Clusia* sp. (B) y *V. triphyllum* (C).

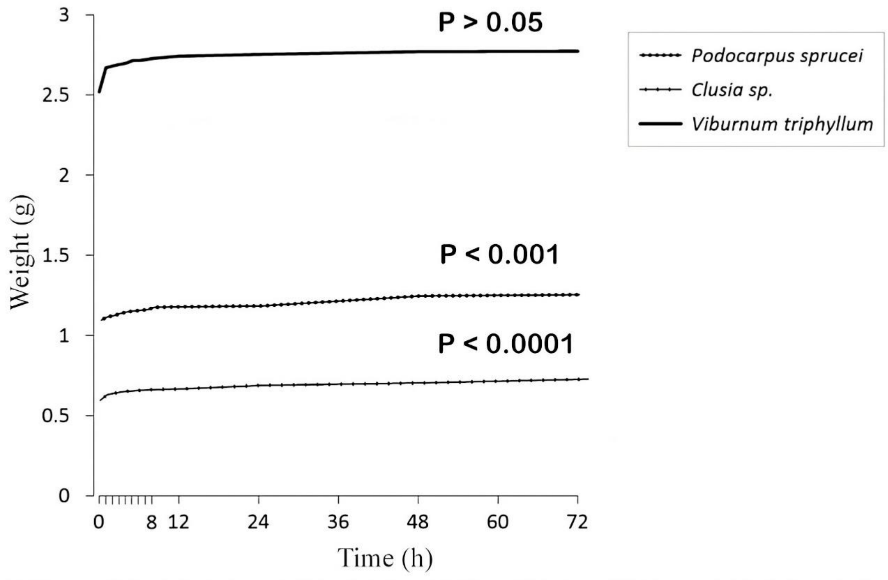

Figure 4. Imbibition of seeds of three native species: *P. sprucei*, *Clusia* sp. and *V. triphyllum*, determined through seed weight from 0 to 72 hours immerse in water.

Imbibición de tres semillas de tres especies nativas: *P. sprucei*, *Clusia* sp. and *V. triphyllum*, determinado a través del peso de las semillas desde 0 a 72 horas sumergidas en agua.

stratification at 40°C, with 35% germination (Figure 6A). In the species *Clusia* sp., the treatment with the highest percentage of germination was the Control with 92%, followed by the hot stratification treatment at 30°C with 89% germination, however, the treatment with the lowest germination was soaking in GA_3 for 24 hours with 70% (Figure 6B). Finally, in *V. triphyllum*, the highest percentage of germination was obtained in the hot stratification treatment at 30° and 40° with values of 80% and 82% respectively, followed by the acid scarification treatment (sulfuric acid) for 3 minutes with 75%, and the lowest germination value was presented by the treatment soaking in GA_3 solution for 36 hours with 40% (Figure 6C). The seeds of the three species exposed to hot stratification treatment at 70°C and *Clusia* sp. At 40°C did not show germination. There were significant differences in the germination percentage in the three native forest species analyzed ($P < 0.05$) as shown in Figure 6.

The accumulated germination curves as the weeks pass for the species indicated that *Clusia* sp. was the one that obtained the best results and began germination in the shortest time (from 4th week until 18th), while the remaining two (*P. sprucei* and *V. triphyllum*) obtained similar values and their germination was late (from 15th week until 40th) in relation to the first (Supplementary material 2). In addition, to complement the behavior of the three species, we calculated the rate, speed and energy germination, *Clusia* sp. was the species with the highest values in all these metrics (Supplementary material 3), this may reinforce the absence of dormancy and quiescence of the last-mentioned species.

DISCUSSION

The results obtained in the morphological characterization of the seeds of the three species under study have allowed to find variations in the size of the seed, embryo and their classification. In addition, it has been possible to show which is the best pre-germinative treatment species-specific.

Seed morphology. There are no previous publications that have described and characterized the embryo and seeds

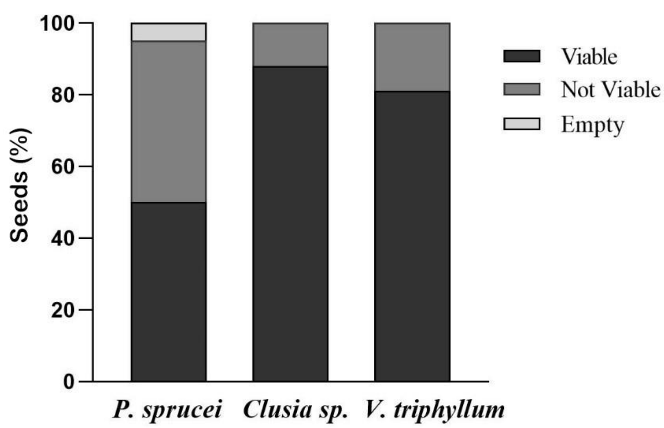

Figure 5. Percentage of viable, non-viable and empty seeds of *P. sprucei, Clusia* sp. and *V. triphyllum.*

Porcentaje de viabilidad, semillas no viables y vacías de *P. sprucei, Clusia* sp. and *V. triphyllum.*

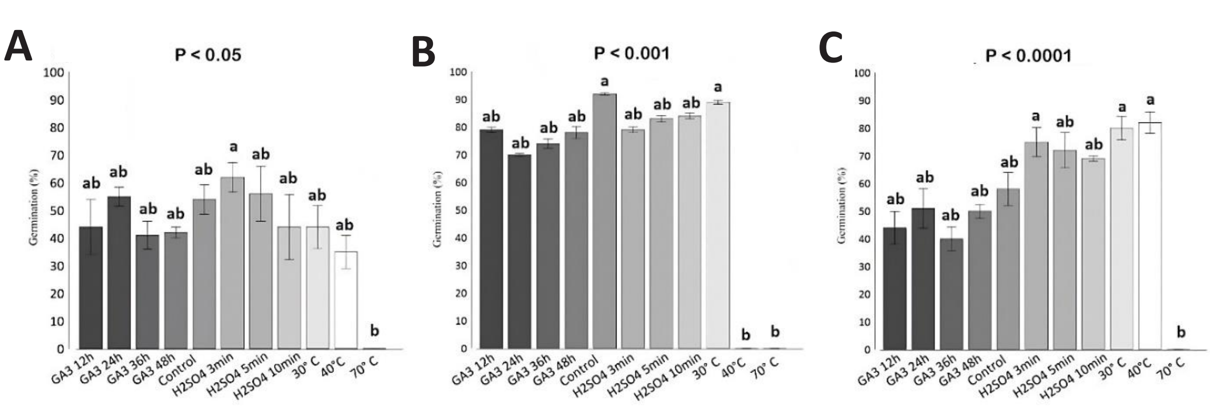

Figure 6. Germination percentage of each species according to the treatments applied. *P. sprucei* (A), *Clusia* sp. (B) and *V. triphyllum* (C).

Porcentaje de germinación por especie acorde a los tratamientos aplicados. *P. sprucei* (A), *Clusia* sp. (B) and *V. triphyllum* (C).

of these species, however, studies on species of the same genus exist. Blendinger (2017) described *Podocarpus parlatorei* seeds (in Argentina and Bolivia), these recorded a length of 12.2 mm x 7.6 mm wide, which is twice the length and width of *P. sprucei*. However, the two species are found in different ecosystems, since *P. parlatorei* is located in the tropical Andes (Catania & Romero, 2017), meanwhile, *P. sprucei* in the high Andean forests, where the climatic conditions are different e.g. higher altitudes and colder temperatures. Furthermore, the radius between the length of the seeds and the length of the embryo was 0.25, it varies with the radius of *P. henkelii* and *P. latifolius* which was 0.37 (Twala & Fisher, 2022).

According to the embryo sizes (Table 1), the embryo of *Clusia* sp. was classified as developed linear, *P. sprucei* has an underdeveloped linear embryo and *V. triphyllum* rudimentary. Therefore, the latter two need more time to germinate due to the fact that it is necessary for the differentiation and maturation of tissues found within the seed to occur and to initiate the emergence of the radicle (Sautu et al. 2006).

Quiescence type. Clusia sp. by having a developed embryo and allowing water to enter through its membrane, can be determined as lacking of quiescence and dormancy (Baskin & Baskin, 2014a). Similarly, *P. sprucei* in the imbibition tests (Figure 4) showed to gain weight significantly ($P <$ 0.001) which suggests that it does not have physical quiescence either; however, seeds with this type of quiescence are mainly attributed to the testa (Apodaca-Martínez et al. 2019; Javed et al. 2022). There is evidence that *P. sprucei* has endogenous (morphological) quiescence similar to another species of the genus Podocarpus, primarily due to its semi-developed embryo (Baskin & Baskin, 2004). The opposite happens with *V. triphyllum*, which behaved in an antagonistic manner (Figure 4); seeds of certain species are structured with components that repel water such as polyphenols, lignin, condensed tannin, cellulose and hemicellulose (Galussi et al. 2015; Ali & Elozeiri, 2017) and are classified as having exogenous (physical) quiescence (Fernández & Gutiérrez, 2003). Likewise, Delgado et al. (2007) mention that species with a rudimentary embryo have endogenous (morphological) quiescence. These two characteristics were presented by *V. triphyllum* (Table 1), which is why it is suggested that this species has exogenous/endogenous quiescence.

Dormancy type. Previous research of Chen et al. (2013), described seeds of Podocarpacea family (*Podocarpus* and *Nageia* genus) and concluded they have morphological dormancy due to their semi-developed embryo which is similar to *P. sprucei*. On the other hand, morphophysiological dormancy is found in rudimentary seeds (Baskin & Baskin, 2014a; Kathpalia & Bhatla, 2018) when the embryo is less than 30% of the size of the seed, so it needs specific conditions for it to develop (Baskin & Baskin, 2014a), in addition, long times to germinate is due to the high ratio between ABA and GA, which prevents the breakdown of their structures (Baskin & Baskin 2004; Baskin & Baskin 2014a) in this study *V. triphyllum* fulfilled with these characteristics (Figure 6C). Seeds with morphophysiological dormancy can be broken through acids or stratifications, (Schlindwein et al. 2019; Baskin & Baskin 2014b) as in the species *Annona squamosa* described a rudimentary embryo (morphophysiological dormancy) which germinates when applying acid stratifications (GA_3) (Martínez et al. 2016).

Pre-germinative treatments. Chemical scarification allows the seed coat to be removed, therefore, when the seed is soaked, the acid allows it to erode the cover, in precise concentrations so as not mistreat the endosperm (Baskin & Baskin, 2004; Hernández Epigmenio et al. 2021). Fahmi et al. (2020) indicate that pre-germinative treatments based on sulfuric acid (H_2SO_4) immersed seeds for 10 minutes, achieved high germination percentages (84%) in *Indigofera zollingeriana*. Likewise, Hernández Epigmenio et al. (2021) in *Guaiacum coulteri* reported the best results with scarification with sulfuric acid (10 minutes), with germinations greater than 80%, although the seeds in scarification do not be prolonged to obtain better results (Yang et al. 2022). Similarly, our study evidenced effectiveness of sulfuric acid for *P. sprucei* and *V. triphyllum* (Figure 6A and 6C).

Clusia sp. and *V. triphyllum* when applying hot stratification at 30°C and 40°C, obtained germination percentages of 89% and 82%, respectively (Figure 6B and 6C). Auge et al., (2015) reported stratification at 35°C in *Arabidopsis thaliana* showing high germination percentages, however, the seeds were incubated also at 10°C. Apparently, combining hot and cold stratification allows a higher germination rate, because it emulates conditions similar to its habitat which could also be applied to the three species under study (Gao et al. 2022; Sano & Marion-Poll, 2021; Auge et al. 2015).

CONCLUSIONS

Our principal finding showed that seed morphological and behavior is species-specific. After analyzing the ratio embryo/seed, *Clusia* sp. had an equilibrated value (~1) this explains the absence of dormancy/quiescence and accelerated germination. On the other hand, *P. sprucei* and *V. triphyllum* registered a opposite ratio embryo/seed (~0.3), probably this is the principal reason to show morphological and morphophysiological dormancy and also, endogenous and endogenous/exogenous acquiescence, respectively. We verify that application of scarification and stratification treatments helped to break dormancy and acquiescence in native seeds. Scarification encouraged *P. sprucei* and *V. triphyllum* to break dormancy, likewise, hot stratification in *V. triphyllum* and *Clusia* sp. These results can help reinforce the information on quiescence dormancy and how to overcome those in these Andean species. Additionally, the knowledge generated in this study will improve the germination capacity of seeds for the production of seedlings promoting the use of these species in restoration programs.

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AUTHORS CONTRIBUTIONS

DSV and PV problem conception, research design, data collection and paper writing. AMH data collection, data analysis and paper writing. GP problem conception, research design, data collection. AM paper writing. XPP research design and paper writing.

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SUPPLEMENTARY MATERIAL

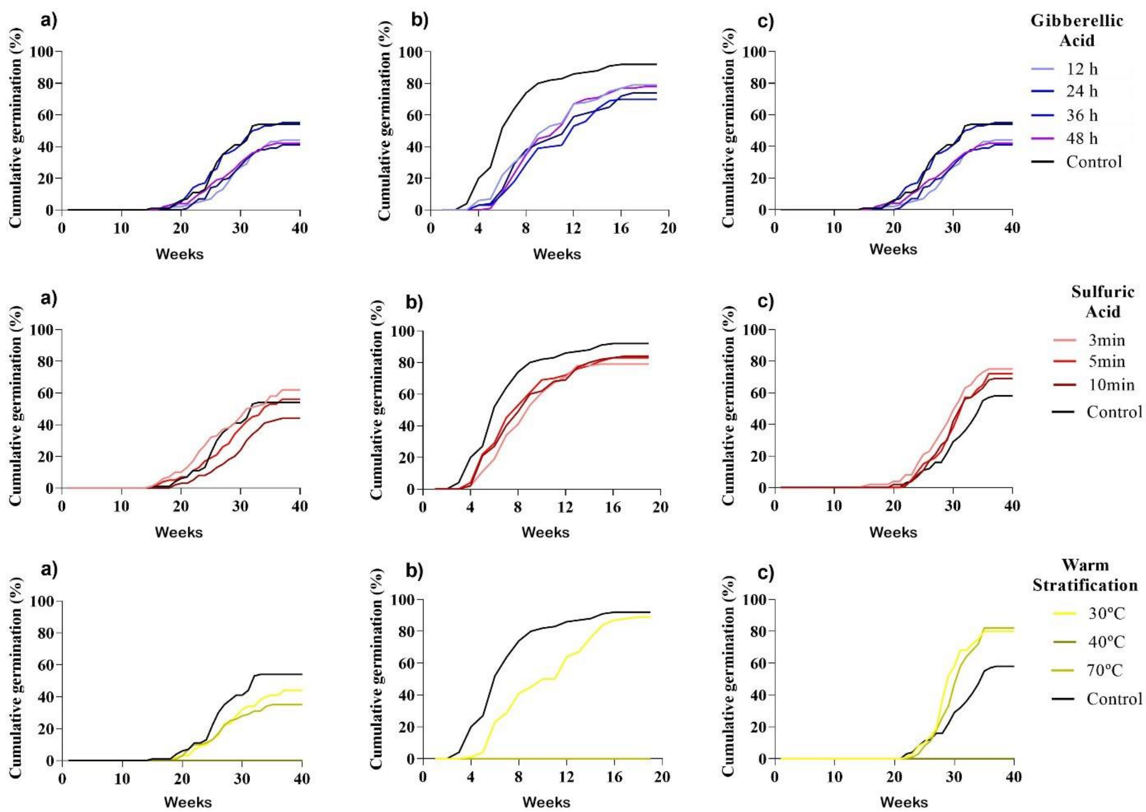
Supplementary material 1. Means of viability percentage for seeds of *P. sprucei, Clusia* sp. and *V. triphyllum.*

Medias de los porcentajes de viabilidad de las semillas de *P. sprucei, Clusia* sp. and *V. triphyllum.*

	P. sprucei		*Clusia* sp.		*V. triphyllum*	
	Viability (%)		Viability (%)		Viability (%)	
	mean	SD	mean	SD	mean	SD
Viable	50 a	6.92	88 a	3.26	81 a	3.82
Not viable	45 ab	11.94	12 ab	3.26	19 ab	3.82
Empty	5 b	1.5	0 b	0	0b	0

Supplementary material 2. Percentage of accumulated germination of seeds per week. a) *P. sprucei*, b) *Clusia* sp., y c) *V. triphyllum*, with the application of different pregerminativo treatments: Gibberellic acid (12, 24, 36 and 48 hours). Sulfuric acid (3.5 and 10 minutes). Warm stratification (30°C, 40°C and 70°C) and control.

Porcentaje de la germinación acumuladas de las semillas por semana. a) *P. sprucei*, b) *Clusia* sp., y c) *V. triphyllum*, con la aplicación de distintos tratamientos pregerminativos: ácido giberélico (12, 24, 36 y 48 horas). Ácido sulfúrico (3, 5 y 10 minutos). Estratificación caliente (30°C, 40°C y 70°C) y control.



Supplementary material 3. Values of germination rate, speed and energy by species.

Tasas de germinación, velocidad y de energía por especies.

	H_2SO_4			GA_3				Stratification			
	3min	5min	10min	12 h	24 h	36 h	48 h	30°C	40°C	70°C	Control
						P. sprucei					
G.rate (1 day^{-1})	0.22	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0	0.21
G.speed (n day^{-1})	4.2	3.4	2.3	2.3	3.5	2.2	2.5	2.5	2.3	0	3.4
G.energy (% day^{-1})	1,186	897.4	563	559.4	958.1	565.1	679.1	647.5	612.5	0	952.8
						Clusia sp.					
G.rate (1 day^{-1})	0.52	0.53	0.52	0.51	0.50	0.51	0.50	0.51	0	0	0.55
G.speed (n day^{-1})	10.6	12.1	11.7	9.9	7.8	8.7	9.0	10.6	0	0	15.8
G.energy (% day^{-1})	851.4	974.7	934.5	781.8	608.2	685.8	704.8	778.28	0	0	1,269.7
						V. triphyllum					
G.rate (1 day^{-1})	0.21	0.20	0.21	0.21	0.20	0.20	0.22	0.21	0.20	0	0.20
G.speed (n day^{-1})	4.1	3.4	3.3	2.3	2.1	1.6	3.3	4.1	3.7	0	2.6
G.energy (% day^{-1})	1,027.4	781.7	783.8	572.2	434.2	333.1	908.2	951.5	827.2	0	590.2

G.rate : Mean germination rate, G.speed: Speed of germination, G.energy: Energy of germination (Timson's index)