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Seed desiccation tolerance and germination of four *Puya* (Bromeliaceae) high-andean tropical species from Colombia

Germinación y tolerancia a la desecación en semillas de cuatro especies altoandinas de *Puya* (Bromeliaceae) de Colombia

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ABSTRACT
Ex-situ seed conservation is one of the most effective tools for biodiversity and genetic diversity protection. In the context of global climate change, *ex situ* conservation has become an important strategy that must be strengthen and integrated with *in situ* conservation strategies. The main objective of this study was to document the desiccation tolerance and germination properties of four species from *Puya* and provide tools that allow the *ex situ* conservation of this and other closely related species. These species are distributed in Páramo, a threatened ecosystem despite of its value as a water reservoir and supplier for urban population. Seed morphological variables and moisture content (MC) were measured before a test of germination was conducted with three different moisture contents (fresh seeds, 10–12 % and 3–5 %). Germination percentage and the mean germination time were evaluated. The germination was above 80 % in all the species with no significant differences when decreasing MC. There were no significant differences in mean germination time. Seed viability for the four species was high, as compared to other Páramo species. The results indicate that all species are orthodox. Therefore, this genus has great potential to be conserved in seed banks, which will contribute to diversity conservation of native species from tropical Andean highlands, and can be implemented in germination protocols for their propagation and reintroduction in restoration programs.

Key words. Andes, *ex situ* conservation, orthodox seeds, Páramo, seed storage.

RESUMEN
La conservación *ex situ* de semillas es una de las formas más efectivas de preservación de la biodiversidad y especialmente de la diversidad genética. En el marco del cambio climático su aplicación es una estrategia importante que se debe fortalecer e integrar con la conservación *in situ*. El objetivo del presente trabajo fue estudiar la tolerancia a la desecación y la germinación de cuatro especies del género *Puya* y proporcionar herramientas para su conservación *ex situ*. Estas especies están distribuidas en el páramo, un ecosistema amenazado a pesar de su valor como reservorio y proveedor de agua para la población urbana. En las semillas se realizó la toma de medidas morfológicas y del contenido de humedad (CH), se realizaron ensayos de germinación en tres contenidos de humedad (semillas frescas, 10–12 % y 3–5 %) y se evaluó el porcentaje de germinación y tiempo medio de germinación. Se registró una germinación superior al 80 % en las cuatro especies y no se encontraron diferencias significativas al disminuir el CH. El tiempo medio de germinación no varió entre especies y la viabilidad fue alta comparada con otras especies de páramo. Los resultados indican que son semillas ortodoxas y las variables morfológicas apoyan esta tendencia. Por tanto, este género presenta un gran potencial para ser conservado en bancos de germoplasma lo que contribuye a la conservación de su diversidad, con las cuales se pueden implementar protocolos de germinación para la propagación y reintroducción en programas de restauración.

Palabras Clave. Almacenamiento de semillas, Andes, conservación *ex situ*, páramo, semillas ortodoxas.

INTRODUCTION

Páramo ecosystem is located in the humid zone over equatorial Andes region between latitudes 11° N and 8° S and altitude between 3000 and 5000 meters, mainly in Venezuela, Colombia, Ecuador, and Northern Peru, but it is also found in some areas in Costa Rica and Panama (Sklenář *et al.* 2005). This ecosystem possesses high biodiversity and endemism with its key biological relevance in the regulation of hydrological cycles, making it an important water reservoir for different regions in South America (Sklenář *et al.* 2005).

In Colombia, Páramos cover only 2.5 % of the territory. Nonetheless, 4700 species of plants have been recorded in this ecosystem, representing 17 % of the country (Marin and Parra 2015). Factors such as anthropogenic pressure, climate change, and reduced resilience after a disturbance are altogether threats of Páramo biodiversity and ecosystem services. For this reason, it is important to preserve their natural diversity through the implementation of *in situ* and *ex situ* conservation strategies. One of the long-term strategies of *ex situ* conservation of plant genetic resources are seed banks (de Viana *et al.* 2009). This strategy has been applied mainly on species of agricultural or commercial use, leading native species to represent less than 1 % of stored taxa in these banks (Rao *et al.* 2007). Furthermore, biological information such as longevity, storage conditions according to desiccation tolerance, germination requirements, and viability (Hong and Ellis 1995), crucial to establish successful *ex situ* conservation plans of native species, is scarce in tropical countries. Additionally, little is known on germination requirements of tropical high mountain species (Baskin and Baskin 2014). Quality and desiccation tolerance information is important, not only for conservation purposes, but also for medium and long-term propagation strategies. Moreover, data about recruitment, natural seed bank dynamics, and reproductive phenology are the basis of restoration and reproductive ecology projects (Vieira and Silveira 2010).

In Colombia, 25 genera and 543 species of Bromeliaceae have been reported (Betancur c2015). *Puya*, represented by 37 species in Colombia from which 26 are endemic, it is also the fifth most diverse genus of the family in the country (Betancur c2015). This genus is mainly distributed in the high Andean forests and Páramos, between the 2500 and 4500 m of elevation, and 90 % of its species are under thread of extinction (Betancur c2015). Detailed information about reproductive cycles are a cornerstone in the implementation of conservation plans of endemic and threatened species (Schemske *et al.* 1994). Therefore, given the scarce knowledge of reproductive biology about these species, procuring such information is needed to advance in conservation plans of the genus *Puya*. The main goal of this study is to evaluate germination, viability, and desiccation tolerance of four Colombian species of *Puya*, and elucidate if there are significant physiological tendencies among this group of closely related species.

MATERIAL AND METHODS

Study location and species

Seeds were collected in four Páramos in Cundinamarca, Colombia from 2014 to 2015 (Fig. 1). These localities have different levels of anthropogenic disturbance, although potato (*Solanum tuberosum* L.)

cultivation and cattle breeding are the more important. Four *Puya* species were studied (Table 1): *Puya* sp. nov. aff. *horrida*, an undescribed species currently undergoing publication process (Betancur, pers. com.), *Puya nitida* Mez, *Puya santosii* Cuatrec, and *Puya trianae* Baker. According to the IUCN Red List criteria, *P. nitida* and *P. santosii* are Near threatened (NT), whereas *P. trianae* is Least concern (LC) (Betancur and García 2006). The conservation status for the new species has not been assessed. All the species are native and endemic to Colombia except *Puya trianae*, which is also distributed in NW Venezuela (Betancur c2015). The four species are monocarpic acaulescent rosettes, mostly found in frailejón-grassland plant communities proper of andean Páramos (Varadarajan 1990, Betancur 2000, Mora *et al.* 2007), common in poorly drained soils and peatlands (Vargas 2002, Pedraza-Peñaloza *et al.* 2004). The identity of target taxa was verified by vouchering specimens at the herbarium of the Bogotá Botanical Garden (JBB).

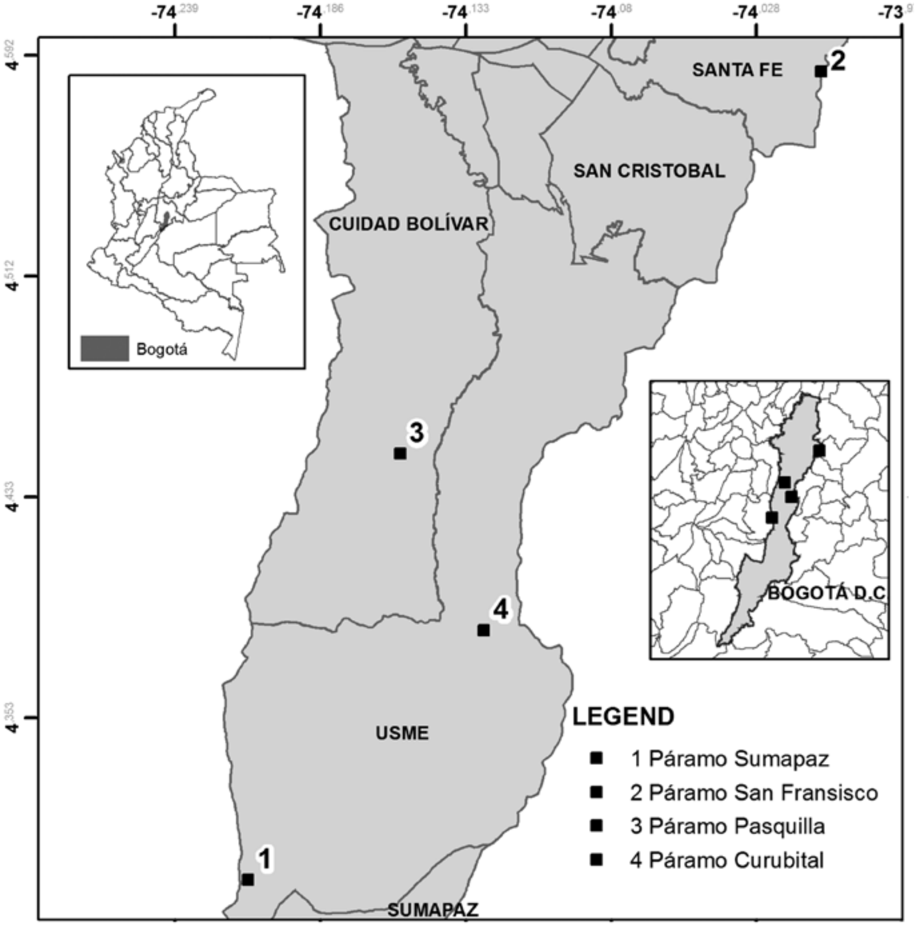

Figure 1. Location of seed sources for the species from Puya in Bogotá, Cundinamarca, Colombia, South America.

Table 1. Dates of collection and location of *Puya* species studied.

Species	Sampled location	Collection date	Coordinates	Altitude (m a.s.l)
Puya sp. nov. aff. *horrida*	Curubital Páramo, Bogotá D.C	11-dec-14	4° 22' North 74° 06' West	3244
P. nitida	San Francisco Páramo, Choachí	14-may-15	4° 34' North 73° 59' West	3172
P. santosii	Pasquilla Páramo, Bogotá D.C	03-dec-14	4° 25' North 74° 11' West	3660
P. trianae	Sumapáz Páramo, Bogotá	26-may-15	4° 10' North 74° 13' West	3725

Seed collection and measurements

Mature fruits from a minimum of five individuals per species and 500 seed per individuals were collected. The seeds were extracted manually in the Germination Laboratory at Bogotá Botanical Garden. Only mature seeds without visible damage were selected following the criteria by Ayala-Cordero *et al.* (2004).

Four features were measured in ten seeds per species: embryo length, endosperm length, body seed length, and overall seed length. The latter includes the dispersal appendix. The measurements were taken through the images obtained under a Motic® SMZ-168 stereoscope (Hong Kong, China) and were processed with the software Motic Images Plus 2.0 (Hong Kong, China).

Quality and desiccation tolerance tests

Moisture content (MC) was assessed on 3 ± 0.5 grams of seeds at 150°C during seven minutes, using a moisture analyzer OHAUS MB45 (Parsippany, Nueva Jersey). Germination tests were carried out in a Thermoline germination chamber (New South Wales, Australia) under controlled conditions with a 12 hours photoperiod, 20/10 ± 2.5 °C, and mean humidity of 75 ± 5 % following the criteria by Pérez-Martínez *et al.* (2014). Both variables were monitored with Data Logger EBCHQ 94150 (China). The germination of fresh seeds was evaluated at initial moisture content (MC_i) and after desiccating the seed to 5.0 ± 2.0 % MC. Due to high MC_i in fresh seeds, an additional experiment was carried out on *P. nitida* and *P. trianae,* with MC at 10 ± 2.0 %. Decrease in MC was reached through a silica-based desiccation chamber with a 2:1 ratio (silica: seeds) and the equation $FSW = SSW\times[(100 - MC_i)/(100 - MC_o)]$ where FSW is the Final Seed Weight, SSW is Starting Seed Weight, MC_i initial moisture content, and MCo is object moisture content after desiccating (in this case 10 or 5 %) (Rao *et al.* 2007).

Four replicates per experiment were done, using 50 seeds each. Before setting the experiment, seeds were disinfected with 5 % sodium hypochlorite solution (Muñoz and Ackerman 2011), and then sowed into Petri dishes with double filter paper. Germination experiments were recorded every third day over two months until there was no more germination. A germination event was recorded as positive whenever the radicle emerges through the seed coat (≥2 mm) (Salisbury and Ross 1992).

Finally, viability was determined in seeds that did not germinate after completion of the previous experiment. For this purpose, a seed was considered viable if its embryo was white and rigid (Buhler *et al*. 2001, Sawma and Mohler 2002, Nurse and DiTommaso 2005). The tetrazolium test was not used in this study given that standardization was not possible with this technique.

Data analysis

Germination percentage (GR) and mean germination time (MGT) were calculated through the following equations (Tompsett and Pritchard 1998, Ranal and Santana 2006):

$$GR = \left(\frac{N}{Ns}\right) * 100$$

$$MGT = \sum_{t=1}^{k} ni\ ti \Big/ \sum_{t=1}^{k} ni$$

Where, N is number of germinated seeds, N_s total number of seeds, *ni* number of germinated seeds at the i–th data measurement; *ti* is time (in days) at the i-th measurement; and *k* is germination test extent (in days).

The normality test (Shapiro–Wilk, $P < 0.05$) was conducted on each data group. If the null hypothesis was not rejected, ANOVA and Tukey tests were applied, but if normality was rejected, a Kruskal–Wallis non-parametric analysis was applied, followed by a measure comparison analysis to evaluate differences. Comparisons were made both among and within species. Statistical analysis was performed using StatGraphics® Centurion XVI version 16.1.11.

RESULTS

Morphological measurements and moisture content

All four species have three-locule capsule fruits, with color ranging from olive–green to light brown at immature stage and to dark brown while ripening. Fruits contain numerous dark brown, ovate seeds with a rough surface and a beige winged edge surrounding the entire seed, which gives the dispersal unit a triangular shape. Inside, approximately 80 % of the seed is endosperm and 20 % is an ovate, smooth surfaced, white embryo (Fig. 2). Regarding the morphometric variables, there were significant differences between species (Table 2). Seeds of *P. nitida* and *Puya* sp. nov. aff. *horrida* are smaller than *P. santosii* and *P. trianae*, both in dispersal unit, seed and embryo. *P. trianae* presented the largest seed and dispersal unit size. Concerning MC, *P. santosii*, *Puya* sp. nov. aff. *horrida*, *P. nitida* registered a value below 15 %; 10.8, 11.8 and 14.3 % respectively, whereas *P. trianae* had 30.5 % MC.

Germination and desiccation tolerance

The four species had high germination rates under all MC evaluated. However, there were significant differences of GR at 5 % of MC ($P = 0.04$). *Puya nitida* was the only species with a GR below 80 % while the rest of species showed a GR near 100 %. There were not differences between other MCs ($P = 0.61$).

Regarding species, there were differences in GR at different MC in *P. nitida* ($P = 0.03$) and *Puya* sp. nov. aff. *horrida* ($P = 0.02$). In the case of *P. santosii* ($P = 0.14$) and *P. trianae* ($P = 0.41$), there were not significant differences (Fig. 3). However, in either species there was no decreasing on GR as MC decreased.

In the case of MGT at MC_i there were significant differences among the studied species. *P. trianae* presented the highest MGT (48 days), while the remaining species registered an average of 26 days. At 5 % of MC there were also significant differences

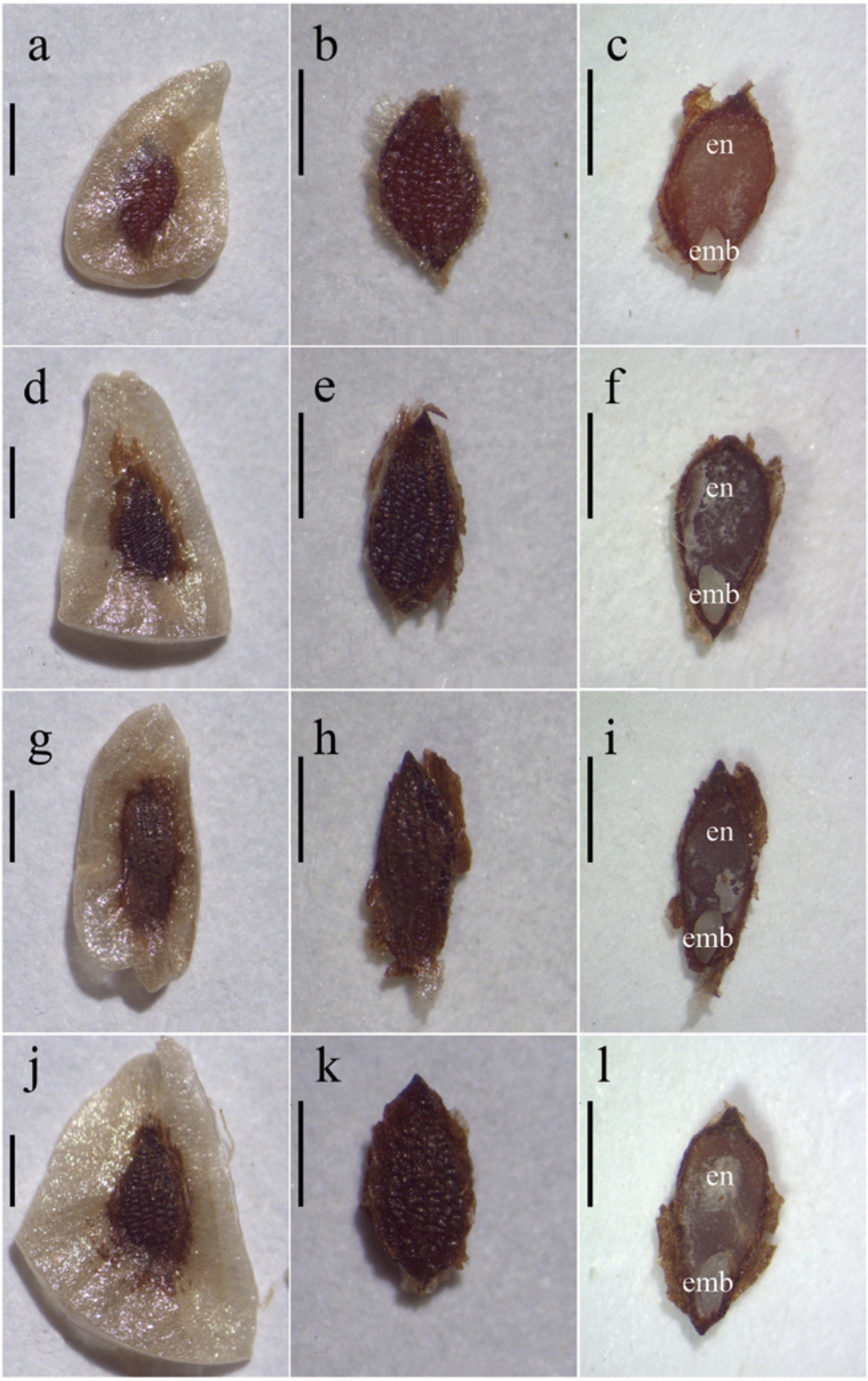

Figure 2. Seed of the *Puya* species, details of: dispersal unit, seed and longitudinal cut of the seed. **a.**, **b.**, **c.** *P. nitida*. **d.**, **e.**, **f.** *Puya* sp. nov. aff. *horrida*. **g.**, **h.**, **i.** *P. santosii*. **j.**, **k.**, **l.** *P. trianae*. en = endosperm, emb = embryo. Scale bars = 1 mm.

Table 2. External and internal morphological values for four species from *Puya*. Measurements followed by the same letters are not significantly different according to Tukey test ($P < 0.05$). SE = standard error (n =10).

Species	**Morphology (mm ± se)**			
	Length	**Width**	**Length**	**Width**
	Dispersal appendix		**Seed**	
Puya nitida	3.433± 0.14 a	1.987±0.05 a	1.763±0.09 a	0.917±0.04 a
Puya sp. nov. aff. *horrida*	3.535±0.16 ab	2.274±0.06 a	1.717±0.07 a	0.986±0.02 ab
Puya santosii	3.966±0.08 b	1.955±0.07 b	2.029±0.04 b	0.894±0.02 ab
Puya trianae	4.487±0.22 c	2.782±0.09 c	2.116±0.06 b	1.008±0.04 b
	Endosperm		**Embryo**	
Puya nitida	1.088±0.05 a	0.628±0.01 a	0.438±0.03 a	0.228±0.00 a
Puya sp. nov. aff. *horrida*	1.071±0.06 a	0.856±0.02 a	0.410±0.01 a	0.279±0.01 b
Puya santosii	1.226±0.05 a	0.625±0.02 b	0.558±0.02 b	0.278±0.01 b
Puya trianae	1.221±0.05 a	0.738±0.03 c	0.569±0.02 b	0.337±0.01 c

($P < 0.05$); *P. trianae* showed the highest MGT with 47 days while *P. santosii* showed the lowest with 26 days. For *P. nitida* and *Puya* sp. nov. aff. *horrida* the mean MGT was of 36 days. Within species there were significant differences in MGT at each MC for *P. nitida* ($P = 0.01$), *Puya* sp. nov. aff. *horrida* ($P = 0.03$) and *P. santosii* ($P = 0.01$) which increased its MGT as the MC decreased (Fig. 4). Seed viability for the four species was high: *P. santosii*, 97 %; *Puya* sp. nov. aff. *horrida*, 94 %; *P. trianae*, 92 % and *P. nitida,* 76 %.

DISCUSSION

The high germination rates reported here for the four species of *Puya* agrees with previous experiments under light laboratory conditions for *P. raimondii* (Vadillo *et al.* 2004), *P. nitida* (Franco 2014), *P. trianae* (Pérez-Martínez *et al.* 2014); and are slightly greater than those registered for *P. santosii* (Pérez-Martínez *et al.* 2014). It is worth noting that the germination rates values for other *Puya* species such as *P. cryptantha*, *P. trianae, P. santosii,* and *P. bicolor* are lower under field or greenhouse conditions (28 % - 50 %, according to Mora *et al.* (2007) and Pico (2014 data not publ.)), which is related to non-controlled environmental factors. Despite of the lower germination rate registered for *P. nitida*, in general the values presented here are considerably higher than those previously reported for other páramo species (Pérez-Martínez *et al.* 2014). One of the biggest limitations in restoration projects are the availability of native plant material (Murcia and Guariguata 2014); these results evidenced the efficiency of propagation in controlled laboratory conditions for all four species studied which can be linked with propagation protocols for restoration projects.

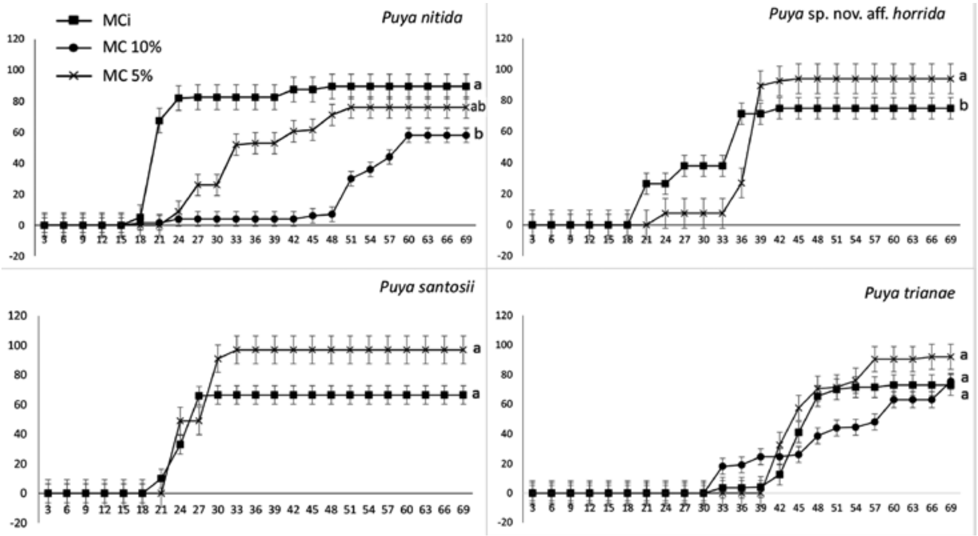

Figure 3. Germination curves at different moisture contents (MCi, MC 10 %, MC 5 %) for four species of *Puya*. The curves with the same letter are not significantly different according to the comparison test ($P < 0.05$). Perpendicular lines denote standard error (n = 4).

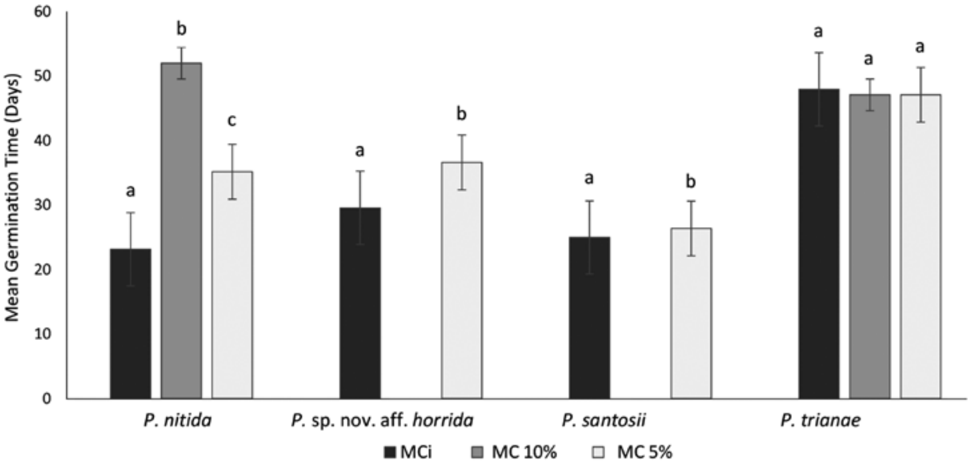

Figure 4. Mean germination time (Days) of four species from *Puya* at different moisture content (MCi, black bar; MC 10 %, grey bar; MC 5 %, white bar). Same letters indicate that these were not significantly different between the species according to Tukey test ($P < 0.05$). Perpendicular lines indicate the standard error (n = 4).

This is the first known study of seed desiccation tolerance for high Andean tropical species. Here is demonstrated that germination was not affected by decreasing seed moisture content in the studied species of *Puya*; on the contrary, GR increased when seeds were at 5 % of MC. This leads to conclude that seeds have an orthodox behavior and are tolerant to low moisture contents (Kermode and Finch-Savage 2002, Nkang *et al*. 2003).

The increase in the mean germination time while moisture content decreases agrees with expectations, since decreasing on MC of seeds also diminishes metabolic activity, taking more time to re-absorb the water needed to start germination (Tweddle *et al.* 2003). Nonetheless, increase on average MGT was six days, which do not imply a significant deceleration on the germination curve.

Although Hong and Ellis (1988) demonstrated that in Meliaceae all desiccation intolerant species have a high moisture content (20.3 – 52.5 %); in this study initial moisture content was not related with the result of desiccation tolerance. This agrees with Daws *et al.* (2006), who found that 104 orthodox species presented MC_i range from 9.1 to 61.6 %.

Regarding seed morphology and morphometry, bigger seeds of *P. trianae* agree with its higher MGT linked to higher volume to imbibe. Heavy seeds with thin coats have been widely reported as poor tolerant to desiccation (Tweddle *et al.* 2003, Pritchard *et al.* 2004, Daws *et al.* 2005, 2006); these relations must be evaluated in contrasting ecosystems such as Páramo, where several morphological traits such as small size seeds, are usually constant. In turn, despite of the humid nature of Páramo, it is worth noting that seeds were collected during dry season a fact that is linked to anemochory of *Puya* and has been referenced in other desiccation tolerant species (Tweddle *et al.* 2003, Pritchard *et al.* 2004, Daws *et al.* 2005). Gathering information from different species and families from Páramo ecosystem will allow researchers to find tendencies within the ecosystem, as well as the development of probabilistic tools to improve our understanding of desiccation tolerance (Daws *et al.* 2006).

In conclusion, the four species evaluated have the potential to be conserved in germplasm banks, and is probable that we can extend the desiccation toleration type to the whole genus, contributing to diversity conservation of native species of Páramo, an endangered ecosystem. Germination protocols can be implemented due to both high viability and germination rate in controlled conditions, a process that will contribute to species propagation and reintroduction for restoration programs. Besides, in order to define seed desiccation tolerance, it would be useful to evaluate variables to foreseen seed physiological and evolutionary responses; variables that may vary at species, genus, family or ecosystem level. For this reason, it is recommended to continue evaluating desiccation tolerance of different species from various Páramo environments to find probabilistic tendencies and models that allow us to rapidly determine desiccation tolerance and to become a tool for decision making process for ex-situ conservation.

AUTHOR´S CONTRIBUTION

MCH and LVPM participate in all steps of the project.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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