GAYANA BOTANICA Gayana Bot. (2024) vol. 81, No. 1, 22-31

ORIGINAL ARTICLE

Reproductive variation and germination capacity of *Tillandsia landbeckii* Phil. in the southernmost *Tillandsia* dune of the Atacama Desert

Variación reproductiva y capacidad de germinación de *Tillandsia landbeckii* Phil. en la duna más meridional de *Tillandsia* en el Desierto de Atacama

Andrea P. Loayza[1,2,*], Claudia Bavestrello-Riquelme[3], Rodrigo S. Rios[1] & Pedro León-Lobos[4]

[1]Instituto Multidisciplinario de Investigación y Postgrado, Universidad de La Serena, 1720256 La Serena, Chile.
[2]Instituto de Ecología y Biodiversidad (IEB), 7800003 Santiago, Chile.
[3]Laboratorio de Biotecnología de Plantas, Centro de Investigación Intihuasi, Instituto de Investigaciones Agropecuarias INIA, Colina San Joaquín s/n, La Serena 1700000, Chile.
[4]Grupo de Especialidad Recursos Genéticos, Centro de Investigación Regional La Platina, Instituto de Investigaciones Agropecuarias, Avenida Santa Rosa 11610, La Pintana, Santiago 8831314, Chile.
*Corresponding author: aloayza@userena.cl

ABSTRACT

Tillandsia landbeckii Phil. is a rootless epiphyte that grows on dunes of the Atacama Desert. There is little information on its natural history, particularly concerning its reproductive ability. To better understand whether sexual reproduction can contribute to recruitment in *T. landbeckii*, we examined its basic reproductive traits and developed a germination protocol. We marked and censused plants, as well as collected seeds from four localities along an inland-coast gradient of the southernmost *Tillandsia* dune in Chile. Seeds were tested for viability and used for germination trials using four culture mediums. Plants vary in size, capsule size and number of seeds per capsule along the gradient, with larger plants occurring inland. Both the probability of flowering and of producing capsules increased with plant size. However, seed viability was low and did not differ across localities. Germination trials were successful, but also yielded relatively low germination probabilities. In summary, our results revealed plant size-related variation in *T. landbeckii*'s reproductive capacity. In spite of this variation, however, low seed viability supports previous findings that sexual reproduction scarcely contributes to recruitment in this population; hence propagation of this species may eventually be necessary to promote genetic diversity.

Keywords: Bromeliaceae, epiphyte, germination protocol, fecundity, sand dunes.

RESUMEN

Tillandsia landbeckii Phil. es una epífita sin raíces que crece en las dunas del Desierto de Atacama. Existe poca información sobre su historia natural, especialmente en lo que respecta a su capacidad reproductiva. Para comprender mejor si la reproducción sexual puede contribuir al reclutamiento en *T. landbeckii*, examinamos algunos rasgos reproductivos básicos y desarrollamos un protocolo de germinación. Marcamos y censamos las plantas, además de recolectar semillas de cuatro localidades a lo largo de un gradiente de costa hacia el interior en la duna más meridional de *Tillandsia* en Chile. Determinamos la viabilidad de las semillas y realizamos ensayos de germinación con cuatro medios de cultivo diferentes. Las plantas variaron en tamaño, tamaño de la cápsula y número de semillas por cápsula a lo largo del gradiente, siendo las plantas más lejos de la costa, las más grandes. Tanto la probabilidad de floración como la de producción de cápsulas aumentaron con el tamaño de la planta. Sin embargo, la viabilidad de las semillas fue baja y no difirió entre las localidades. Los ensayos de germinación fueron exitosos, pero en general las probabilidades de germinación fueron relativamente bajas. En resumen, nuestros resultados revelaron una variación relacionada con el tamaño de la planta en la capacidad reproductiva de *T. landbeckii*. A pesar de esta variación, la baja viabilidad de las semillas respalda hallazgos previos que indican que la reproducción sexual apenas contribuye al reclutamiento en esta población; por lo tanto, eventualmente podría ser necesario propagar esta especie para promover su diversidad genética.

Palabras clave: Bromeliaceae, dunas, epífita, fecundidad, protocolo de germinación.

INTRODUCTION

With approximately 730 species, the genus *Tillandsia* is the most species-rich genus of the Bromeliaceae (WFO Plant List 2023). Most of the species in this group are epiphytic, obtaining water and nutrients from fog (Zotz 2013). One such species, *Tillandsia landbeckii* Phil., is a functionally rootless plant that forms monospecific stands known as *Tillandsia* dunes or "tillandsiales" in the hyper-arid Atacama Desert of northern Chile (González *et al.* 2011; Latorre *et al.* 2011; Westbeld *et al.* 2009). Atmospheric fog plays a vital role in controlling the growth of *T. landbeckii* plants (González *et al.* 2011; Latorre *et al.* 2011), which are able to survive in this environment through a series of specialized adaptations including closely knitted, aciculate leaves covered with highly specialized trichomes, and a Crassulacean Acid Metabolism (Belmonte *et al.* 2022; Benzing & Bennett 2000; Raux *et al.* 2020; Rundel *et al.* 1997). However, the aridity of the Atacama Desert may restrict *T. landbeckii*'s reproductive ability, especially in terms of seed production and germination.

Little is known about the reproductive ability of *T. landbeckii*. Rundel (Rundel *et al.* 1997) proposed that this species may propagate asexually via ramets from a single or a few genets. While this ability enables *T. landbeckii* to colonize suitable dunes, it results in populations with low genetic variability, a phenomenon that has been reported at the edges of *T. landbeckii*'s distribution (Merklinger *et al.* 2020). Nevertheless, plants can produce flowers and seeds, the latter presumably via autogamy or cleistogamy (Till 1992a, 1992b), and there is evidence of sexual reproduction in the central populations (Merklinger *et al.* 2020). However, since average annual precipitation in the southern and northern limits of *T. landbeckii*´s distribution ranges from < 30 mm to < 1 mm, respectively, seeds rely solely on fog water for establishment; therefore, it is unlikely that natural germination is a prevalent occurrence.

To date, there are very few studies on *T. landbeckii* and little is known about its ecology (Alfaro *et al.* 2021; Contreras *et al.* 2022). Furthermore, a recent editorial (Koch *et al.* 2022) stressed the lack of biological data available for this species. Currently, one of the major threats to the Tillandsiales of the Atacama Desert are changes in fog dynamics, which these ecosystems rely on. Such changes may have already affected the survival of many *Tillandsia* dunes (del Río *et al.* 2021; García *et al.* 2021). In this context, it becomes increasingly important to obtain relevant information on *T. landbeckii*, particularly with respect to reproductive aspects, as well as to develop protocols for the propagation and conservation of this species. So far, no attempts have been made to germinate *T. landbeckii* seeds, thus there is no information regarding its germination requirements, nor basic natural history data, such as natural levels of seed viability. Therefore, in this study, we set out to examine basic reproductive traits of *T. landbeckii*, including seed production, viability and seed number per capsule at four localities of the most southern *Tillandsia* dune in the Atacama Desert. Because genetic data has revealed that in this population plant reproduction is almost exclusively clonal (Merklinger *et al.* 2020), we expected that only a low percentage of plants in these localities would produce fruit capsules, and that levels of seed viability would be low across sites. Additionally, we develop a seed germination protocol for this species by comparing the efficiency of different culture media.

MATERIALS AND METHODS

SITE DESCRIPTION

We surveyed and collected seeds of *T. landbeckii* from four localities along an east-west gradient of the *Tillandsia* dune located at approximately 20 km inland from the town of Caldera in Chile. These localities, namely S1, S2, S3, and S4, are positioned from east to west and decrease in altitude from 750 to 360 m above sea level (Table 1). The mean annual temperature in this area is around 15.5 °C, and fog is the main source of water in this ecosystem. Other than *T. landbeckii*, only a few plant species are found in this system, these include *Skytanthus acutus* (Apocynaceae), *Atriplex deserticola* (Chenopodiaceae) and *Ephedra breana*. (Ephedraceae).

Plant characterization and seed collection

We established three 50x2 m plots at each of the four localities. In each plot, we counted and measured all *T. landbeckii* individuals, characterizing their size in terms of basal area by measuring the length of the longest and shortest axis for each plant. Most plants at our study site are distinct individuals with a cushion-like shape (Fig. 1), thus we calculated their basal area using the formula for the area of an ellipse. In some cases, however, cushions tended to bind forming small bands, making it difficult to distinguish individual plants. In such cases, we measured the longest and shortest axis of distinct plant bands, and considered them as a single individual.

Table 1. Site characteristics of the four localities where *T. landbeckii* plants were sampled / Características de las cuatro localidades donde se muestrearon las plantas de *T. landbeckii*.

Site	Distance from the shore (km)[1]	Elevation (m.a.s.l.)	Geographical Coordinates		Mean plant density[2]
			S	W	
S1	34	750	27°10´11”	70°29´30”	34.7
S2	29	628	27°10´49”	70°30´55”	47
S3	23	471	27°08´11”	70°35´51”	58.3
S4	17	360	27°05´49”	70°39´30”	38.7

[1] Euclidean distance.
[2] Number of *T. landbeckii* plants per 100 m^2 plot.

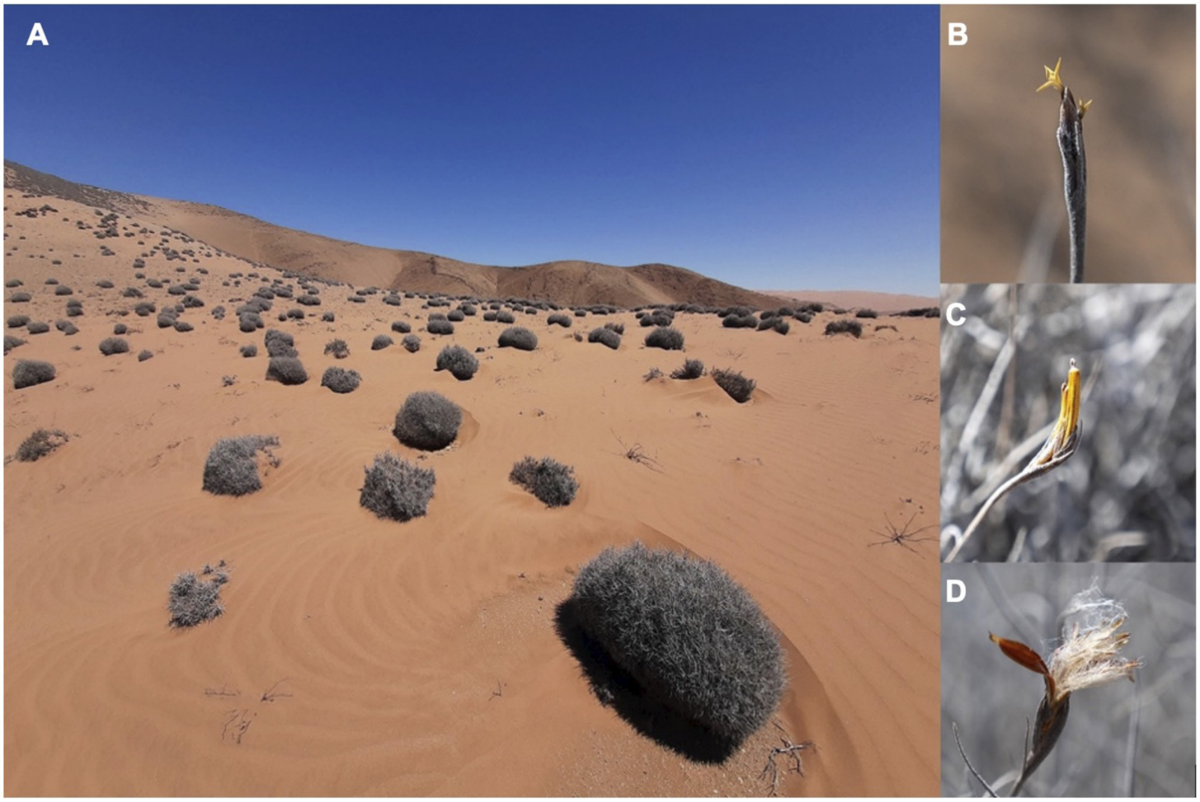

Figure 1. A. Plants of *T. landbeckii* at the study area. B. Open flower. C. Mature seed capsule before opening. D. Open capsule showing plumose seeds adapted to wind dispersal. / Plantas de *T. landbeckii* en el área de estudio. B. Flor abierta. C. Cápsula madura antes de abrirse. D. Cápsula abierta con las semillas plumosas adaptadas para la dispersión por el viento.

To determine whether plants were able to flower and produce seeds, we recorded the presence of flowers or flower buds and mature seed capsules for each individual during December 2020, January, February, April, and May 2021 (Fig. 1). We also collected mature capsules from all plants in the plots during the same period, to examine differences in capsule size and the number of seeds produced per capsule at each locality. The capsules were stored in small plastic containers (50 ml Falcon tubes) and preserved at 4 °C until they were processed at Universidad de La Serena. Here, 20 capsules per locality were randomly selected for measuring and seed counting. Additionally, we collected seeds from open capsules in the field, which were stored as described above and used for the seed germination trials.

Seed viability

To assess seed viability, we collected fresh seeds during April 2022 from all localities, except S4, where no plants had mature capsules. We randomly selected 100 intact seeds from each locality and placed them in a 1% tetrazolium chloride solution (2,3,5-tryphenyl tetrazolium chloride, TTC) for 48 h at 30 °C. Seed viability was evaluated by examining seeds under a microscope. We used two different criteria to estimate viability: a conservative approach in which only completely stained seeds were classified as viable, and a more lenient approach in which partially stained seeds were also considered viable.

Germination trials

To conduct these experiments, we pooled seeds from all four localities. The seeds were sterilized by rinsing them in a Tween-20® solution for three minutes with continuous shaking, followed by three rinses with sterile distilled water (sdw). After that, the seeds were rinsed for three minutes (shaken gently for one minute and left still for two) with 70% ethanol. Next, they were rinsed three times with sdw, followed by a rinse with 1% chlorine, and then three final rinses with sdw. The seeds were then resuspended in 1 ml of sdw at 4 °C for 72 h before sowing.

We assessed the effect of four MS (Murashige and Skoog) culture media (Table 2) on seed germination. In the first trial, we prepared seven 60 mm × 15 mm Petri plates with each of the four media (N=7), sowed eight seeds per plate (N=56 seeds/medium), and sealed each plate with Parafilm M™. Based on the results from the first trial, we repeated the essay only with MS Activated Carbon, sowing eight seeds in each of 50 Petri plates (N=400 seeds). Both trials were conducted in a laminar flow hood with appropriate laboratory sanitation practices, and seed sowing was performed in a growth chamber at 21 °C with a 16/8h photoperiod. Seeds were monitored every two days until germination ceased; this process took 60 days in the first experiment and 30 days in the second. We defined germination as the point at which the seeds had swelled and started to emerge.

Table 2. Culture media used for *T. landbeckii* germination experiments / Medios de cultivo utilizados para los experimentos de germinación de *T. landbeckii*.

Reagents (for 500 ml)	MS 1	MS 2	MS 3	MS Activated Carbon
Murashige & Skoog (MS 519) with vitamins	2.2 g	2.2 g	1.1	2.2 g
Saccharose	15 g	15 g	5 g	15 g
6-Benzylaminopurine BAP (Citokyne)	500 ul	n/a	n/a	500 ul
Indole-3-butyric acid IBA (Auxine)	n/a	500 ul	n/a	500 ul
SEM (secondary esblishment medium)	n/a	n/a	0.25 g	n/a
Casein hydrolysate	0.1 g	n/a	n/a	0.1 g
Ascorbic acid (0.05 g/ml)	25 ul	n/a	n/a	25 ul
Plant Preservative PPM	500 ul	n/a	n/a	500 ul
Activated carbon (antioxidant)	n/a	n/a	n/a	0.5 g
Agar Agar	4 g	4 g	4 g	4 g

Statistical analyses

We first assessed whether the proportion of fruiting plants (i.e., with capsules) differed among localities with a contingency test. Second, we examined if plant size differed among the four sampled localities using linear model with log-transformed values of plant area to meet normality requirements. Differences among groups were examined using estimated marginal means. Third, we determined whether the probability of flowering and fruiting varied with plant size using a mixed effects logistic regression model, in which the binary response variables were, whether a plant produced (1) flowers (yes/no) and (2) mature seed capsules (yes/no). The independent variable was plant size, and each locality was considered as a random factor in the model. We examined whether the size of mature capsules and the number of seeds per capsule varied among localities using a generalized linear model (GLM) with a Gaussian and Poisson distribution, respectively. In both cases, differences among groups were evaluated through estimated marginal means. Finally, we used a contingency test to determine if there were any differences in the proportion of viable seeds among the localities.

To assess the effect of the different culture media on the probability of germination, we conducted a Cox Proportional hazards model nested within each petri dish. Here, the dependent variable is the hazard function, which describes how the hazard (in this case, the "risk" of a seed germinating) changes over time, and the effect parameter describes how this hazard is related to the medium culture. For these analyses, all comparisons were made against germination rate in MS3 because it is the simplest medium (see Table 2). To examine how the germination probability of *T. landbeckii* changes with time in the MS Activated Carbon during the second trial, we used an accelerated failure time model. All statistical analyses were performed using the R statistical environment (R Core Team 2022).

RESULTS

Seed production and viability

We marked a total of 536 plants across all four localities (S1=104, S2=143, S3=175, S4=116). The proportion of plants with capsules differed among localities (χ^2=66.95, df = 3, P < 0.001), but it was overall low, with the highest percentages of fruiting plants in S3 and S1 (29.1% and 12.5%, respectively), followed by S2 (4.1%). No plants in S4 produced capsules.

Mean plant size differed among localities ($F_{3,532}$=19.61, P<0.0001); plants from the site nearest to the coast (S4) were smaller than those from the other localities (Fig. 2A). Both the probability of flowering and producing mature seed capsules increased with plant size (Figs. 2B, 2C). Specifically, a one unit increase in plant area was associated with a 3.43 and 1.44 unit increase in the expected log odds of producing flowers and mature seed capsules, respectively (Table 3).

Table 3. Mixed effects logistic regression for flower and mature capsule status (1=present, 0=absent) of *T. landbeckii* plants based on their size (area [m^2]). / Regresión logística de efectos mixtos para los estados de flor y cápsula madura (1= presente, 0= ausente) de las plantas de *T. landbeckii* en función de su tamaño (área [m2]).

Fixed effects				
Flower				
Intercept	-0.6458	0.4547	0.56	0.52 (0.22-1.28)
Size	3.4347	0.4667	<0.001	31.02 (12.43-77.43)
Mature seed pod				
Intercept	-4.4805	0.8997	<0.001	0.01 (0.002-0.027)
Size	1.4365	0.5284	<0.001	4.21 (1.49-10.50)
Radom effects (Locality)				
Flower			**Mature seed pod**	
Variance	0.7402		Variance	2.025
Std. Dev	0.8604		Std.Dev	1.423

[1] Odds ratio showing lower and upper CI in parenthesis.

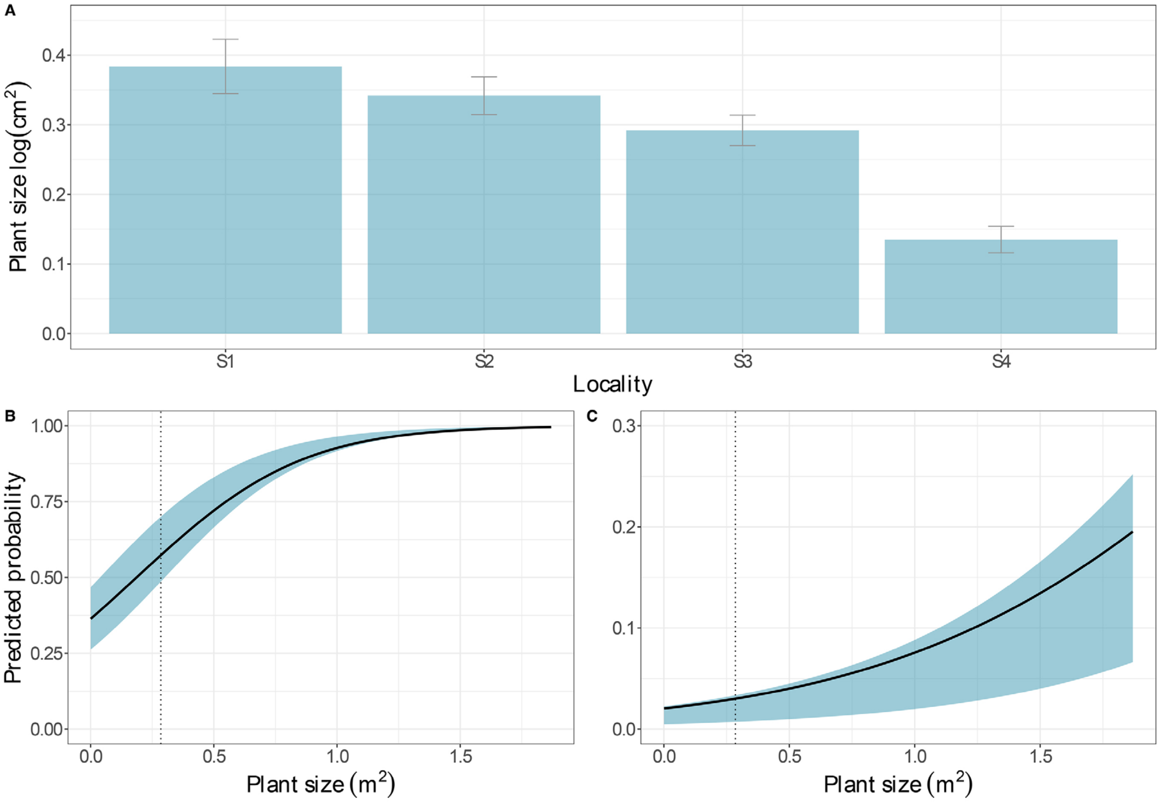

FIGURE 2. A. Bar plot showing differences in size distributions of *T. landbeckii* individuals at the four localities. Error bars represent ± 1 standard error and lowercase letters indicate differences among groups based on the GLM model. B. Probability of producing flowers in relation to plant size using a mixed effects logistic regression model. C. Probability of producing mature seed capsules in relation to plant size using a mixed effects logistic regression model. The dashed lined shows mean plant area across localities. / A. Gráfico de barras mostrando las diferencias en las distribuciones de tamaños de individuos de *T. landbeckii* en las cuatro localidades. Las barras de error representan ± 1 error estándar y las letras en minúscula indican diferencias entre grupos en base al modelo de GLM. B. Probabilidad de producir flores en función al tamaño de la planta utilizando un modelo de regresión logística de efectos mixtos. C. Probabilidad de producir cápsulas maduras en función al tamaño de la planta utilizando un modelo de regresión logística de efectos mixtos. La línea punteada muestra el área promedio de las plantas en las diferentes localidades.

Capsule size differed among localities (Dev=1338, df=3,75, $P<0.001$); capsules from the site nearest to the coast (S4) were smaller than from all other localities, except S2 (Fig. 3A). The number of seeds per capsule ranged between 3-64. The mean number of seeds per capsule varied among all localities (Dev=183.29, df=3,75, $P<0.001$); with capsules from S4 and S3 having the fewest and highest number of seeds, respectively (Fig. 3B).

The proportion of viable seeds did not differ among localities, either when considering stained (χ^2=4.67, df = 2, P = 0.1) or partially stained (χ^2=4.00, df = 2, P = 0.13) seeds as viable. The mean percentage of viable seeds in the former scenario was 19.5% (± 8.43 sd), whereas in the latter, it increased to 42% (± 9.4 sd).

GERMINATION TRIALS

All germination media successfully triggered germination of *T. landbeckii* seeds (Fig. 4). On average, however, a higher percentage of seeds germinated on the MS4 and MS Activated Carbon mediums (44.9%±22.5 and 44.9%±19.2, respectively), than in the MS2 and MS3 mediums (30.6%±15.3 and MS4=34.7%±7.6, respectively). These differences translate into marginal differences in the probability of germination among treatments (χ^2_{Wald}=6.95, gl=3, P=0.07). Specifically, the probability of germination in the MS Activated Carbon and MS1 were 46% and 58% higher, respectively compared to the reference medium (MS3) (Fig. 4). There were no differences in the probability of germination between MS3 and MS2. In the MS Activated Carbon, *T. landbeckii* seeds began germinating on day 5, and by day 29, the probability of germination was 0.29 (95% CI, 0.333-0.244).

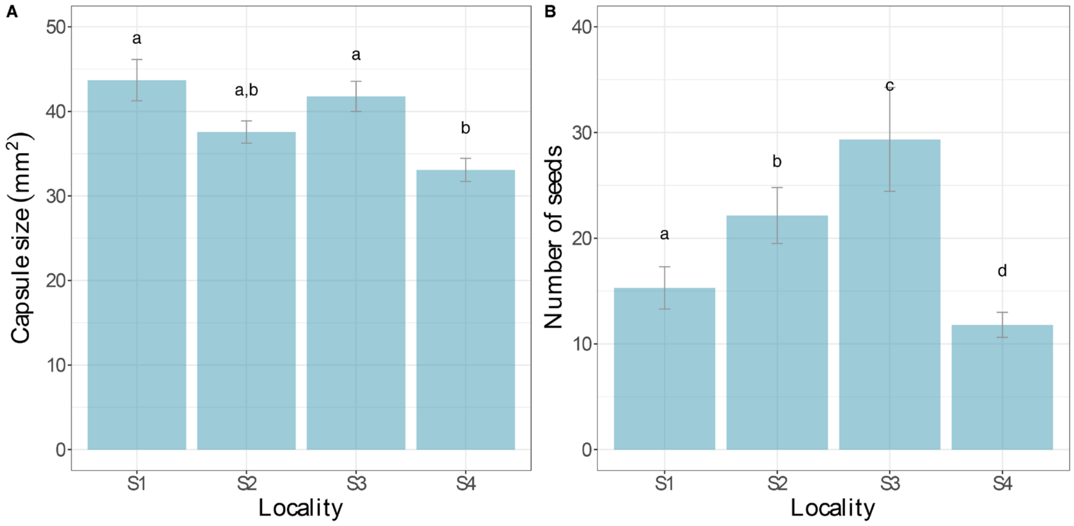

Figure 3. A. Capsule size of *T. landbeckii* individuals at the four localities sampled. B. Mean number of seeds in capsules from different localities. Error bars represent ± 1 standard error and lowercase letters indicate differences among groups based on GLM models. / A. Tamaño de la cápsula de individuos de *T. landbeckii* en las cuatro localidades. Las barras de error representan ± 1 error estándar y las letras en minúscula indican diferencias entre grupos basadas en los modelos de GLM.

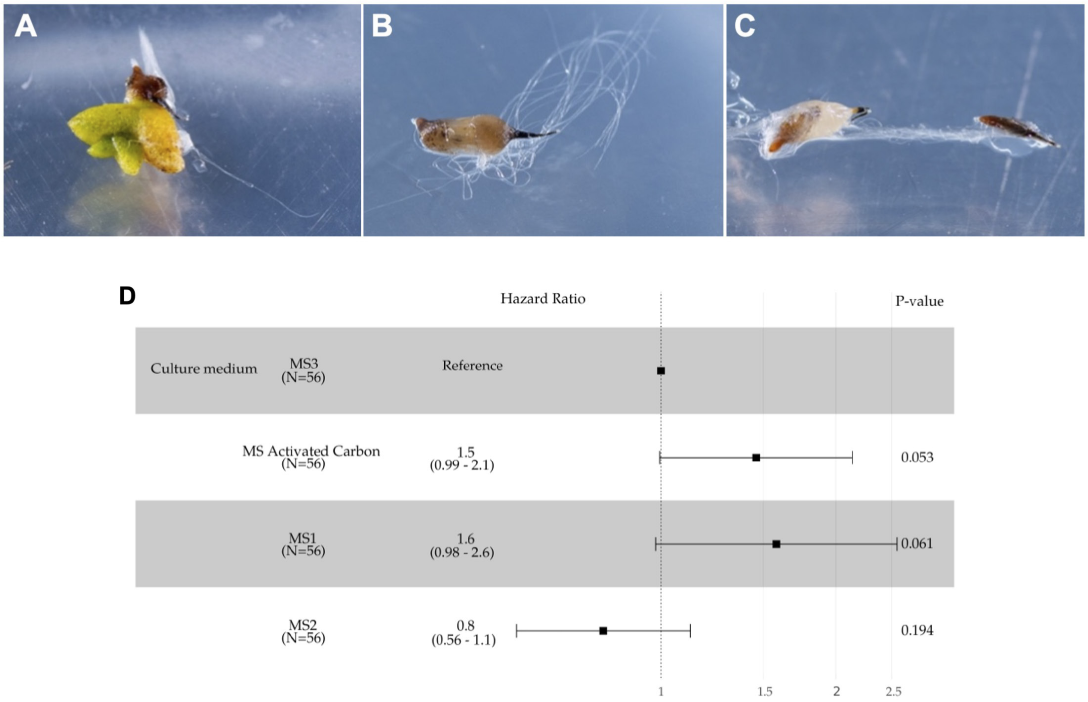

Figure 4. A. Early *T. landbeckii* seedling. B. Swollen seed in the process of germinating. C. Comparison of a germinating (left) and a non-germinating seed (right). D. Forest plot of the Cox proportional hazards regressions of germination clustered by plate replicate. The plot shows seed germination probabilities (hazard ratio, HR) and 95 % CI from seeds in each culture medium compared to those in MS3. The germination probability of a seed in MS3 is standardized to 1 and denoted by the dashed vertical line. An HR > 1 indicates an increased germination probability, whereas an HR < 1 indicates a decreased probability. / A. Plántula temprana de *T. landbeckii*. B. Semilla hinchada en proceso de germinación. C. Comparación de una semilla en proceso de germinación (izquierda) y una semilla no germinante (derecha). D. Gráfico de sobrevivencia de las regresiones de riesgos proporcionales de Cox de la germinación agrupada por placa. El gráfico muestra las probabilidades de germinación de las semillas (función de riesgo, RR) y un IC del 95 % en comparación con las semillas en medio de cultivo MS3. La probabilidad de germinación de una semilla en MS3 se estandarizó a 1 y se indica con la línea vertical punteada. Un RR > 1 indica una mayor probabilidad de germinación, mientras que un RR < 1 indica una menor probabilidad.

DISCUSSION

Our findings reveal that along an east-west gradient of the southernmost *Tillandsia* dune of the Atacama Desert, *T. landbeckii* plants exhibit variation in size, capsule size, and the number of seeds per capsule. In addition, we observed that both the likelihood of flowering and of producing mature capsules increased with plant size. However, despite these dissimilarities, overall seed viability was low and did not differ among localities. Germination experiments, while effective, yielded relatively low germination rates (<50%), confirming the low seed viability detected in the seeds.

Plants decreased in size, had smaller capsules and fewer seeds per capsule with proximity to the shore and decreasing elevation. *T. landbeckii* primarily relies on fog for nutrient and water supply (Westbeld *et al.* 2009). Therefore, its distribution is influenced by the spatial dynamics of the fog mantle (Borthagaray Peradotto & Marquet 2010; García *et al.* 2021; Latorre *et al.* 2011), which typically occurs along the coast between 600 and 1000 m a.s.l. (Cereceda *et al.* 2008; Latorre *et al.* 2011). Although in this study, we did not quantify fog water input in our sampled localities, *T. landbeckii* plants closer to the shore likely receive less moisture than those further East, which inevitably results in less water and nutrient input to the former. Given that atmospheric nutrient supply is a significant driver of plant growth in *T. landbeckii* (González *et al.* 2011), lower nutrient input nearer to the shore is expected to result in slower growth and smaller plant size. Empirical evidence regarding the relationship between nutrient limitation and growth, was reported by Hernández and collaborators (1999) for *Tillandsia guatemalensis*. These authors found that the growth rate of this epiphytic bromeliad increased in response to NPK fertilization. Similarly, as in other vascular epiphytes (Laube & Zotz 2003; Zotz & Richter 2006), nutrient input may also determine reproductive output in *T. landbeckii*. In particular, it has been suggested that P is the most limiting macronutrient for reproduction in vascular epiphytes (Benzing 2008; Zotz & Richter 2006). Accordingly, P enriched plants of *Werauhia sintenisii*, an ephiphytic bromeliad of the subfamily Tillandsioideae, produced more seeds per fruit and per plant, and had a higher frequency of flowering in two consecutive years than controls, suggesting that fertilized plants overcome the cost of reproduction more readily than non-fertilized plants (Lasso & Ackerman 2013). Hence, the observed pattern of larger capsule size, and higher number of seeds per capsule in *T. landbeckii* with increasing altitude and distance to the shore, may be partly be explained by differences in P fluxes from atmospheric fog (González *et al.* 2011). Future studies, should examine how *T. landbeckii* vital rates change along fog gradients, and test whether P limits its reproduction in the field along the gradient.

We found a positive relationship between plant size in *T. landbeckii* and both the probability of flowering and the probability of producing mature seed capsules. While reproductive size thresholds have been reported for some long-lived semelparous bromeliads (Veldhuisen *et al.* 2022), there is limited information available for the Bromeliaceae in general. Although further research is required to comprehend the underlying mechanisms behind these patterns, several ecophysiological parameters (such as photosynthetic capacity and stomatal regulation) related to carbon gain in epiphytes (Schmidt *et al.* 2001; Schmidt & Zotz 2001; Zotz *et al.* 2001), as well as to nutrient allocation towards reproduction (Zotz 2000), have been reported to be size-dependent. Therefore, it is possible that larger *T. landbeckii* plants have more resources for reproduction. Moreover, as *T. landbeckii* obtains all of its nutrients and moisture from its specialized leaves, it follows that larger individuals should have higher acquisition rates than smaller ones. However, detailed studies are necessary to unravel the effects of size-related variation on reproduction and performance of *T. landbeckii*.

Tillandsia landbeckii is an epiphytic plant that predominantly reproduces asexually along its distribution range in Chile (Flores-Palacios *et al.* 2015). This mode of reproduction is also prevalent in the area where this study was conducted (Merklinger *et al.* 2020). This pattern suggests that seeds do not contribute significantly to population recruitment. However, it is unclear whether this is due to extrinsic (e.g., water availability) or intrinsic (e.g., seed production or viability) factors. Our conservative estimates of seed viability indicate that less than 20% of *T. landbeckii* seeds in this population are viable, suggesting that seed viability can strongly limit recruitment. Data on seed viability are only available for a few *Tillandsia* species (Chilpa-Galván *et al.* 2018; García-Suarez *et al.* 2006; Martelo-Solorzano *et al.* 2022; Sosa-Luría *et al.* 2012) and these estimates are highly variable, ranging from *ca.* 100% to <40%. Low seed viability of some species of *Tillandsia* has been attributed to either incomplete embryo formation or damaged embryos (García-Suarez *et al.* 2006; Sosa-Luría *et al.* 2012). The causes for this remained unexplored, but may be related to environmental conditions. If so, we would expect variation in seed viability across sites and years. This hypothesis is partly supported by the fact that viability of *T. landbeckii* seeds collected from the area in 2016 was close to 60% (P. León-Lobos, unpublished data).

The germination of *T. landbeckii* was higher in media that included BAP, casein hydrolysate and ascorbic acid.

BAP is known to provide additives that enhance cell division and growth, and bromeliad seeds germinated in media supplemented with cytokinins produce more shoots from each embryo (da Silva *et al.* 2009; Fischer & Zimmer 1988). Similarly, casein hydrolysate has been reported to be effective for seed germination of some orchids (Li *et al.* 2018). A shortcoming of our study, is that we did not produce seedlings for transplants following germination. Consequently, it will be necessary in future studies to develop protocols that promote seedling survival and growth, as well as efficient transplant protocols. Such protocols can help the propagation of *T. landbeckii*, which may become particularly important considering the decreasing presence of fog across many *Tillandsia* dunes in Chile, and its effects on the long term survival of these ecosystems (del Río *et al.* 2021).

CONCLUSIONS

Here we present evidence of size-related variation in *T. landbeckii*´s reproductive capacity, as well as a geographic pattern in size variation. Larger plants, which are more common inland, exhibit a higher probability of producing mature capsules and producing more seeds per capsule. However, despite this variation, seed viability is uniformly low, suggesting sexual reproduction is unlikely to contribute significantly to recruitment in this population. These findings, combined with previous genetic research, suggest that in vitro propagation of this species may eventually be necessary to promote genetic diversity.

ACKNOWLEDGMENTS

The authors would like to thank Gustavo Rios and Alvaro Lever for help during fieldwork. Photographs of *T. landbeckii* in the field were taken by Alvaro Lever. Funding: This research was funded by a research grant from Compañía Minera del Pacífico. Data Availability Statement: Data will be made available in a repository. Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Alfaro, F.D., Manzano, M., Almiray, C., García, J.-L., Osses, P., del Rio, C., Vargas, C., Latorre, C., Koch, M.A., Siegmund, A. 2021. Soil bacterial community structure of fog-dependent *Tillandsia landbeckii* dunes in the Atacama Desert. Plant Systematics and Evolution 307(5): 1-11.

Belmonte, E., Arriaza, B., Arismendi, M., Sepúlveda, G. 2022. Foliar anatomy of three native species of *Tillandsia* L. from the Atacama Desert, Chile. Plants 11(7): 870.

Benzing, D.H. 2008. Vascular epiphytes: general biology and related biota. Cambridge University Press.

Benzing, D.H., Bennett, B. 2000. Bromeliaceae: profile of an adaptive radiation. Cambridge University Press.

Borthagaray, A.I., Fuentes, M.A., Marquet, P.A. 2010. Vegetation pattern formation in a fog-dependent ecosystem. Journal of Theoretical Biology 265(1): 18-26.

Castro Hernández, J., Wolf, J.D., García-Franco, J.G., González-Espinosa, M. 1999. The influence of humidity, nutrients and light on the establishment of the epiphytic bromeliad *Tillandsia guatemalensis* in the highlands of Chiapas, Mexico. Revista de Biología Tropical 47(4): 763-773.

Cereceda, P., Larrain, H., Osses, P., Farías, M., Egaña, I. (2008). The spatial and temporal variability of fog and its relation to fog oases in the Atacama Desert, Chile. Atmospheric Research 87(3-4): 312-323.

Chilpa-Galván, N., Márquez-Guzmán, J., Zotz, G., Echevarría-Machado, I., Andrade, J. L., Espadas-Manrique, C., Reyes-García, C. 2018. Seed traits favouring dispersal and establishment of six epiphytic *Tillandsia* (Bromeliaceae) species. Seed Science Research 28(4): 349-359.

Contreras, S., Landahur, M., García, K., Latorre, C., Reyers, M., Rethemeyer, J., Jaeschke, A. 2022. Leaf wax composition and distribution of *Tillandsia landbeckii* reflects moisture gradient across the hyperarid Atacama Desert. Plant Systematics and Evolution 308(1): 1-13.

da Silva, A.L.L., Franco, E.T.H., Dornelles, E.B., Bortoli, C.L.R., Quoirin, M. 2009. In vitro multiplication of *Vriesea scalaris* E. Morren (Bromeliaceae). Iheringia, Série Botânica 64(2): 151-155.

del Río, C., Lobos-Roco, F., Latorre, C., Koch, M.A., García, J.-L., Osses, P., Lambert, F., Alfaro, F., Siegmund, A. 2021. Spatial distribution and interannual variability of coastal fog and low clouds cover in the hyperarid Atacama Desert and implications for past and present *Tillandsia landbeckii* ecosystems. Plant Systematics and Evolution 307(5): 1-23.

Fischer, G., Zimmer, K. 1988. Regeneration of germinating seeds in vitro. International Symposium on Propagation of Ornamental Plants 226, 615-618.

Flores-Palacios, A., Bustamante-Molina, A.B., Corona-López, A.M., Valencia-Díaz, S. 2015. Seed number, germination and longevity in wild dry forest *Tillandsia* species of horticultural value. Scientia Horticulturae 187: 72-79.

García, J.-L., Lobos-Roco, F., Schween, J.H., del Río, C., Osses, P., Vives, R., Pezoa, M., Siegmund, A., Latorre, C., Alfaro, F. 2021. Climate and coastal low-cloud dynamic in the hyperarid Atacama fog Desert and the geographic distribution of *Tillandsia landbeckii* (Bromeliaceae) dune ecosystems. Plant Systematics and Evolution 307(5): 1-22.

García-Suarez, M.D., Rico-Gray, V., Molina-Aceves, N., Serrano, H. 2006. In-vitro germination and clonal propagation of endemic *Tillandsia califanii* Rauh (Bromeliaceae) from Mexico. Selbyana 27(1): 54-59.

González, A.L., Fariña, J.M., Pinto, R., Pérez, C., Weathers, K.C., Armesto, J.J., Marquet, P. A. 2011. Bromeliad growth and stoichiometry: responses to atmospheric nutrient supply in fog-dependent ecosystems of the hyper-arid Atacama Desert, Chile. Oecologia 167(3): 835-845.

Koch, M.A., Quandt, D., Siegmund, A. 2022. Living at its dry limits: Tillandsiales in the Atacama Desert. Plant Systematics and Evolution 308(2): 11.

Lasso, E., Ackerman, J.D. 2013. Nutrient limitation restricts growth and reproductive output in a tropical montane cloud forest bromeliad: findings from a long-term forest fertilization experiment. Oecologia 171(1): 165-174.

Latorre, C., González, A.L., Quade, J., Fariña, J.M., Pinto, R., Marquet, P.A. 2011. Establishment and formation of fog-dependent *Tillandsia landbeckii* dunes in the Atacama Desert: Evidence from radiocarbon and stable isotopes. Journal of Geophysical Research 116: G03033.

Laube, S., Zotz, G. 2003. Which abiotic factors limit vegetative growth in a vascular epiphyte? Functional Ecology 17(5): 598-604.

Li, Y.-Y., Chan, C., Stahl, C., Yeung, E.C. 2018. Recent advances in orchid seed germination and micropropagation. In: Lee, YI., Yeung, ET. (Eds.) Orchid Propagation: From Laboratories to Greenhouses—Methods and Protocols, pp. 497-520. Springer Protocols Handbooks. Humana Press, New York, NY.

Martelo-Solorzano, A.M., Lidueña-Pérez, K.I., Corredor-Prado, J.P. 2022. Seed's morpho-anatomy and post-seminal development of Bromeliaceae from tropical dry forest. Rodriguésia 73: e02122020.

Merklinger, F., Zheng, Y., Luebert, F., Harpke, D., Böhnert, T., Stoll, A., Koch, M., Blattner, F., Wiehe, T., Quandt, D. 2020. Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. Global and Planetary Change 184: 103076.

WFO Plant List. 2023. Retrieved 10/10/2023 from https://wfoplantlist.org/taxon/wfo-4000038422-2023-12?page=1

Raux, P. S., Gravelle, S., Dumais, J. (2020). Design of a unidirectional water valve in Tillandsia. Nature Communications 11(1): 1-7.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Rundel, P.W., Palma, B., Dillon, M.O., Sharifi, M.R., Nilsen, E.T., Boonpragob, K. 1997. *Tillandsia landbeckii* in the coastal Atacama Desert of northern Chile. Revista Chilena de Historia Natural 70: 341-349.

Schmidt, G., Stuntz, S., Zotz, G. 2001. Plant size: an ignored parameter in epiphyte ecophysiology? In: Eduard Linsenmair, K., Davis, A.J., Fiala, B., Speight, M.R. (Eds.) Tropical Forest Canopies: Ecology and Management, pp. 65-72. Springer.

Schmidt, G., Zotz, G. 2001. Ecophysiological consequences of differences in plant size: in situ carbon gain and water relations of the epiphytic bromeliad, *Vriesea sanguinolenta*. Plant, Cell & Environment 24(1): 101-111.

Sosa-Luría, D., Chávez-Servia, J.L., Mondragón-Chaparro, D., Estrada-Gómez, J.A., Ramírez-Vallejo, P. 2012. Viabilidad y germinación de semillas de seis especies de *Tillandsia* (Bromeliaceae) de Oaxaca, México. Revista Fitotecnia Mexicana 35(SPE5): 37-42.

Till, W. 1992a. Die Untergattung Diaphoranthema von Tillandsia. 4, Teil: Das *Tillandsia recurvata* Aggregat. Die Bromelie 1(92): 15-20.

Till, W. 1992b. Systematics and evolution of the tropical-subtropical *Tillandsia* subgenus Diaphoranthema (Bromeliaceae). Selbyana: 88-94.

Veldhuisen, L., Lino, M.C.G., Bodine, E., Aguirre-Santoro, J., Jabaily, R. 2022. Description of life history and reproductive size thresholds in three high elevation *Puya* (Bromeliaceae). bioRxiv: 2022.02.18.480924 https://doi.org/10.1101/2022.02.18.480924

Westbeld, A., Klemm, O., Grießbaum, F., Sträter, E., Larrain, H., Osses, P., Cereceda, P. 2009. Fog deposition to a *Tillandsia* carpet in the Atacama Desert. Annales Geophysicae 27: 3571-3576.

Zotz, G. 2000. Size dependence in the reproductive allocation of Dimerandra emarginata, an epiphytic orchid. Ecotropica 6(1): 95-98.

Zotz, G. 2013. The systematic distribution of vascular epiphytes–a critical update. Botanical Journal of the Linnean Society 171(3): 453-481.

Zotz, G., Hietz, P., Schmidt, G. 2001. Small plants, large plants: the importance of plant size for the physiological ecology of vascular epiphytes. Journal of Experimental Botany 52(363): 2051-2056.

Zotz, G., Richter, A. 2006. Changes in carbohydrate and nutrient contents throughout a reproductive cycle indicate that phosphorus is a limiting nutrient in the epiphytic bromeliad, *Werauhia sanguinolenta*. Annals of Botany 97(5): 745-754.

Received: 09.09.23
Accepted: 22.11.23

Editor: Alfredo Saldaña