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Morphological characterization of *Tillandsia recurvata* seeds (Poales: *Bromeliaceae*

Caracterización morfológica de semillas de *Tillandsia recurvata* (L.) L. (Poales: Bromeliaceae)

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ABSTRACT

Tillandsia recurvata (L.) L., an epiphyte with wide distribution, is commonly considered a parasitic plant. It possesses both ecological and utilitarian relevance; recently, medicinal properties against cancer and diabetes have been attributed to it, which has spurred interest in its propagation. In this context, the present study describes the seed's morphological characteristics and evaluates the effect of seed size on viability. The predominant color identified was N199 Grey-Brown C (59 %) in the seed and NN155 White Group C (60 %) in the coma (seed hairs), according to the Royal Horticultural Society colour charts. The average length was 2.96 mm, with a width of 0.27 mm and a seed area of 0.59 mm^2. The coma, weighing 1.02 mg, represents the seed's largest mass. Viability (tetrazolium test) was 27.8 %, and eight pigmentation patterns were distinguished. Germination reached only 10.8 %, with no statistically significant differences found between the responses to different seed sizes. The germinative process was interrupted at stage two-three without seedling formation. It is concluded that the comprehensive morphological characterization carried out constitutes a critical step for the propagation and conservation programs of this species with medicinal potential.

KEY WORDS: Conservation, endosperm, viability, ball moss, medicinal.

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RESUMEN

Tillandsia recurvata (L.) L., epífita de amplia distribución, suele ser considerada planta parásita. Posee relevancia tanto ecológica como utilitaria; recientemente, se le han atribuido propiedades medicinales contra el cáncer y la diabetes, lo cual ha impulsado el interés por su propagación. En este contexto el presente estudio describe en las características morfológicas de la semilla y evalúa el efecto de su tamaño en la viabilidad. Se identificó el color predominante N199 Grey-Brown C (59 %) en la semilla y NN155 White Group C (60 %) en la coma, con las cartas Royal Horticultural Society. La longitud promedio fue de 2.96 mm, con un ancho de 0.27 mm y 0.59 mm de área de semilla. La coma con 1.02 mg representa la mayor masa de la semilla. La viabilidad (tetrazolio) fue del 27.8 %, y se distinguieron ocho patrones de pigmentación. La germinación fue de sólo 10.8 %, sin encontrar diferencias estadísticamente significativas entre las respuestas a diferentes tamaños de semillas. El proceso germinativo se interrumpió en etapa dos-tres sin formación de plántulas. Se concluye que la caracterización morfológica integral llevada a cabo constituye un paso crítico para los programas de propagación y conservación de esta especie con potencial medicinal.

PALABRAS CLAVE: Conservación, endospermo, viabilidad, heno motita, medicinal.

Introduction

The great richness of forests is contributed to by epiphytic species, whose importance can be observed from ecological, faunistic, ethnobotanical, and even pharmacological perspectives (Lucena et al., 2013; Lowe et al., 2020; García-Campoy et al., 2024). Some members of the Bromeliaceae family belong to this group, and some also tolerate severe drought, resist frost, and are distributed across various habitats. These plants exhibit significant variation in size, as well as the time required to reach the reproductive stage (Benzing, 2000); while some species complete their cycle in a year or two, others are reported to take nearly 100 years (Huaman-Arqque et al.,2022). *Tillandsia recurvata* (L.) L. is an epiphytic plant commonly known as *heno motita*, *gallito*, *paxtle*, *roseta*, among other names (Flores et al., 2011; Pérez, 2021). It is distributed from the southern United States to Argentina (Véliz, 2010; Pérez, 2021), adapting to various stressors such as solar radiation, water availability, and nutrient scarcity, and even thriving on power lines (Benzing, 2023).

Its roots are short-lived and lack the anatomical structures needed to retain absorbed water. Water uptake is performed by specialized trichomes that cover the entire leaf surface

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(Lucena et al., 2013). Its flowers are autogamous and self-pollinate; they do not secrete nectar, do not emit fragrance, and do not possess brightly pigmented appendages (Benzing, 2023). According to the description by Martelo-Solorzano et al. (2022), the seeds are narrowly fusiform with a mature brown testa. They present plumose appendages (the coma), formed by numerous whitish filiform hairs, and these appendages create a structure similar to a double parachute. This aids in dispersal (Benzing, 2000), as this adaptation increases the surface-to-volume ratio, reducing the rate of fall. These seeds are reported to have the lowest amount of endosperm and embryos with starch reserves and vascular bundles, which makes them different from most other *Tillandsia* species (Martelo-Solorzano et al., 2022). According to Fernández et al. (1989), the seeds rapidly lose their viability due to the low accumulated reserves. Other authors suggest that the absence of endosperm reserves could be an adaptation to the ephemeral availability of water (necessarily liquid water), allowing them to germinate faster and require less time for seedling establishment (Montes-Recinas et al., 2012). In contrast, others only report imbibition without seedling formation (Martelo-Solozano et al., 2022). Another phenomenon mentioned in the species is vivipary—germination within the fruit while it is still attached to the plant (Pérez-Noyola et al., 2020). Furthermore, when studying the possible relationship between the species' presence and the death of some phorophytes (host trees), the presence of fungi from the genera *Fusarium*, *Alternaria*, *Pestalotia*, *Phoma*, *Aspergillus*, and *Bipolaris* was identified (Amaro, 2017), these fungi could potentially influence the seeds during the germination process, thereby diminishing the number of individuals.

This plant stores rainwater and also intercepts water from the fog generated by evaporation (Guevara-Escobar et al., 2011), y his capability makes it so prolific that it is even considered opportunistic (a weed) due to its ecoflexibility (Benzing, 2023), however, this does not guarantee its stability in the environment, chemically, this species contains terpenes, flavonoids, and cinnamic derivatives, and some isolated compounds illustrate the species' potential as a source of biomolecules of medicinal interest (Lucena et al., 2013; Lowe et al., 2020), which are consequently extracted from the wild; among its other current uses, it is notable as an ornamental plant, a bioindicator for assessing air quality, as fodder for feeding caprines, and as an alternative for organic substrates, to mention a few (Flores et al., 2011; INIFAP, 2018; Mejía, 2017; De La Mora, 2020; Lowe et al., 2020), the medical field has identified significant potential in this species as an anticancer, anti-tumor, anti-angiogenic, antidiabetic, antimicrobial, antispasmodic, antipyretic, anti-inflammatory, and wound-healing agent, among others, with formulas based on this species even reaching patent status (Lowe et al., 2012; Lowe et al., 2014; Lowe et al., 202; García-Campoy et al., 2024). Nonetheless, all these studies have been conducted using specimens extracted from the natural environment (wild-collected) rather than cultivated ones. Consequently, there is no clear evidence regarding the species' life cycle, given that, like many bromeliads, it reproduces both sexually and asexually, producing offsets (or 'pups') that can result in individuals resembling perennial specimens.

The characterization of agricultural varieties has traditionally been based on morphological descriptors, including seed shape, color, and size (Morales-Morales et al., 2019). Seed shape is determined by the type of ovule from which it was formed, as well as the growth pattern it followed and the position it occupies within the fruit (Soblechero et al., 2005). The position and

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characteristics of seeds can sometimes evidence or influence the germination percentage, hence, the objective of this study is to characterize the seeds of *T. recurvata* and observe whether any of these characteristics influence their viability or germination potential, thereby exploring potential methods for management, conservation, or further studies concerning this species.

Material and Methods

The seeds of *T. recurvata* were collected from the university campus in Chapingo, State of Mexico. Specimens were first randomly collected from various host plants (or phorophytes) such as cacti, colorines (*Erythrina americana*), ash trees (*Fraxinus spp.*), and privet (*Ligustrum spp.*). The collected specimens were mixed and maintained in a greenhouse while awaiting fruit dehiscence (or fruit rupture). Following the random dehiscence, the seeds were collected and stored at room temperature (20- 30 °C) in plastic containers until data collection. The variables to be determined were:

Color. The color of both the coma (plumose appendages) and the seed was determined. They were placed under a stereoscopic microscope, and the Royal Horticultural Society (RHS) Colour Charts were used for identification.

Coma length. The comas (plumose appendages) were manually twisted to facilitate photographic capture. Subsequently, the photographs were processed using ImageJ® software (Available at https://imagej.net/ij/).

Weight. Seed with Coma. The weight of 100 seeds was determined using an Adventurer Pro AV64C analytical balance (NJ, USA) with a precision of 0.0001g, with five replicates. From each of the five groups of 100 seeds, 10 seeds were randomly selected to form five separate groups of 10 seeds. Each of these 10-seed groups was weighed individually, and the data were recorded. Seed without Coma. Following the procedure above, the coma was carefully removed from the seeds to weigh the seed and the coma separately, both in the 100-seed and 10-seed groups.

Number of seeds in 1 mg. One milligram of seeds was weighed, and the corresponding number of seeds was quantified. This process was carried out with five replicates, from which the average was obtained.

Seed length, width, and area. Once the coma was removed from the seeds, photographic capture was repeated, and the images were processed using ImageJ® software.

Classification. For the variables coma length, seed length, seed width, and surface area, the data were grouped into class intervals using Sturges' rule to observe their distribution. The formula used was: $K=1+3.32*\log N$, Where: K= number of class intervals. N= number of simple elements. Log= Common logarithm base 10. Furthermore, because not all resulting class intervals had a sufficient number of seeds to perform the viability and germination tests, the data were

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further grouped into three size ranges: small (2 - 2.75 mm), medium (2.75 - 3.5 mm), and large (3.5 - 4.25 mm).

Viability test. Base bioassay. Initially, three different concentrations of triphenyltetrazolium chloride (0.2 %, 0.5 %, and 1 %) were applied to a sample of 30 seeds. These seeds were randomly selected, and each concentration group was placed in a Petri dish with 10 seeds. A preliminary soaking (or pre-soaking) was performed for 12 hours. Subsequently, the different tetrazolium concentrations were added, and the samples were evaluated at 12, 24, and 48 hours post-application to identify the most suitable concentration for the remaining classified seeds.

Viability assessment. Once the concentration yielding the best results was identified, the technique was applied to the seeds classified by length. Thirty seeds were chosen from each length classification group: small (2 - 2.75 mm), medium (2.75 - 3.5 mm), large (3.5 - 4.25 mm), each size group was thus composed of three replicates of 10 seeds each. The 0.2 % tetrazolium solution was added to these groups, these seeds were previously soaked for 12 hours, and evaluations following the tetrazolium application were conducted at 12, 24, and 48 hours. Data regarding the seed staining were recorded. It should be noted that the coma was removed from the seeds before subjecting them to the tetrazolium viability test, although they were whole seeds (no cuts were made), they were not subjected to temperatures above ambient conditions. The interpretation of the tetrazolium test to determine seed viability was performed according to the methodology proposed by Moreno (1984) for small grass seeds. For this purpose, a diagram was created from the obtained photographs, showing the different levels of seed staining. The viability percentage was determined for each treatment, and the data collected after 48 hours were used for statistical analysis.

Germination. Desinfection. The seeds were placed in filter paper envelopes, and the seeds were washed with 70 % alcohol (ethanol) for three minutes. Subsequently, they were rinsed twice with sterile water. Disinfection was then performed using 10 % commercial bleach for 20 minutes. Upon completion of this time, three rinses with sterile water were carried out.

Sowing. The seeds were placed in Petri dishes containing filter paper according to each treatment (length and coma presence/absence); these were maintained under a 16:8 (light:dark) photoperiod and kept at room temperature for their respective evaluations. Removing the coma was necessary to obtain the seed lengths (small, medium, and large). In the case of seeds *with* the coma, they were not categorized by size; they were only randomly selected.

Experimental design. A completely randomized experimental design was employed for both the tetrazolium viability and germination tests. For the viability test, there were three treatments (Small, Medium, and Large), with three replicates each (each experimental unit consisted of one Petri dish with 10 seeds). The evaluation was conducted by counting the seeds that exhibited staining. For the germination test, in addition to the three size treatments (Small, Medium, and Large), an additional treatment or control group (seed with coma) was included. Statistical analysis. A one-way Analysis of Variance (ANOVA) was performed to assess the effect of the treatments on the average pigmentation or germination. Following the ANOVA, descriptive statistics were calculated. Response variables. The experiment was evaluated every third day starting from its establishment and concluded 45 days after its establishment. During the evaluations, the following data were recorded: Germination. A seed was considered germinated if it showed seed imbibition and was subsequently tracked to determine the stages leading to seedling formation. Contamination percentage. This was calculated by counting the number of seeds exhibiting any visible symptoms of fungal or bacterial infestation. Germination percentage. This was estimated based on the quantity of germinated seeds in the Petri dishes. Necrosis percentage. These are the seeds that visibly turned dark or brown, without showing mycelium or signs of contamination throughout the duration of the germination test.

Results and Discussion

Seed coat color. Four distinct hues were identified (Figure 1a); the variation was reflected in color intensity (saturation), with some exhibiting intense coloration and others slightly lighter. The largest proportion corresponds to the color N199 Grey-Brown C (59 %), followed by N199 Grey-Brown D (37 %). In third place are the seeds that showed a seed coat color of 164 Greyed-Orange A (3 %), and only a few presented the color 165 Greyed-Orange A (1 %). This general color range signifies a brownish hue. In contrast, Lidueña & Martelo (2018) mention that the color of the seed coat is light yellow.

Coma color. The coma (plumose appendages) also showed four distinct hues (Figure 1b), all within the White Group. Over half of the comas are represented by the color NN155 White Group C (60 %), while the color NN155 White Group B accounts for 33 %, the NN155 White Group A comprises only 7 %, and the NN155 White Group B represents less than 1 %.

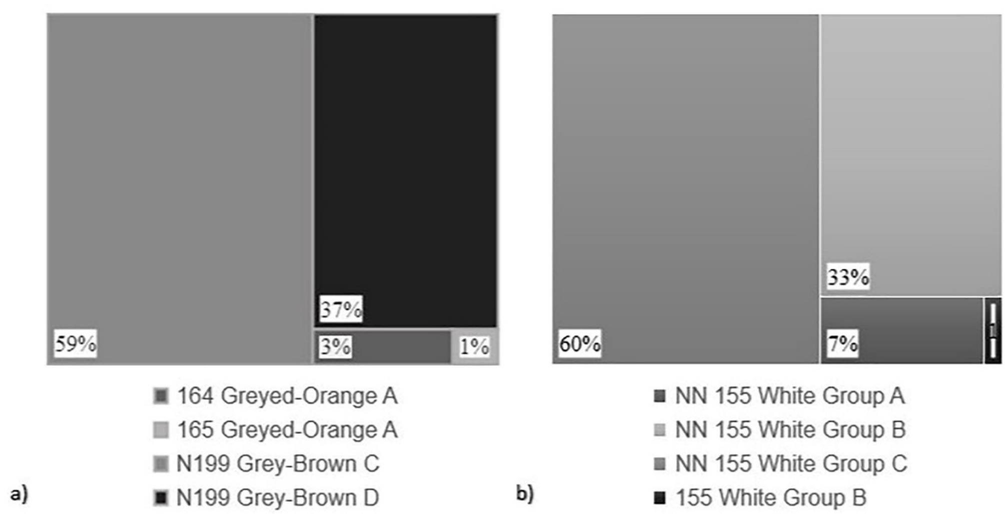

Figure 1. Color shades observed in seeds of *Tillandsia recurvata,* a) seed coat and b) coma, with color charts from the Royal Horticultural Society.

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Weight. The majority of the seed's weight was found to be concentrated in the coma (Table 1), this demonstrates the significant importance of this structure for the seeds of this species, as previously mentioned, the coma features a characteristic double parachute that functions in dispersal (Benzing, 2000), provides greater adherence to bark (Amaro, 2017), and may aid in water capture, however, information regarding the aerodynamic properties of these structures is lacking (Wester & Zotz, 2011).

Table 1. Dimensional variables obtained in the characterization of *Tillandsia recurvata* (L.) L. seeds.

Response variable	Maximum	Minimum	Average
Weight of 100 whole seeds (mg)	15	14.5	14.68
Weight of 100 seeds without coma (mg)	6.6	4.8	6.12
Weight of 100 comas (mg)	10.4	6.7	7.76
Weight of 10 whole seeds (mg)	1.7	1.3	1.4
Weight of 10 seeds without coma (mg)	0.6	0.3	0.4
Weight of 10 comas (mg)	1.2	0.8	1.02
Seeds per milligram (number)	10	7	8.2
Seed length (mm)	4.4	1.88	2.96
Coma length (mm)	23.8	6.75	16.9
Seed width (mm)	0.48	0.10	0.27
Seed area (mm^2)	1.14	0.26	0.59

Coma length. Ten class intervals were obtained (Figure 2a) ranging from a minimum of 6.76 mm to a maximum of 23.8 mm (Table 1), the largest quantity of comas was quantified in interval 7 (17 – 18.7 mm), accounting for 25 %, followed by interval 6 (15.3 – 17 mm) with 20 %, the lowest percentage of comas was found in interval 1 (6.7 – 8.5 mm), with less than 1 %. Seeds are frequently dispersed in clumps that adhere to the branches of surrounding trees, the number of seeds that reach the canopies depends on the distance between neighboring trees, as well as the wind intensity, however, the largest proportion of seeds remain on the same tree (Bernal et al., 2005), although it is unknown whether the length of the coma directly influences the dispersal distance, Martelo-Solorzano et al. (2022) describe the coma as having a structure similar to a double parachute, due to the fusion of the testa. The largest proportion of the sample (over 80 %) falls within the range of 13.6 mm to 22.1 mm, with the remainder distributed between 6.7 mm and 13.6 mm and 22.1 mm and 23.9 mm.

Seed length. The length observed in the sample (Table 1) corresponds, on average, with the findings reported by Martelo-Solorzano et al. (2022), who documented similar lengths. Ten class intervals were obtained (Figure 2b), the highest percentage of seeds are located in interval 4 (2.6 – 2.9 mm), concentrating 22 % of the total sample, conversely, interval 1 (1.8 – 2.1 mm) and interval 10 (4.2 – 4.5 mm), when summed, reach only 2.4 %, the range of lengths is wide, making it highly probable to encounter seeds of different sizes, including some that fall below or above the intervals mentioned here.

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Seed width. The seed width ranged from 0.10 mm to 0.48 mm, as indicated in Table 1, of the ten class intervals, 26 % of the seeds are located in interval 5 (0.25 – 0.29 mm), while interval 3 (0.18 – 0.22 mm) and interval 7 (0.33 – 0.37 mm) each represent 13 %; conversely, the interval that groups the smallest proportion of seeds is interval 10 (0.45 – 0.49 mm), with less than 1 %. Almost the entire sample (89.2 %) falls within the width range of 0.18 mm to 0.37 mm for one of the seed faces. 4 % is concentrated from 0.1 mm to 0.18 mm, while 6.8 % corresponds to the width of seeds located between 0.37 mm and 0.49 mm.

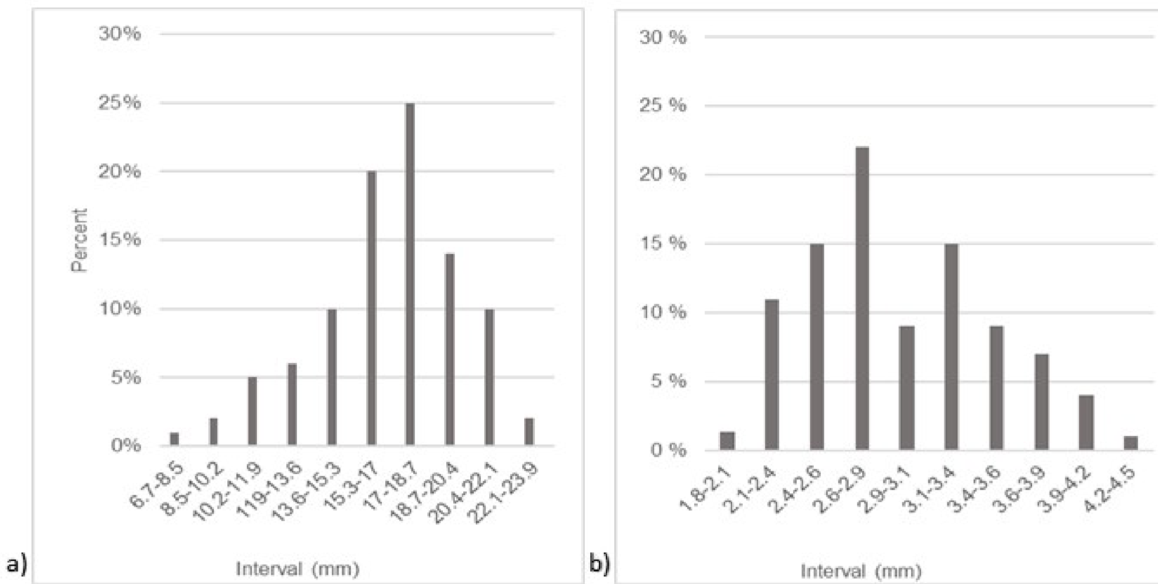

Figure 2. Seed distribution of *Tillandsia recurvata* (L.) L., in the intervals identified by a) coma length and b) seed length.

Seed area. An average area of 0.59 mm² was observed (Table 1), the largest quantity of seeds, representing 21 %, is found in interval 4 (0.53 – 0.61 mm²). In contrast, interval 9 (0.97 – 1.06 mm^2) and interval 10 (1.06 – 1.15 mm^2) each represent less than 1 %. 62 % of the seeds range from 0.44 mm^2 to 0.7 mm^2, likewise, 22 % are represented by seeds with areas from 0.7 mm^2 to 0.97 mm^2, and 12.8 % are found between 0.35 mm^2 and 0.44 mm^2, the remaining 3.2 % falls into the first interval and the last two.

Tetrazolium viability test. The seeds subjected to the tetrazolium test exhibited different staining patterns over time. Eight distinct staining patterns were observed in the seeds (Figure 4):

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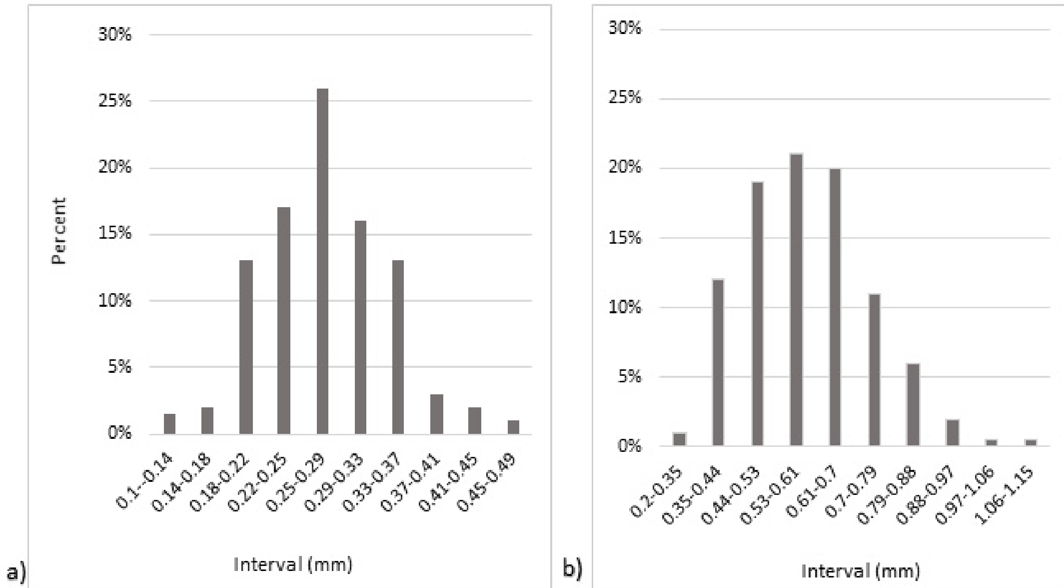

Figure 3. Seed distribution intervals of *Tillandsia recurvata* (L.) L., according to a) seed width and b) surface area.

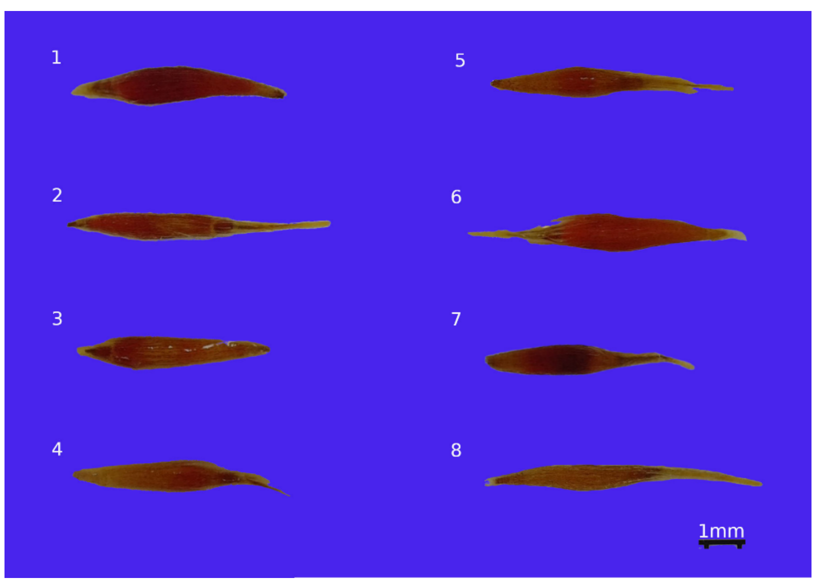

Figure 4. Staining patterns observed in seeds of *Tillandsia recurvata* (L.) L. 48 hours after adding 0.2 % tetrazolium solution.

No. 1. Complete staining of the embryo. No. 2. Unstained area in part of the embryo. No. 3. Unstained central part of the embryo. No. 4. Slight staining at one end. No. 5. Central area of the embryo stained. No. 6. Faint or pale red staining. No. 7. Embryo with dark red color. No. 8. No staining.

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After 12 hours, an opaque reddish coloration was observed (Figure 5), after 24 hours, the color change was evident, most seeds exhibited either total or partial staining (the largest proportion of seeds stained during this time interval), at 48 hours, the red color intensified, and the color became homogenized in those that had previously been partially stained. The final data (48 hours) were subjected to a one-way Analysis of Variance (ANOVA), which revealed a statistically significant difference among the three treatments (F (2,6) =5.6, p = 0.042); therefore, the seed classification demonstrated differences and, overall, low viability (Figure 6a), the small seeds showed the lowest viability at 16.7 %, followed by medium seeds at 30 %, and large seeds at 36.7 %, the overall general viability percentage was only 27.8 %.

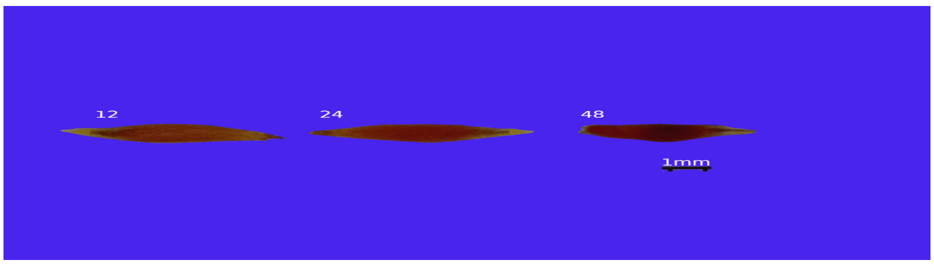

Figure 5. Staining levels observed in seeds of *Tillandsia recurvata* (L.) L. at 12, 24, and 48 hours after adding 0.2 % tetrazolium solution.

Germination. The analysis results indicated that there was no statistically significant difference in the average germination among the treatments (F (3,8) = 1.79, p = 0.227). The seeds showed high variability regarding the number of seeds that germinated and the time required, overall, lower germination viability percentages were observed compared to those obtained in the tetrazolium test (Figure 6a and b), a decrease of 55 % was observed in small seeds, 80 % in medium seeds, and 52 % in large seeds. The small seeds exhibited the lowest germination percentage, reaching barely 6.7 %, although medium seeds presented the exact same percentage, this does not align with the trend observed in the viability test, however, the result for large seeds, which showed 10 %, did align with the expected trend, the only seeds that reached 20 % were the seeds with the coma. This response could be attributed to the coma retaining moisture for a longer period, despite daily water addition, as the paper remained visibly dehydrated.

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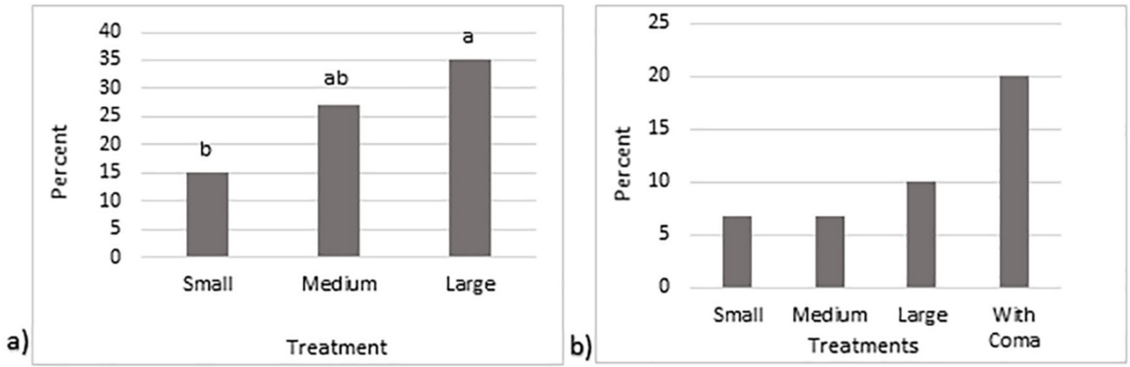

Figure 6. Percentages of *Tillandsia recurvata* (L.) L. seeds, viable according to a) the tetrazolium test and b) the germination test.

Montes-Recinas et al. (2012) classified the germination of *T. recurvata* into two stages: swollen seeds with a ruptured seed coat (Stage 1) and chlorophyllous embryos (Stage 2). In the current test, neither testa rupture nor the formation of structures was observed only seed swelling and a color change were noted, which could indicate chlorophyllous embryos (Figure 7), considering the description by Luján-Santiago et al. (2024) for *T. usneoides*, these seeds would fall between Stage 2 and Stage 3. However, seed enlargement was observed, the characteristic curve described by those authors for Stage 3 was not visible. These results contrast sharply with those reported by Fernández et al. (1989), who obtained 97 % – 100 % germination under both light and dark conditions with seeds collected at the time of impending dehiscence, which was maintained for 4 – 5 months, although lower percentages were obtained with seeds harvested and stored in the same year, no germination occurred in seeds harvested 1 – 5 years earlier, thus, the fact that the seeds were stored might have affected the germination potential at the time of the germination test, even though they were stored for no more than five months. Conversely, Bernal et al. (2005) observed that seeds under natural environmental conditions (within the canopies of four tree species) germinated between 0 % and 7.5 % of the total seeds evaluated, which is similar to the percentage observed in this study.

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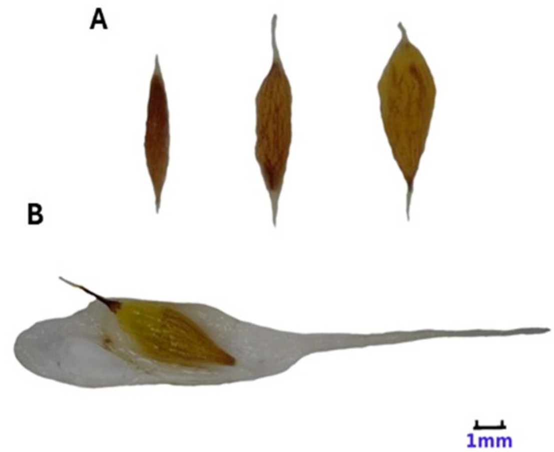

Figure 7. ***Tillandsia recurvata*** **seeds undergoing germination, showing A) different stages of imbibition without a coma, B) seed with a coma.**

As previously mentioned, contact between the seed and liquid water is indispensable during the germination process, since water vapor is insufficient to ensure complete germination, this is because tolerance to desiccation decreases once the seed has germinated (Montes-Recinas et al., 2012), this factor may explain the low percentages obtained, as the oscillation in temperatures caused the filter paper to occasionally fail to remain moist, and the lack of moisture led some seeds to exhibit desiccation symptoms, furthermore, authors like Gastón de Iriarte (2017) suggest that temperature plays a crucial role by influencing the enzymes that regulate the speed at which biochemical reactions occur within the seed, given that enzymatic activity takes place between minimum and maximum temperatures, another characteristic of the *T. recurvata* seed, observed by Martelo-Solorzano (2022), is its low endosperm content, which could affect latency or necessitate an agent to promote its germination, they also report low germination rates between 14 and 28 days and, similarly, without seedling formation, another factor that can affect seed germination is the presence of certain compounds, such as phytol and/or palmitic acid (Valencia-Díaz et al., 2010).

The germination process was prolonged up to 24 Days After Sowing (DAS) for most of the seeds, and up to 33 DAS for the large seeds. The small seeds germinated at 24 DAS, while the medium seeds germinated at 12 DAS, the large seeds were the ones that extended their germination period, ranging from 15 DAS up to 33 DAS. Although the germination period for the large seeds was broader than that of the coma seeds, the latter showed the highest germination percentage, with the majority occurring at 12 DAS.

Contamination. The contamination rate was low, with a maximum of 10 % for both the small and large seeds, while the medium seeds and those with the coma presented 6.7 % contamination.

Contamination began starting from 9 Days After Sowing (DAS) and extended up to 18 DAS, afterward, it only spread across the seeds already contaminated; the fungus completely covered some of the seeds. Small seeds exhibited the highest contamination percentage at 12 DAS (6.7 %), while at 18 DAS it was lower (3.3 %), medium seeds showed initial signs of contamination from 9 DAS, as did the seeds with the coma, however, unlike the medium seeds, the seeds with the coma did not exhibit contamination in new, previously uncontaminated seeds, large seeds presented homogeneous contamination from 12 DAS until 18 DAS. Therefore, although the washing technique may have been correct (as it is implemented for tissue culture), the repeated opening and closing of the Petri dishes for evaluation and watering could have caused contamination by environmental agents, a different watering strategy should be sought for subsequent experiments.

Necrosis. The small seeds exhibited the lowest necrosis percentage (3.3 %). In contrast, the seeds with the coma reached up to 33.3 % necrosis, resulting in the highest number of necrotic seeds. Medium seeds showed 13.3 % necrosis, while large seeds showed twice that amount (26.6 %). For the large seeds and those with the coma, the highest necrosis percentages occurred at 18 DAS, the medium seeds, conversely, showed two dates when seed necrosis became evident, 18 and 21 DAS, the small seeds manifested the lowest necrosis percentage (3.3 %) and only at 21 DAS. Necrosis, or cell darkening, oxidation, or death, is commonly reported in tissue culture; therefore, it may be due to various factors, including the chemicals used during washing (either the product itself or the exposure time for contamination reduction), the oxidation of phenolic compounds, or environmental conditions (Díaz-Lezcano et al., 2000; Hoang et al., 2020; Verona-Ruiz et al., 2020; Lujan-Santiago et al., 2024).

Conclusions

An effect of the size characteristic on the germination of *T. recurvata* seeds was observed; the large seeds (3.5 – 4.25 mm) had the highest viability (36.7 %), but only 10 % germination. While the medium size indicated a higher viability percentage in the tetrazolium test, it presented the same germination results as the small seeds. It was also observed that the presence of the coma doubled the germination rate; however, the overall rate remained very low (20 %). Nevertheless, given that this is a wild species, the high seed dispersal helps ensure adaptation across different locations. A notable difference was that the seeds with the coma also promoted rapid germination (one week), although larger seeds without the coma were available, their germination period was prolonged by nearly a month.

The findings confirm existing descriptions of morphological characteristics, including the predominance of the coma and the wide variation in seed dimensions. The tetrazolium viability test showed a low overall viability of 27.8 % and indicated that larger seeds tend to be more viable. However, germination percentages were significantly lower, reaching only 10.8 %, with a notable interruption in the seedling development process, which aligns with reports noting the lack of endosperm as a limiting factor. It was observed that seeds with the coma, despite not being classified by size, yielded the highest germination rates, suggesting that the coma plays

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a crucial role beyond dispersal, possibly by retaining the necessary moisture for germination. Conversely, the variability in germination results and the high incidence of necrosis in some treatments (especially in seeds with the coma) indicate that factors such as desiccation, storage, and handling technique can negatively affect germination and early seedling development. Therefore, the morphological characterization and evaluation of the germination potential of *T. recurvata* are essential steps for developing propagation strategies that support its conservation and medicinal use, while minimizing extraction from the wild. The results demonstrate the need to optimize germination protocols, accounting for variables such as substrate, moisture supply, and temperature, to overcome the inherent challenges posed by the limited nutritional reserves in these seeds. Future studies should focus on establishing *in vitro* culture protocols or controlled conditions that favor germination and seedling establishment, thereby ensuring the sustainable use of this species.

Author contributions

“Conceptualization of the work, EFVH, DAR; methodology development, EFVH, DAR; software handling, EFVH, DAR, MDJH; experimental validation, EFVH, DAR, MDJ; results analysis, EFVH, DAR, MDJH; data curation, EFVH, DAR, MDJH, RSC, MTCL; writing and manuscript preparation, EFVH, DAR, MDJH, RSC, MTCL; writing—review and editing, EFVH, DAR, MDJH, RSC, MTCL; project administration, EFVH, DAR, MDJH; funding acquisition, EFVH.

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Conflicts of interest

The authors declare no conflicts of interest.

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