



Original Papers

Seed desiccation tolerance and effect of flooding on the propagation of *Senecio carbonellii* (Asteraceae): implications for conservation

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Abstract

Senecio carbonellii, an endemic plant of Colombia, is classified as critically endangered in the wetlands of Bogotá D. C. This study aims to determine the desiccation tolerance and longevity of *S. carbonellii* seeds and evaluate the effect of flooding on seed germination and plant growth to provide information for the development of conservation strategies for the species. For this purpose, we assessed the germination, viability (using tetrazolium testing), and longevity of desiccated seeds compared to seeds with initial moisture. We also evaluated the effect of flooding on the emergence and growth of seedlings and plants under nursery conditions. Our findings indicated that the seed viability and germination percentages were significantly higher in the seeds with initial moisture compared to desiccated seeds. However, we observed that viability is maintained even after storing the seeds for more than two years, indicating that the seeds partially tolerate desiccation and that they are likely to have an intermediate storage behavior. Furthermore, flooding depth reduced the stem number, diameter, and length of plants. The results revealed that flooding negatively affects emergence and growth in the nursery.

Key words: germination, growth, nursery, submergence, wetlands.

Resumen

Senecio carbonellii es una planta endémica de Colombia, que está categorizada en peligro crítico en los humedales de Bogotá D.C. Con este estudio se busca determinar la tolerancia a la desecación y la longevidad de las semillas de *S. carbonellii* y evaluar el efecto de la inundación en la germinación y el crecimiento de las plantas con el fin de brindar información para el desarrollo de estrategias de conservación de la especie. Para esto se evaluó la germinación, la viabilidad (con la prueba de tetrazolio) y la longevidad de semillas desecadas en comparación con semillas con humedad inicial. También se evaluó el efecto de la inundación en la emergencia y crecimiento de las plántulas y plantas en condiciones de vivero. Nuestros resultados indican que el porcentaje de viabilidad y germinación de la semilla fue significativamente mayor en las semillas con humedad inicial en comparación con las semillas desecadas. Sin embargo, observamos que la viabilidad se mantiene luego del almacenamiento de las semillas por más de dos años, lo que indica que las semillas toleran parcialmente la desecación y que es probable que tengan un comportamiento de almacenamiento intermedio. Por otra parte, la profundidad de inundación redujo el número de tallos, el diámetro y la longitud de las plantas. Los resultados revelaron que la inundación afecta negativamente la emergencia y el crecimiento en vivero.

Palabras clave: germinación, crecimiento, vivero, sumergencia, humedales.

Introduction

Wetlands are strategic ecosystems in water regulation, nutrient cycling, pollutant removal, and carbon sequestration (Ramsar Convention

on Wetlands 2018). These ecosystems provide services related to water supply, food provision, climate regulation, and climate change mitigation (Millennium Ecosystem Assessment 2005; Ramsar

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Convention on Wetlands 2018). Unfortunately, there has been a reduction in the coverage of natural wetlands, leading to an increased risk of species extinction, a decrease in ecosystem services, and the need for conservation strategies (Ramsar 2005; Ramsar Convention on Wetlands 2018). In Bogotá D.C., wetlands are located at altitudes between 2,600 and 3,490 m.a.s.l. (Herrera *et al.* 2004). The Complex of Urban Wetlands of the Capital District of Bogotá, which is listed in the Wetlands of International Importance of the Ramsar Convention, is one example of a high andean wetland ecosystem (Ramsar 2019; Santa Méndez *et al.* 2020; Alcaldía Mayor de Bogotá 2021).

Senecio carbonellii S. Díaz (Asteraceae), or the marsh daisy, is a species endemic to Colombia and is the only species of aquatic flora classified as critically endangered in the wetlands of Bogotá D.C. Specifically, this species is restricted to the La Conejera Wetland in Bogotá (Acueducto de Bogotá & Fundación Humedal la Conejera 2014; Bernal *et al.* 2020; Fajardo-Gutiérrez *et al.* 2021) in a sector surrounded by forest plantations, crops, and livestock. It is an emergent, rhizomatous, perennial herb that can reach heights of up to one meter and is considered a “Facultative Wetland” plant (Díaz-Piedrahíta 1986; Guzmán Ruiz A. 2012). Additionally, this plant reproduces by seeds and stolons.

Botanical gardens play a fundamental role in plant conservation as they develop studies in biodiversity, seed science, conservation, and ecology (Faraji & Karimi 2020) and carry out actions aimed at *ex situ* conservation of flora, including the consolidation of seed banks, establishment of live plants, and tissue culture (Mounce *et al.* 2017). Due to its endangered and endemic status, *S. carbonellii* is a priority species for conservation in Colombia. To strengthen *ex situ* conservation of this type of plant, Castellanos-Castro *et al.* (2017) proposed the development of propagation protocols. For the propagation of wetland species, variables such as light, temperature, oxygen, pH, soil characteristics, and flooding have been reported to influence dormancy and seed germination, as well as seedling establishment (Kellogg *et al.* 2003; Baskin & Baskin 2014; Wagner & Oplinger 2017; Phartyal *et al.* 2020; Rosbakh *et al.* 2020).

Flooding generates adverse conditions on plants, including hypoxia, the production of toxic compounds, soil compaction, and nitrogen deficiency (Blom & Voesenek 1996). In some species, these conditions reduce flower and seed

production, decrease the number of stems per unit area, increase stem length (Webb *et al.* 2012), and either promote or inhibit seed germination (Casanova & Brock 2000; Kellogg *et al.* 2003; Webb *et al.* 2012; Baskin & Baskin 2014; Phartyal *et al.* 2020; Rosbakh *et al.* 2020). Not all species living in flooded environments are flood tolerant (Bailey-Serres & Voesenek 2008). Flood-tolerant plants have evolved two survival strategies. They can either restrict growth processes by adjusting their metabolism or rapidly elongate their stems, leaves, or petioles to reach the air (Bailey-Serres & Voesenek 2008).

Desiccation tolerance is involved in the maintenance and establishment of plant communities. This strategy is key in seed dispersal, stress resistance, and the preservation of soil seed banks (Marques *et al.* 2018). The seeds of some aquatic plants can be conserved in *ex situ* seed banks as they tolerate desiccation (Dalziel *et al.* 2019). In Bogotá, it has been reported that some wetland species generate seed banks in the soil (Montenegro-S *et al.* 2006; Romero *et al.* 2017). However, it remains unknown how these seeds behave in response to desiccation. Given that *S. carbonellii* is an endangered species found solely in the La Conejera Wetland in Bogotá, the aim of this investigation is to study the conservation potential of *S. carbonellii* in a seed bank and assess how flooding affects plant propagation in nursery conditions. This information will facilitate the development of effective conservation and reintroduction strategies for this species.

Materials and Methods

Study area and seed source

The evaluated plant material was collected from La Conejera Wetland, situated in Bogotá D.C. (04°45'44.6"N, 74°06'23.1"W, 2,536 m.a.s.l.). A single specimen was collected and archived in the Botanical Garden of Bogotá (JBB) Herbarium and assigned the herbarium stamp 32391. On April 19, 2021, fruits of the *S. carbonellii* were collected and achenes were separated from the capitulum during seed cleaning. The collected seeds were subsequently preserved in the seed bank collection with accession code BSJBB-245 (Fig. 1).

Two laboratory experiments (Experiments 1 and 2) were carried out to determine the desiccation tolerance and longevity of *S. carbonellii* seeds. Additionally, to assess the impact of flooding on the establishment of *S. carbonellii*, we conducted Experiments 3 and 4, in which we evaluated

emergence and growth under different submergence levels. For this purpose, we used plants propagated at the Botanical Garden of Bogotá in the La Conejera Wetland.

Seed quality

After seed collection, we evaluated the quality of a subsample of 40 seeds with the cut test. The proportion of filled seeds was evaluated using the following formula (DiSacco *et al.* 2020):

Proportion of filled seeds = N° of filled seeds / N° of cut seeds.

Taking into account this value, oversowing was carried out so that the trials had approximately

15 seeds with embryo. The following formula was used to calculate the rate for oversowing (DiSacco *et al.* 2020):

Oversowing = seeds needed for the trial / proportion of filled seeds.

Experiment 1: seed tolerance to desiccation

Four viability trials were conducted to statistically compare the differences in seed viability between two groups: one group with Moisture Content initial (MCi) corresponding to fresh seeds and another group with Moisture Content object (MCo) corresponding to seeds that had been dehydrated to 15% Relative Humidity



Figure 1 – a-e. *Senecio carbonellii* – a-b. habitat; c. inflorescence; d. fruit; e. seed. Source: Photos by Liliana Martínez.

equilibrium (RHe) and stored in a freezer at a temperature of -20 °C until use (Gold *et al.* 2004; Seaton *et al.* 2013; DiSacco *et al.* 2020). Two trials were conducted using a germination chamber to evaluate germination while the remaining two trials assessed seed viability using the tetrazolium chloride test (TZ).

Germination trials in germination chamber:

Two germination trials were carried out. The first trial evaluated the germination of seeds with MCi while the second evaluated the germination of seeds with moisture content object MCo. Before sowing, the seeds were disinfected with 1% sodium hypochlorite for five minutes and subsequently washed with water (Muñoz & Ackerman 2011). The seeds were sown in four Petri dishes with an agar-agar medium. Each replicate was a Petri dish planted with 21 seeds each, taking into account the need for oversowing. Then, the dishes placed under controlled conditions within a germination chamber with a 12-hour photoperiod, temperature of 20/10 ± 2.5 °C, and humidity of 75 ± 5% (Thermo Scientific Precision Model 818 Incubator). Germination, defined as the emergence of a radicle protrusion measuring at least 2 mm through the surrounding tissues, was monitored three times a week (Bewley *et al.* 2013; DiSacco *et al.* 2020).

The Germination Percentage (GP) and Mean Germination Time (MGT) of each trial were calculated based on the following formulas (Tompsett & Pritchard 1998; Ranal & Santana 2006):

$$GP = \left(\frac{N}{N_s} \right) * 100 \quad MGT = \sum_{t=1}^k ni \, ti / \sum_{t=1}^k ni$$

Where N is the number of germinated seeds and N_s the total number of seeds, ni is the number of seeds germinated at each collection of germination data, ti is the time (in days) of the respective data collection, and k refers to the duration of the entire test, also measured in days.

Viability test with tetrazolium:

The tetrazolium test was performed on four replicates, each consisting of 21 seeds from both the MCi and MCo treatments. The seeds were hydrated with water for 24 hours, then 1% tetrazolium was added, and the seeds were placed in an oven at a temperature of 40 °C in dark conditions for 24 hours. Then the number of viable

seeds (characterized by a red-stained embryo), unstained seeds (when the embryo does not stain), and empty seeds (which lack an embryo) were recorded (DiSacco *et al.* 2020).

Experiment 2: seed longevity

The viability of seeds stored for 24.5 months (688 days) in the seed bank (accession number BSJBB-245) was evaluated using the tetrazolium viability test in accordance with the methodology previously described above (MCo_L).

Experiment 3: flooding effect on seedling emergence and growth

Experiment 3 was carried out in the aquatic plant nursery of the Botanical Garden of Bogota (04°39'57.85"N, 74°5'55.93"W) with relative humidity of 73.74%, average daily temperature of 16.62 °C (Data Logger EBCHQ 94150 and Thermo Scientific Orion Start A329 series), water pH of 6.67, and water electrical conductivity of 24.29 µScm⁻¹.

On May 14, 2021, two treatments were established: water depth of 0 cm and 4 cm relative to the substrate level (Mcfarland & Shafer 2011; Wagner & Oplinger 2017). In a plastic tray with 160 alveoli, four replicates of 21 seeds were sown. These seeds had been previously disinfected with 1% sodium hypochlorite for five minutes (Muñoz & Ackerman 2011). The seeds were sown on coconut coir substrate and covered with river sand (approx. 3 mm thick) to prevent movement during immersion. To avoid substrate loss, a 2 cm × 2 cm square of geotextile was placed at the bottom of each alveolus (Fig. 2). Throughout the experiment, the substrate was saturated, and the trays were covered with 75% black polystyrene and transparent plastic. Emergence of seedlings was evaluated three times a week for 91 days, and the shoot height of each plant was recorded, measuring the length from the base of the stem to the apex of the leaves. Seedling was considered the stage between seed germination and the emergence of the first true leaves (Larson *et al.* 2015). The measurement was made every 15 days for 10 weeks. The Emergence Percentage (EP) and mean emergence time (MET) were calculated.

Experiment 4: effect of flooding on plant growth and development

On May 22, 2018, the experiment was started employing a simple randomized design. A total of

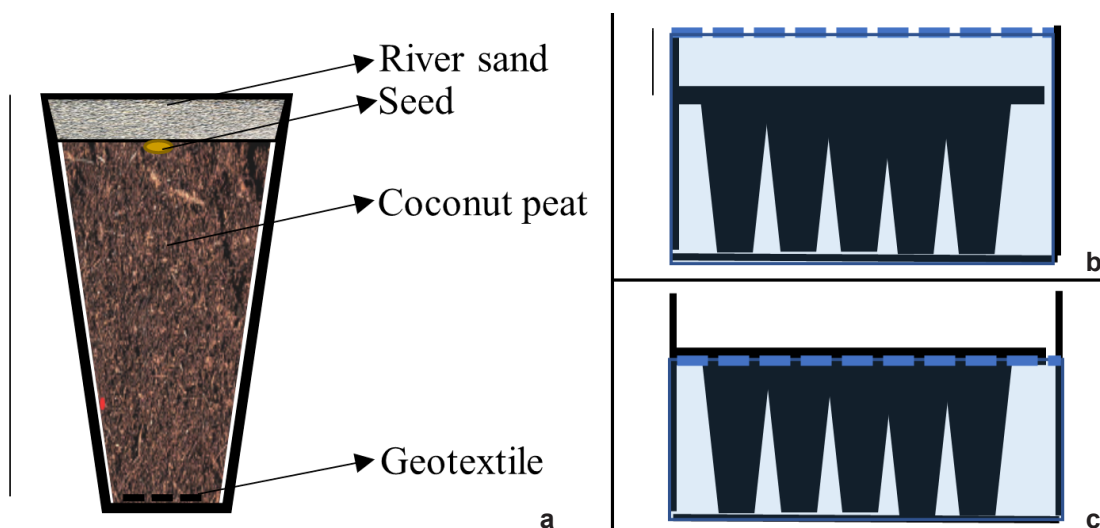


Figure 2 – a-c. Diagram of seed sowing in nursery – a. substrate and seed sowing; b. flooding treatment of 4 cm above the substrate surface; c. non-flooding treatment: the water level is at the same level as the substrate surface.

33 plants were transplanted into 28 × 20 cm plastic bags that contained a substrate composed of 70% black soil and 30% peat, with perforations at the base. The plants were randomly placed at different locations in a tank measuring 2 m long by 1 m wide by 0.4 m deep. To create different depth levels, the plants were placed on plastic baskets. The evaluated treatments were (Fig. 3): 1) Not submerged: the substrate was not in contact with the water in the tank, the soil was irrigated by sprinkling three times a week, 2) Partially submerged: the substrate remained saturated, and bags were submerged to a depth of 13 cm, and 3) Submerged: the substrate remained 7 cm below the water level. Before submitting the plants to each treatment, the height was measured, the number of initial stems was quantified, and the basal diameter of the stems

in each plant was measured. The plants used in the experiment ranged in height from 0.2 to 1 m and from 5 to 51 stems. The evaluated plants had several stems and more than a couple of true leaves.

Stem length, stem diameter, and stem-emergence rate

The plant height was recorded every 6 weeks for 18 weeks, the number of new stems was quantified, and their diameter and length were measured. To calculate the rate of stem emergence, the initial number of stems present on each plant on May 22 (the start of the experiment) was considered as the initial count, and the final number of stems observed after 83 days (12 weeks) was taken as the total number of stems. The following equation was used to calculate the stem emergence rate and determine the growth of the plants over time (Price & Munn 1999):

$$TRAP = \frac{\ln NT_2 - \ln NT_1}{t_2 - t_1}$$

Where TRAP is the relative rate of stem emergence, NT_2 is the number of stems at time 2 (t_2), NT_1 is the number of stems at time 1 (t_1), t_1 is time 1 (May 22) and t_2 is time 2 (August 15).

Data analysis

In Experiments 1, 2, and 3, normality (Shapiro Wilk) and homogeneity of variance (F-test) tests were performed for the variables: percentage viability, GP, EP, MGT, MET, and the

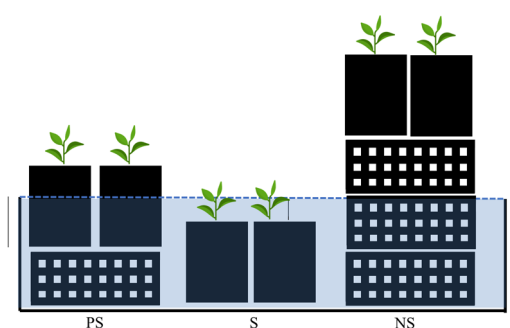


Figure 3 – Diagram of flooding treatments in nursery. Partially Submerged (PS), Submerged (S), and Not Submerged (NS).

height for each desiccation (MCi and MCo) and flooding (0 cm and 4 cm) treatments. Subsequently, a Student's t-test was performed to evaluate significant differences between treatments. To determine the difference between treatments (MCi, MCo and MCo_L, an ANOVA was performed followed by a Tukey test.

In Experiment 4, a longitudinal profile analysis was carried out (Feys 2016) for the variables that did not meet the assumptions of normality and/or homoscedasticity (stem length and diameter), in which treatments were the between-subjects factor, and sampling date was the within-subjects factor. For this, nonparametric rank-based tests were used following an F1-LD-F1 design (Noguchi *et al.* 2012). After the differences between factors were identified, the same profile analysis was used to determine the difference between treatments.

Statistical analyses and plots were performed in R software (R Core Team 2021), using the packages “stats” (R Core Team 2021), “car” (Fox & Weisberg 2019), “nparLD” (Noguchi *et al.* 2012) and “ggplot2” (Wickham 2016).

Results

Seed tolerance to desiccation

The evaluation of the proportion of filled seeds revealed that seven out of 10 seeds of *S. carbonellii* have embryos. Seeds with initial moisture content (MCi) showed a higher percentage of viability with the tetrazolium test [$t(6) = 5.3997$, $p = 0.001664$] and germination test [$t(6) = 3.495$, $p = 0.0129$] compared to the desiccated seeds (MCo) (Fig. 4). Regarding MGT, there were no significant differences between the two treatments used; however, in both treatments the majority of seeds germinated within the first 15 days (Fig. 4c).

Seed longevity

Regarding seed longevity, the seeds with MCi (52.4%) presented a higher viability in comparison to the seeds with the MCo (20.2%) and MCo_L (32.1%) treatments. However, the viability of dry seeds was maintained (MCo_L 32.1%) after 24.5 months of storage. The analysis of variance (ANOVA) revealed significant differences between the evaluated treatments [$F(2,9) = 12.27$, $p = 0.002$]. The viability percentage was significantly higher in the MCi treatment compared to the MCo ($p = 0.021$) and MCo_L ($p = 0.033$) treatments (Fig. 4a).

Effect of water level

on seedling emergence and growth

The emergence percentage for plants in the 0 cm treatment (29.76%) was significantly higher than plants in the 4 cm treatment (5.95%) according to the results of the t-student test [$t(6) = 4.3992$, $p = 0.004572$] (Fig. 5a). Regarding the mean germination time (MGT), only two replicates (of four) germinated in the flooding (4 cm) treatment. Moreover, a shorter germination time was observed in the 0 cm treatment (Fig. 5b). In the 0 cm treatment, emergence occurred between 10 and 50 days, while in the 4 cm treatment it occurred between 20 and 70 days (Fig. 5c).

Regarding growth, differences were observed in plant height in relation to water level, with plants reaching the highest values in the 0 cm treatment (7.74 cm) compared to the 4 cm treatment (1.5 cm) (Fig. 5d). In the 0 cm treatment, the highest percentage of survival was observed after 10 weeks, corresponding to 71% (17 plants) while in the 4 cm treatment, only 8% of the plants reached survival (2 plants). Due to the reduced number of plants in the 4 cm treatment, it was not possible to perform the proposed statistical tests.

Effect of water level

on plant growth and development

The establishment phase of *S. carbonellii* was notably impacted by the extent of substrate submersion. As the proportion of submerged soil increased, there was a corresponding decrease in stem-emergence rate and in the number of new stems generated on a per-pre-existing stem basis (Fig. 6). Plants grown in partially or fully submerged soil displayed significantly smaller stem diameters compared to those in non-submerged soil (Fig. 7a). This pattern persisted consistently across time. In other words, regardless of the point in time, plants in non-submerged soil consistently exhibited larger stem diameters than those in the other two treatments. In addition, plant diameter remained relatively constant over time regardless of treatment. Specifically, by July 15 (after 54 days), plants in all treatments had reached their maximum diameter, and this diameter did not change significantly over time (Fig. 7a). Regardless of sampling time, plants in the non-submerged or partially submerged substrates had greater stem length relative to plants growing in the submerged substrate. In contrast to diameter, plant length increased with time regardless of the treatment to which they were exposed (Fig. 7).

Discussion

Seed desiccation tolerance and longevity

The seeds of *S. carbonellii* do not tolerate desiccation as their viability decreases significantly when their moisture content is reduced, displaying a recalcitrant behavior (Fig. 4). In aquatic plants, it has been found that tolerance to desiccation depends on the species, and this response is not necessarily linked to traits such as seed size, the

number of seeds per fruit, phylogenetic group, or niche preference (Hay *et al.* 1999). Some species inhabiting ephemeral wetlands have evolved the capacity to tolerate desiccation which allows them to maintain viability during periods of drought (Tuckett *et al.* 2010; Dalziell *et al.* 2019). In contrast, the viability of recalcitrant seeds (such as those of *S. carbonellii*) is adversely affected by drought conditions (Hay *et al.* 1999).

The fact that the germination of desiccated seeds (MCo_L) did not decrease over storage time

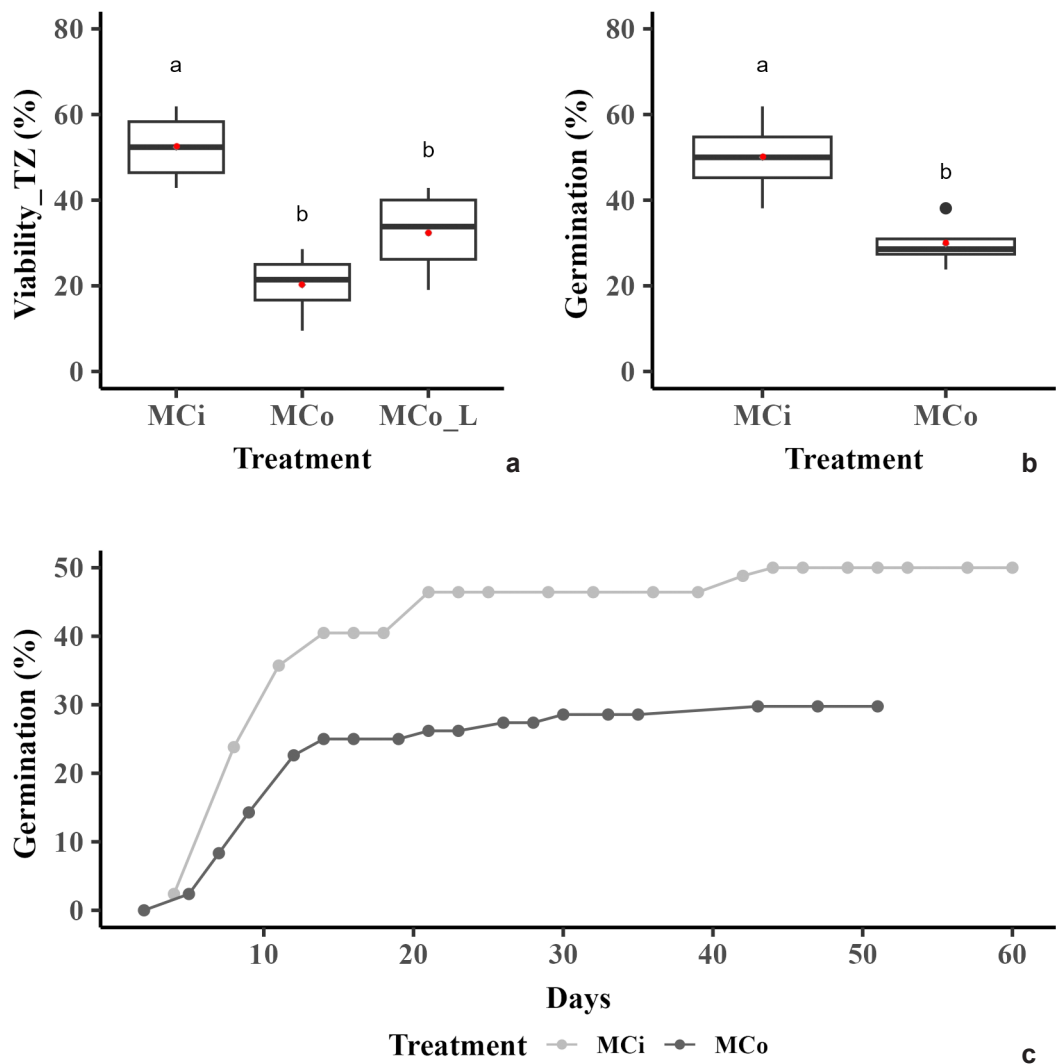


Figure 4 – a-c. Viability and germination percentage in laboratory tests – a. viability percentage in the tetrazolium (TZ) test. Mean values of treatments with different letters are significantly different according to Tukey’s test ($p < 0.05$); b. germination percentage; c. cumulative germination percentage over time. Mean values of treatments with different letters are significantly different according to the t-student test for independent samples ($p < 0.05$). Initial moisture content (MCI), object moisture content (MCo), Seeds stored 24.5 months (MCo_L). Red dots indicate the mean value.

contradicts what has been reported for recalcitrant seeds, which tend to lose viability when subjected to drying and storage (Walters *et al.* 2013). This would indicate that *S. carbonellii* seeds may have an intermediate behavior (Hong *et al.* 1996; Vargas *et al.* 2014). Another possibility is that the seed collection had included immature seeds, which could have impact seed viability during the drying process (Hong *et al.* 1996). Therefore, it is recommended to evaluate the desiccation tolerance of *S. carbonellii* seeds at different

moisture contents and storage temperatures to better understand their response to desiccation (Hay *et al.* 1999; Pammenter *et al.* 2002).

Effect of flooding on seedling emergence and growth

The results indicate that emergence and growth of *S. carbonellii* seedlings are reduced by flooding, specifically by lack of oxygen. Water depth has been reported to have a negative effect on wetland plant germination (Wagner & Oplinger

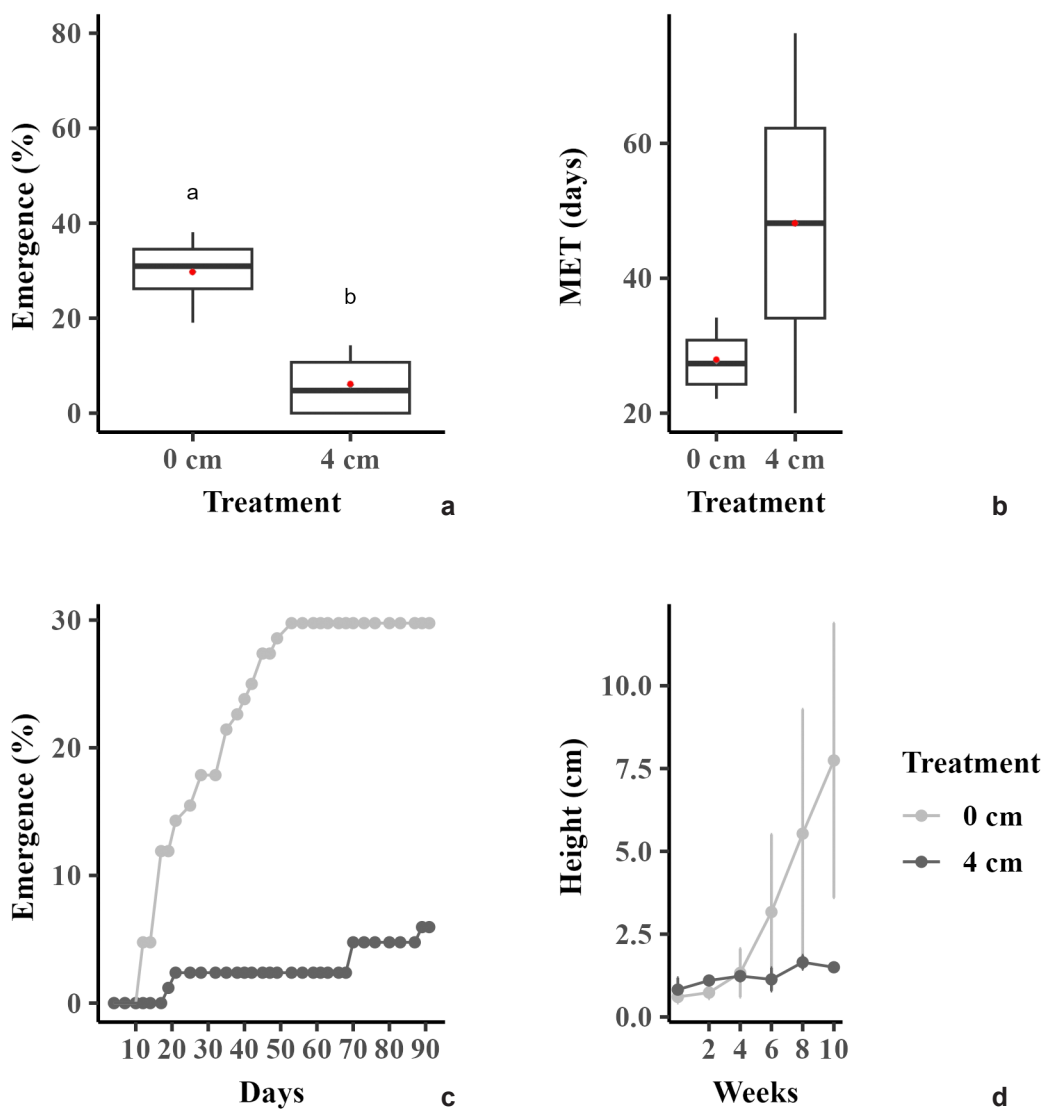


Figure 5 – a-d. Seedling emergence and height in nursery – a. emergence percent $n = 4$; b. mean emergence time in nursery, 0 cm ($n = 4$) and 4 cm ($n = 2$). Red dots indicate the mean value. Different letters indicate significant differences between tests according to the Student's t-test for independent samples ($p < 0.05$); c. cumulative emergence over time; d. seedling height over time. 0 cm ($n = 17$) and 4 cm ($n = 2$).

2017), and the absence of oxygen inhibits or diminishes germination in various wetland species including obligate and facultative species (Fraser *et al.* 2014). This behavior has also been reported in edge species (Rosbakh *et al.* 2020). In line with these observations, the current study demonstrates that seed immersion led to a reduction in the percentage of emergence and an increase in the mean emergence time (MET) for *S. carbonellii*. (Fig. 5), which may be related to flooding pulses that influence recruitment, germination and seedling growth (Van der Hammen *et al.* 2008).

In wetland plants, the seedling stage is particularly vulnerable because most seedlings are unable to thrive under anaerobic conditions. Low oxygen levels can adversely affect seed and seedling respiration, limit nutrient uptake, hinder oxygen storage in specialized tissues such as aerenchyma (Cronk & Fennessy 2001), and generate anoxia stress (Pan *et al.* 2022). Additionally, under such conditions, toxic compounds such as Fe, Mn, and H_2S are produced, soil compaction occurs, and low nitrogen concentrations are present due to increased denitrification (Byun *et al.* 2017). However, if a seed successfully germinates and survives in flooded conditions, it is most likely to establish itself (Fraser *et al.* 2014). It has been reported that vegetative reproduction is predominant in wetland

plants and that clonal structures are more successful than seedlings (Cronk & Fennessy 2001). For *S. carbonellii*, it was observed that flooding limits both germination and survival as only 8% of germinated seeds manage to survive under this condition. Germination and survival rates may be related to the type of seed dispersal, since it has been reported that some *Senecio* species in the Andes disperse by nautochoric means, that is, their seeds float on water (Melcher *et al.* 2000). This type of dispersal has been reported in emergent wetland species as the adaptation allows them to disperse through the water until they reach the shoreline where there is a higher probability of germination and establishment (Soons *et al.* 2017). On the other hand, the fact that flooding limits the establishment of the seeds could indicate the importance of vegetative reproduction in the persistence of this species.

Effect of water level on plant growth and development

Many wetland species exhibit morphological adaptations in response to water levels, including leaf promotion and stem elongation (Coops *et al.* 1996; Bailey-Serres & Voesenek 2008; Deegan *et al.* 2012; Byun *et al.* 2017). This response has been reported in species habiting environments with prolonged but relatively shallow flooding

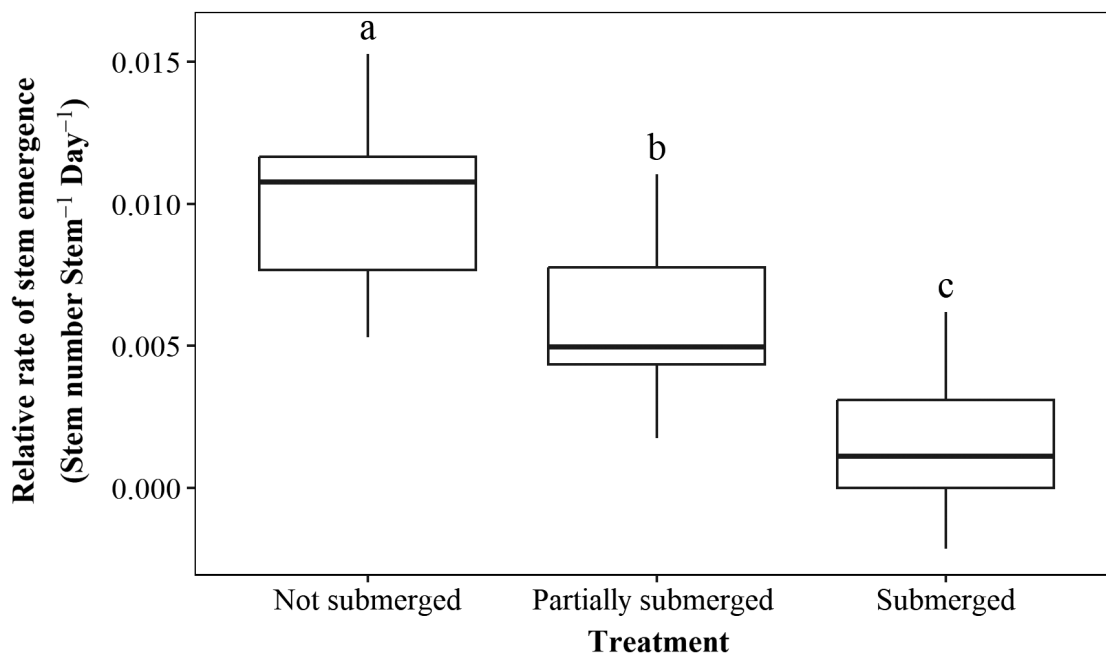


Figure 6 – Stem-emergence rate of the evaluated treatments (N = 9). Mean values of treatments with different letters are significantly different according to Tukey's test ($p < 0.05$).

(Bailey-Serres & Voesenek 2008). However, when flooding is deep or ephemeral, plants often adopt a growth suppression strategy by adjusting their metabolism and conserving energy (Bailey-Serres & Voesenek 2008). This behavior has been documented in *Rorippa sylvestris* (L.) Besser and *Rumex acetosa* L., where the plants minimize growth and conserve carbohydrate reserves during flooding events (van Veen *et al.* 2013; Akman *et al.* 2012). *S. carbonellii* employs a similar strategy as indicated by the shorter stem length in the submerged treatment. It appears that *S. carbonellii* is not tolerant of permanent flooding as its establishment and growth were significantly reduced when its seeds, seedlings, or plants were continuously submerged (Figs. 5, 6 and 7). This aligns with the plant's natural habitat where the species is found only on elevated mounds but not in the flooded areas between these mounds.

Conservation implications

The seeds of *S. carbonellii* partially tolerate desiccation, so their conservation in *ex situ* seed banks is possible. The possible intermediate seed storage behavior would imply that seeds can survive under drought conditions (Tweddle *et al.* 2003; Leck & Brock 2000), and raises the possibility that this species may form seed banks in the soil. Although progress has been made in understanding the storage of *S. carbonellii* seeds, it is still important to determine the appropriate storage methodologies to maintain seed viability so that they can be used in future ecological restoration processes (De Vitis *et al.* 2020).

However, persistent flooding in its habitat limits germination, growth, and establishment of the species. Considering the above, the conservation challenges facing *S. carbonellii* in Bogotá are largely associated with its habitat and life history. It is necessary to implement conservation actions in the La Conejera wetland to ensure the preservation of the existing populations. Furthermore, knowledge of the germination requirements and seedling performance is essential for formulating effective conservation strategies for this species (Fraser *et al.* 2014). Recommendations for the propagation of this species in nurseries include the use of humid substrates and avoiding flooding. When considering *S. carbonellii* establishment in wetland ecosystems, it is recommended to select areas with permanent humidity (but not saturated) to enhance the probability of survival and successful establishment.

Although it is necessary to combine *in situ* and *ex situ* methodologies to preserve *S. carbonellii*, efforts should be aimed at the conservation of its ecosystem, the plant's reintroduction in other wetlands, and the study of seed banks in the soil. To further strengthen long-term conservation, ongoing research should focus on understanding seed behavior under different humidity and temperature conditions as well as conducting studies on the establishment in *ex situ* conditions. This approach will be pivotal in developing effective conservation strategies for *S. carbonellii*.

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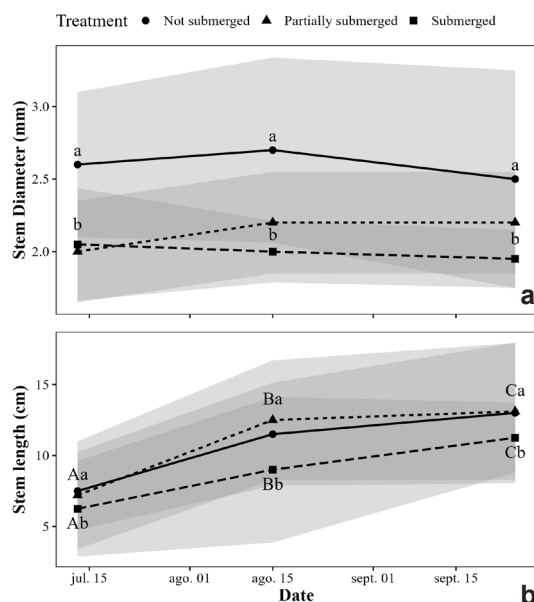


Figure 7 – a-b. Change over time of new stems in nursery – a. change in diameter of new stems; b. change in length of new stems (N = 94 for Not Submerged, N = 89 for Partially Submerged and N = 26 for Submerged). Each dot represents the median and the shaded ribbon represents the interquartile range for each treatment at its respective date. Medians with different letters indicate that there is a significant difference between treatments ($p < 0.05$). Lower-case letters indicate differences among treatments, and upper-case letters indicate differences between sampling dates.

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Data availability statement

In accordance with Open Science communication practices, the data of this study are available on request from the corresponding author. The data of experiment 4 are openly available in Catalogador del Jardín Botánico de Bogotá at <http://catalogador.jbb.gov.co:8090/app/resource?r=001_bio-em_sc_2018032>.

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