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ARTICLE

Conservation and propagation of *Drosera schwackei*: cryopreservation, *in vitro* germination, and development of an ornamental carnivorous species

Conservação e propagação de *Drosera schwackei*: criopreservação, germinação in vitro e desenvolvimento de uma espécie carnívora ornamental

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Abstract: *Drosera schwackei* (Droseraceae) is a carnivorous species with high ornamental potential, but no established protocols exist for its cultivation and conservation. This study aimed to develop a protocol for in vitro germination, plant development, and cryopreservation of *D. schwackei* seeds. The experiment was conducted in a completely randomized design with a 2 x 4 factorial scheme (two concentrations of sodium hypochlorite and four immersion times), with four replications, each containing 30 seeds. The seeds were cultivated in different concentrations of MS medium (MS1/3, MS1/2, MS100%, and control with agar/water). Two experiments were carried out to evaluate the in vitro development of seedlings: 1) Testing different salt concentrations of MS medium (1/3, 1/2, and 100%); 2) MS1/3 medium supplemented with different concentrations of BAP (6-benzylaminopurine). For cryopreservation, seeds were stored in cryotubes and immersed in liquid nitrogen for 1, 6, 12, 24, 48, and 120 hours, with a control group inoculated on the same day. Disinfestation with 2% sodium hypochlorite for 10 minutes effectively stimulated germination. MS1/3 and MS1/2 media favored seed germination and seedling growth, while low concentrations of BAP promoted better plant development. The seeds survived cryopreservation for up to 120 hours in liquid nitrogen without impairing plant development or morphology. *Sphagnum* moss proved an efficient substrate for acclimating *D. schwackei* seedlings from in vitro cultivation.
Keywords: conservation, *Droseraceae*, micropropagation, ornamental plants.

Resumo: *Drosera schwackei* (Droseraceae) é uma espécie carnívora com grande potencial ornamental, mas ainda não há protocolos estabelecidos para seu cultivo e conservação. O objetivo deste estudo foi desenvolver um protocolo para germinação in vitro, desenvolvimento vegetal e criopreservação das sementes de *D. schwackei*. O experimento foi conduzido em delineamento inteiramente casualizado, com esquema fatorial 2 x 4 (duas concentrações de hipoclorito de sódio e quatro tempos de imersão), com quatro repetições, cada uma contendo 30 sementes. As sementes foram cultivadas em diferentes concentrações do meio MS (MS1/3, MS1/2, MS100% e controle com ágar/água). Dois experimentos foram realizados para avaliar o desenvolvimento in vitro das plântulas: 1) Teste com diferentes concentrações de sais do meio MS (1/3, 1/2 e 100%); 2) Meio MS1/3 suplementado com diferentes concentrações de BAP (6-benzilaminopurina). Para criopreservação, as sementes foram armazenadas em criotubos, imersas em nitrogênio líquido por 1, 6, 12, 24, 48 e 120 horas, com um grupo controle inoculado no mesmo dia. A desinfestação com hipoclorito de sódio a 2% por 10 minutos foi eficiente e estimulou a germinação. Os meios MS1/3 e MS1/2 favoreceram a germinação e o crescimento das plântulas, enquanto baixas concentrações de BAP promoveram um melhor desenvolvimento das plantas. As sementes sobreviveram à criopreservação por até 120 horas em nitrogênio líquido, sem prejuízos ao desenvolvimento ou morfologia das plantas. O musgo esfagno mostrou ser um substrato eficiente para a aclimatação das mudas de *D. schwackei* oriundas do cultivo in vitro.
Palavras-chave: conservação, *Droseraceae*, micropropagação, plantas ornamentais.

Introduction

The genus *Drosera* L. (Droseraceae) has approximately 250 described species, present in all continents except Antarctica (Gonella et al., 2015; Fleischmann et al., 2018). In Brazil, 32 species of the genus occur, 18 of which are endemic (Gonella et al., 2022). The genus is renowned for its carnivorous nature, attracting, capturing and digesting insect prey (Darwin, 1875; Juniper et al., 1989).

Based on their carnivorous nature, *Drosera* species have specialized glands on the upper surface of their leaves, which secrete droplets of acidic and enzymatic mucilage, functioning as natural traps to capture their prey (Juniper et al., 1989; Katogi et al., 2022). Due to the beauty of their traps and curiosity about their carnivorous traps, *Drosera* species are highly valued ornamental plants in botanical gardens, generating increasing economic value (Kumar and Mishra, 2021).

However, species of the genus *Drosera* are among the most vulnerable groups to extinction due to habitat fragmentation and destruction (Cross et al., 2020). This vulnerability becomes more severe in the case of endemic species with restricted distribution, such as *D. schwackei* (Diels) Rivadavia.

Drosera schwackei is endemic to the *campos rupestres* (rupestrian fields) vegetation, being restricted to a few locations in Minas Gerais, Brazil, and is threatened with extinction, mainly due to small populations typically found in areas associated with livestock, urban expansion and pollution, and mining activities (Gonella et al., 2022). Preserving endangered species is an urgent task to maintain biodiversity (Cross et al., 2020). This, combined with the low propagation rate of these plants in their natural habitat, justifies the *in vitro* propagation of carnivorous plants as a strategy for *ex-situ* conservation.

In vitro germination is the most common method for establishing plant material and offers advantages such as more viable and responsive

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explants, making it suitable for propagating ornamental plants (Mehbub et al., 2022). Thus, using *in vitro* cultivation techniques can improve germination rates in less time, using less space to produce more uniform seedlings with better phytosanitary quality.

Drosera schwackei faces significant challenges due to the limited knowledge of seed technology, with the absence of established cultivation protocols for this species in the literature highlighting the need for a standardized *in vitro* germination procedure. To optimize *in vitro* cultivation, the MS medium (Murashige and Skoog), rich in macronutrients, micronutrients, and vitamins (Safitri et al., 2024), is commonly used, often in combination with growth regulators such as 6-Benzylaminopurine (BAP), a synthetic cytokinin frequently applied in plant culture media (Ruan and Yi, 2023). Studies suggest that lower concentrations of MS and BAP may yield better results for other Drosera species (Jayaram and Prasad, 2007).

In addition, recognizing the need for conservation, especially of plant species threatened with extinction, cryopreservation techniques have been considered a promising tool for the long-term storage of plant genetic resources (Ruta et al., 2020). This method enables the sustainable and theoretically indefinite preservation of plant tissues, garnering increasing attention in recent years (Mosa et al., 2023).

Another relevant aspect is acclimatization for *ex vitro* cultivation, particularly in the case of *Drosera* species, which thrive in environments with high light intensity, water saturation, and low nutrient availability conditions that provide them with a significant competitive advantage (Ellison and Gotelli, 2009). However, to ensure proper acclimatization, evaluating and testing the most suitable substrates for each species is essential.

Considering the importance of *Drosera schwackei* for biodiversity conservation and its potential for ornamental use, coupled with the scarcity of studies on propagation and conservation protocols, as well as the challenges it faces due to its limited distribution and environmental threats, this study aims to develop a protocol for in vitro germination, plant development, and seed cryopreservation of *D. schwackei* to ensure its long-term conservation.

Material and Methods

The experiments were conducted at the Seed Laboratory of the Department of Agronomy and the Tissue Culture Laboratory of the Department of Forest Engineering at Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). *D. schwackei* seeds collected at Biribiri State Park (PEBI) from March to May 2018 were used. The seeds were collected and stored in a cold chamber (10 ºC and 50% RH).

Seed disinfestation

The experiment was carried out in a completely randomized design in a 2 x 4 factorial scheme (two concentrations of sodium hypochlorite and four immersion times), with four replications containing 30 seeds per repetition.

Seeds were taken to the laminar flow, and disinfestation started by immersion in a 70% (v v^{-1}) ethanol solution for one minute. Then, treatments were carried out which consisted of different times (1, 3, 5 or 10 minutes) of immersion in sodium hypochlorite solution (NaOCl), in two concentrations of active chlorine 1.0% or 2.0% (v v^{-1}), followed by four rinses of the seeds in distilled and sterilized water. After disinfestation, the germination test was conducted. A total of 30 seeds from each treatment were distributed in a Petri dish with half-strength MS hormone-free medium. The dishes were maintained at 25 °C ± 2 °C with a 16-hour photoperiod and a photon flux density of 36 µmol m^{-2} s^{-1}. The following variables were evaluated: contamination (fungal and bacterial), germination (G%), and the germination speed index (GSI). The number of contaminated and germinated seeds was evaluated daily for 30 days (Maguire, 1962).

In vitro germination

First, disinfestation was carried out by immersing the seeds in a 70% (v v^{-1}) ethanol solution for one minute, followed by immersion in 2.0% (v v^{-1}) sodium hypochlorite solution for 10 minutes. Subsequently, four rinses of the seeds were performed in distilled and sterilized water. Seeds were introduced in different concentrations of the MS medium (MS1/3; MS1/2; MS100% of the salts and the control - agar/water), supplemented with 30 g L^{-1} of sucrose and 7 g L^{-1} of agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 20 minutes.

After introduction, the seeds were kept in a growth chamber with a photon flux density of 36 µmol m^{-2} s^{-1}, temperature of 25 °C ± 2 °C, and a photoperiod of 16 hours. The experiment was carried out with four treatments and four repetitions, one Petri dish with 50 seeds per repetition. The germination evaluation was performed daily for 30 days, recording the percentage of germinated seeds (G%) in each treatment and the germination speed index (GSI) calculated according to Maguire (1962). After 30 days, the seedlings' aerial part length was evaluated with a digital caliper.

Seedling development

The seedlings obtained from the germination test were subcultured in MS medium because it is the most suitable and used essential tissue culture medium for plant regeneration from tissues and callus. Two experiments were carried out to verify the seedling *in vitro* development: 1) Different salt concentrations of the MS medium (1/3; 1/2 and 100%) and 2) Culture medium 1/3MS salt strength, supplemented with different concentrations of BAP (6-benzylaminopurine). In the first experiment, the seedlings, when reaching 1 ± 0.5 cm in height, were subcultured in test tubes containing MS hormone-free medium, with different concentrations of salts (1/3; 1/2; 100% and the control - water/agar), with four replicates per treatment.

In the second experiment, the seedlings were subcultured in test tubes with 1/3 MS salt strength supplemented with 0.2 mg L^{-1} of auxin (naphthalenoacetic acid-ANA) and four concentrations of BAP (6-benzylaminopurine). The treatments consisted of BAP concentrations (0.0, 0.5, 1.0, and 1.5 mg L^{-1}) with four replications per treatment.

In both experiments, each repetition consisted of six test tubes containing one seedling, totaling 96 tubes. The culture media were supplemented with 30 g L^{-1} of sucrose and 7 g L^{-1} of agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121 ºC for 20 minutes. After transplanting the seedlings in the laminar flow, the tubes were transferred to a growth room with a temperature of 25 °C ± 2 ° C and a photoperiod of 16 hours for 6 weeks. Following that time, the height of the aerial part, number of leaves, number of roots, length of the most significant root, and number of shoots were recorded.

Acclimatization of tissue-cultured plants

The plants from the first phase of the *in vitro* development experiment, at 16 weeks, were transferred to plastic cups (50 mL) containing four different types of substrates arranged in a tray. The substrates used were Plantmax® (commercial substrate), coconut fiber with vermiculite (70% coconut fiber + 30% vermiculite), rice husks with vermiculite (70% rice husks + 30% vermiculite), and the sphagnum moss. The tray was initially covered with plastic film to increase the relative humidity. The plants were irrigated daily with distilled water and pre-conditioned in an incubator (Bio-Oxygen Demand-BOD) under a temperature of 25 °C and constant light for 60 days.

Seed cryopreservation

The water content of the seeds, before being frozen in liquid nitrogen (-196 ºC), was evaluated. The evaluation was conducted using the greenhouse method at 105 °C for 24 hours (Brazil, 2009), using five subsamples with 100 seeds each.

The seeds of *D. schwackei* were stored in cryotubes, immersed in a canister containing liquid nitrogen, and kept for 1, 6, 12, 24, 48, and 120 hours, except for the control group inoculated on the same day. After the completion of each storage period, cryotubes containing the seeds were removed from liquid nitrogen and thawed at room temperature for one hour. The next step was disinfestation in laminar flow. The seeds were immersed for one minute in ethanol (70%) and for ten minutes in sodium hypochlorite (2.0% active chlorine) in the sequence; four washes were carried out with sterile water.

The seeds were introduced in Petri dishes with half-strength MS hormone-free medium, supplemented with 30g L^{-1} of sucrose and 7 g L^{-1} of agar. The pH of the medium was adjusted between 5.6 and 5.8 and then sterilized in an autoclave for 20 min at 121 ºC. The experimental units were maintained in a growth room at 25 ° C ± 3 ºC under white fluorescent light (photon flux density of 36 µmol m^{-2} s^{-1}) and a photoperiod of 16 hours for 30 days.

The germination percentage of the seeds was evaluated for 30 days after inoculation. The analyzed variables comprised germination percentage (G%) and germination speed index (GSI). The experimental design was completely randomized, with four replications of 25 seeds each.

Statistical analysis

The experiments were subjected to analysis of variance (ANOVA), and the means were compared to each other by the Tukey test at 5% probability. The statistical analysis was performed using the program "R" version 3.5.0.

Results

There was no significant difference between treatments with different concentrations and immersion times with sodium hypochlorite for the contamination analysis. There was an interaction between the factors of immersion time and sodium hypochlorite concentration for germination percentage and germination speed index (Table 1). A higher percentage of germination with increasing concentrations of hypochlorite and immersion time can be verified.

Table 1. *In vitro,* germination (G%) and germination speed index (GSI) of *D. schwackei* seeds are a function of sodium hypochlorite concentrations (% of active chlorine) and different immersion times.

Immersion time (min)	Sodium hypochlorite concentrations			
	1% of active chlorine		2% of active chlorine	
	G%	GSI	G%	GSI
1	70 a	1.37 b	85 a	1.74 b
3	85 a	1.73 a	95 a	2.11 ab
5	73 a	1.63 ab	93 a	2.03 ab
10	80 a	1.60 ab	99 a	2.44 a
CV (%)	9.3	8.56	7.95	10.62

Means followed by the same letters in the column do not differ by Tukey's test ($p \leq 0,05$).

Higher GSI values were observed in treatments with higher concentration and immersion time. The active chlorine concentrations did not affect the vigor of *D. schwackei* seeds measured by GSI (Table 1). The seeds started germinating on the 10th day in all treatments. The highest percentage of seed germination was observed in the medium MS1/2 and MS1/3 of the salts (Table 2).

Table 2. *In vitro* Germination (G%), germination speed index (GSI), and height of the seedling aerial part (mm) are functions of the different salt concentrations of the MS medium.

Medium	G (%)	GSI	Seedling height (mm)
Agar/water	86 ab	3.34 a	2.94 c
MS1/3	93 a	3.25 a	4.39 a
MS1/2	96 a	3.56 a	3.61 b
MS100%	77 b	2.32 b	2.79 c
CV (%)	7.78	9.63	6.92

Means followed by the same letters in the column do not differ by Tukey's test ($p \leq 0,05$).

Regarding the length of seedlings, there were differences in the results according to the salt concentrations. In the culture medium without salt supplementation (agar/water) or at its highest concentration (MS100%), the seedling showed less growth (Table 2). The seedlings showed a more significant increase in the medium MS1/2 and MS1/3 of the salts. It is observed that the presence of salts and carbohydrates proved necessary for the maintenance of the seedling for an extended period (Fig. 1).

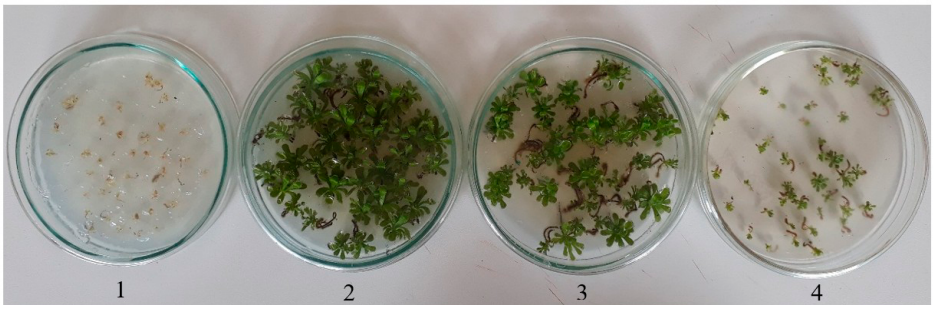

Fig. 1. Seedling growth as a function of the concentration of salts in the MS medium at 30 days of cultivation.
1 - Agar/water; 2 - MS1/3; 3 - MS ½ and 4 - MS100% of the salts.

The cryopreserved seeds of *D. schwackei* showed germination above 80% for all treatments except for the control. Germination was high with 1, 6, and 12 hours of cryopreservation.

Regarding the beginning of germination, all treatments, including time 0 (non-cryopreserved seeds), started germination at the same time, from the 11th day. Descriptively, the treatment with the lowest GSI was control (Table 3).

Cryopreservation did not interfere with plant development and morphology in any storage period. The plants emitted roots and leaves with an intense green color (Fig. 2).

Table 3. *In vitro* Germination (G%) and germination speed index (GSI) of *D. schwackei* seeds stored in liquid nitrogen (-196 °C) for 0, 1, 6, 12, 24, 48 and 120 hours.

Time (hour)	G (%)	GSI
0	75 b	1.44 b
1	95 a	2.01 a
6	93 a	1.64 ab
12	91 a	1.88 ab
24	90 ab	1.83 ab
48	90 ab	1.73 ab
120	89 ab	1.74 ab
CV (%)	7.50	11.74

Means followed by the same letters in the column do not differ by Tukey's test ($p \leq 0,05$).

Fig. 2. Normal seedlings obtained from the germination of *D. schwackei* seeds stored in liquid nitrogen (-196 °C) for 120 hours. A - radicle; B - hypocotyl; C - cotyledons; D - tegument; and E - aerial part.

Chlorosis and necrosis were not observed in the seedling leaves (Fig. 3), even in the media with the lowest concentrations of macronutrients. The average values recorded for shoot height, number of leaves, and number of roots were significantly higher when the seedlings were grown in the culture medium 1/3MS salt strength (Table 4).

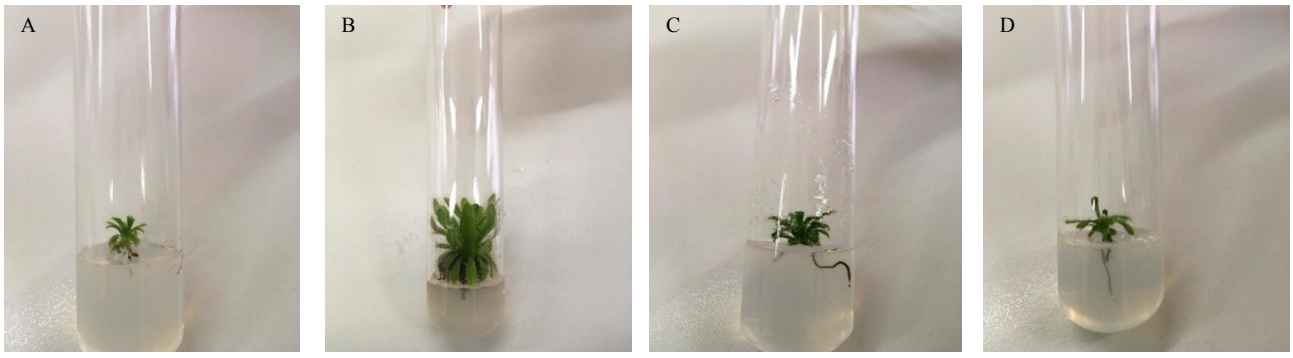

Fig. 3. *In vitro* growth of *D. schwackei* plants at different concentrations of salts in the MS culture medium. A - Agar/water; B - 1/3; C- ½ and D -100%.

Table 4. Effect of salt concentrations of the MS medium on the height of the aerial part (HAP), number of leaves (NL), number of roots (NR), and length of the most significant root (LLR) of *D. schwackei*.

Medium	HAP	NL	NR	LLR
Agar/water	3.10 c	6.12 d	1.37 c	3.81 b
MS1/3	12.74 a	24.66 a	8.79 a	9.30 a
MS1/2	6.80 b	14.87 b	2.58 b	7.89 ab
MS100%	6.04 b	11.99 c	1.87 c	6.55 b

Means followed by the same letters in the column do not differ by Tukey's test ($p \leq 0,05$)

The treatment with the MS1/2 medium provided seedlings with statistically intermediate values for the number of leaves and roots, while the MS100% medium was not beneficial for seedling growth. However, the treatment composed only of agar and water, the height of the aerial part and the number of leaves produced were significantly lower than the other treatments with the MS medium, stating that, even in lower concentrations, macronutrients are fundamental for the development of *D. schwackei* seedlings.

The response of the *D. schwackei* explants to the different concentrations of cytokinin (BAP) are shown in Table 5. With the increase in BAP doses, there was a progressive reduction in the size and number of explants in all variables analyzed in the 1/3MS salt strength. The lowest concentrations of BAP provided better results in the height of the aerial part, number of leaves, number of roots, and length of the most significant root, with a decreasing linear behavior (Table 5 and Fig. 4).

Table 5. Effect of BAP concentrations in 1/3MS salt strength on the height of the aerial part (HAP), number of leaves (NL), number of roots (NR), and length of the most significant root (LLR) of *D. schwackei*.

Treatment	HAP	NL	NR	LLR
0.0 mg L^{-1} de BAP	16.75 a	18.83 a	11.16 a	5.29 a
0.5 mg L^{-1} de BAP	11.44 b	14.67 b	4.29 b	3.00 b
1.0 mg L^{-1} de BAP	8.91 bc	7.62 c	2.46 b	2.03 bc
1.5 mg L^{-1} de BAP	6.66 c	5.37 c	2.25 b	1.33 c

Means followed by the same letters in the column do not differ by Tukey's test ($p \leq 0,05$).

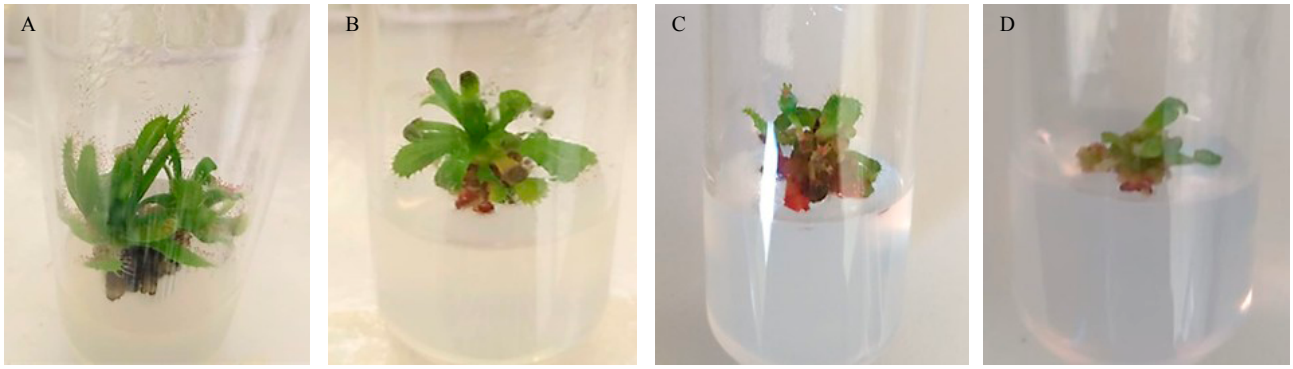

Fig. 4. *In vitro* growth of *D. schwackei* plants at different concentrations of BAP. A - 0.0 mg L^{-1}; B - 0.5 mg L^{-1}; C - 1.0 mg L^{-1} and D - 1.5 mg L^{-1}.

None of the treatments promoted the formation of shoots. When analyzing the height of the aerial part, significant differences were observed with the reduction of the cytokinin concentration; better results were obtained with the medium without the addition of BAP. Treatments without adding BAP were significantly higher than the others for the number of roots and the length of the most significant root.

In the trial evaluating the behavior and survival of *ex vitro* plants, *Sphagnum* moss stood out as the most efficient substrate, promoting a higher survival rate and better development of *D. schwackei* seedlings (Fig. 5). This result highlights its potential for use in the production of this species' seedlings.

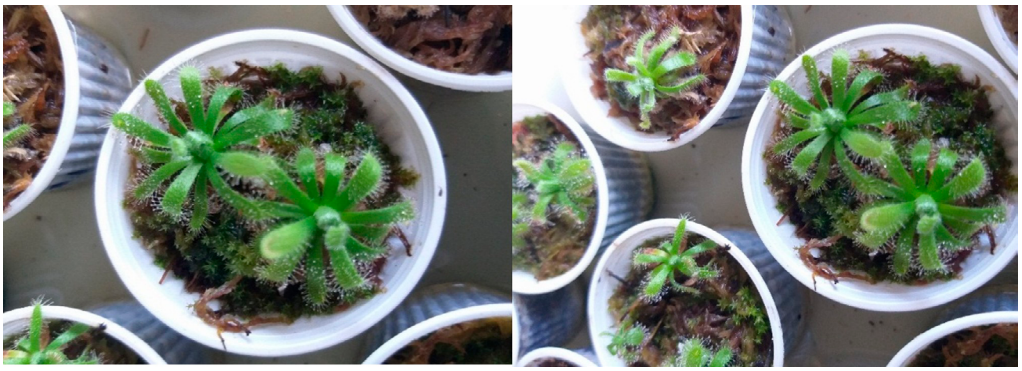

Fig. 5. *D. schwackei* plant acclimatized in *Sphagnum* moss.

Discussion

Seed disinfestation is a critical step for the success of *in vitro* propagation, as any failure in this process compromises the entire material. In this context, treatments with 1.0% and 2.0% active chlorine, with immersion times of 1, 3, 5, and 10 minutes, effectively controlled contamination. According to Mihovilović et al. (2024), sodium hypochlorite is the most efficient product for explant disinfestation. In the present study, the highest germination percentage was observed using 2% active chlorine and a 10-minute immersion, highlighting its effectiveness.

Germination began on the tenth day for all treatments and increased consistently over time. Higher concentrations and longer immersion times were associated with improved disinfestation and germination, as observed by Silva et al. (2014), who achieved 0% contamination in *Zephyranthes sylvatica* (Amaryllidaceae) seeds using 5% sodium hypochlorite for 20 minutes. Sodium hypochlorite, a powerful oxidizer, likely enhances germination by modifying seed coat membrane properties or increasing oxygen availability to the seed (Ahmed et al., 2022). This could explain the superior germination observed with 2% sodium hypochlorite for 10 minutes compared to 1% with shorter immersion times. The combination of higher concentrations and prolonged exposure may have increased the permeability of *D. schwackei* seeds, further promoting germination.

The salt concentrations in the culture medium affected the germination percentage of *D. schwackei*. Similar findings were reported by Pêgo et al. (2013), who observed reduced germination in *Syngonanthus elegantulus* (Eriocaulaceae) seeds, a native species of rupestrian fields, when exposed to higher salt concentrations in MS medium.

The MS medium contains 14 salts in its composition and is the most concentrated, which may have affected the G%, the GSI, and the length of *D. schwackei* seedlings due to the osmotic pressure. The decrease in the concentration of salts provided the highest germination rates—average concentrations of salts in the medium favor seed germination and seedling length. Lower concentrations of salts provide less osmotic pressure than that of a medium rich in nutrients, thus favoring the absorption of water by the seed and, consequently, its germination (Deinlein et al., 2014).

The vigor of the seeds, evaluated by the GSI, also showed different behaviors depending on the concentrations of the culture medium. The lowest GSI was observed in the highest concentration of 100% salts. The results of this study align with those of Reis et al. (2008), who, when evaluating different salt concentrations in MS medium (MS, MS/2, and MS/4), found that lower concentrations increased the germination speed index (GSI) and germination percentage (G%) in *Melissa officinalis* (Lamiaceae) seeds. The authors attributed these findings to the impact of higher salt concentrations on the osmotic potential, reducing the water availability required for the seed imbibition process during germination.

In *in vitro* seedling development agar and water alone are not sufficient to maintain the seedling. The use of carbohydrates and salts is necessary for supplementing the culture medium. The lack of supplementation affected the growth of seedlings and the high content of salts (MS100%) also reduced their growth. These results are similar to those of Pêgo et al. (2013), since studying "everlasting flowers" (species native to rupestrian fields), they observed that decreasing the concentration of salt in the culture medium positively affected the *in vitro* growth of *Syngonanthus elegantulus* and *Comanthera mucugensis* (Eriocaulaceae). Probably due to the adaptation of the "everlasting flowers", as well as *Drosera*, to shallow and poor soils, characteristic of rupestrian fields, in which they are found.

From the results observed by the differences between treatments, the use of culture medium 1/3MS salt strength is recommended, both for the virtuous development presented by the seedlings, as well as for the saving of material and maintenance time of the *in vitro* seedling.

The high production of seeds per fruit associated with the high rate of *in vitro* germination, allows the obtaining of a large number of individuals, without the need for multiplication via induction of side shoots, generally associated with the use of growth regulators (Bertsouklis et al., 2022). Thus, propagation by seeds allows for the maintenance of the genetic variability of the propagated individuals, which become essential for in situ conservation programs through reintroduction into the natural environment.

The water content of *D. schwackei* seeds was 20%. Seeds with a low water content are more conducive to cryopreservation because they reduce the possibility of intracellular ice crystals formation during freezing (Kashyap et al., 2020). The disruption of the endomembrane system results in loss of selective permeability and cell compartmentalization causing damage to plant tissue and making the development of a new plant unfeasible. The data show that exposure to liquid nitrogen (-196 ºC) does not interfere with the physiological quality of seeds preserved for up to 120 hours. Cryopreservation is an effective option for the long-term conservation of plant genetic resources, including ornamental plants, threatened plant species with unorthodox seeds or limited seed availability (Popova et al., 2023).

Studies with native species have found favorable results for the cryopreservation of seeds in *Jatropha curcas* (Euphorbiaceae) (Prada et al., 2015) and *Zephyranthes sylvatica* (Silva et al., 2014). The data presented reiterate that the cryopreservation of *D. schwackei* seeds may be a viable alternative for long-term conservation of the species.

Different studies report the absence of negative effects of cryopreservation on the germination of seeds of several species such as *Drosophyllum lusitanicum* (Drosophyllaceae), *Helianthus annuus* (Asteraceae) and *Hymusloto cephalus* (Zaidi et al., 2010). The results obtained also indicate that the gradual cooling rate is not necessary, because the direct immersion of cryotubes with seeds in liquid nitrogen did not decrease the germination percentages. This fact simplifies the cryopreservation process and significantly reduces costs (Zaidi et al., 2010). Therefore, cryopreservation is a reliable and economical method for conserving *D. schwackei* seeds.

Seedling cultivation in the presence of different concentrations of macronutrients did not negatively influence the survival of *D. schwackei* seedlings, which was 100% in all treatments. Indicating that the seedlings were not deficient in nutrients that regulate metabolic processes, such as nitrogen, phosphorus, potassium, calcium, magnesium and sulfur. However, the influence of different treatments can be observed in all morphological parameters evaluated in the seedlings.

The results recorded for *Cattleya cernua* (Orchidaceae) corroborate the finding that lower concentrations of macronutrients benefit *in vitro* growth and development (Sasamori et al., 2021). Regarding the

formation of the root system in *D. schwackei* seedlings, concentrations of macronutrients lower than the original in the MS medium were beneficial. The use of mediums with reduced concentrations of macronutrients can stimulate the formation and growth of the roots (Jose, 2023).

For *D. schwackei*, the gradual increase in the concentration of salts in the MS medium led to a linear decrease in the morphological parameters evaluated, being the medium with the lowest concentration of salts (MS1/3), considered suitable for the cultivation of this species. The carnivorous habit of *D. schwackei* can be an important factor in the lower requirement of mineral nutrients. In general, nutrients are obtained through the capture and digestion of small insects, thus justifying the observed results. The root system of these plants is underdeveloped, with few branches and many root hairs, probably to increase water absorption.

None of the treatments resulted in shoot formation. Conversely, lowering cytokinin levels increased seedling height. Similar results were observed by Jayaram and Prasad (2007) in *Drosera indica* (Droseraceae). Their study, which tested various concentrations of MS medium (1/4, 1/3, 1/2, and full strength) and BAP (0.01 to 2.0 mg L^{-1}), found that lower levels of cytokinins such as zeatin and kinetin promoted growth, while higher levels inhibited shoot formation.

The plants in the treatments of higher concentrations of BAP, were well developed and with root system. However, with less height of the aerial part and number of leaves. These results suggest a reduction in concentrations of this cytokinin, because, although very important, they can promote the opposite effect, when used in inadequate concentrations. Another suggestion would be to study a balance between cytokinin and auxin, to stimulate and control *in vitro* development.

Cytokinins are used during the *in vitro* multiplication phase, as they promote the breakdown of apical dominance and maximize the number of shoots, favoring propagation. However, in unsuitable concentrations, they can cause characteristic physiological disturbances in plants, such as reduction in elongation, shortening of internodes, vitrification, inhibition of rooting, tufting and others (Rivas et al., 2022).

The inhibitory effects of cytokinins on root regeneration and shoot elongation have been documented in several species (Nazir et al., 2022), possibly due to the phytotoxicity caused by growth regulators (Jan et al., 2020). An alternative to minimize the effects of cytokinins on *in vitro* growth of the aerial part and the rooting of plants is the transfer of the shoots to a nutrient medium free of growth regulator.

The substrates evaluated for acclimatization of plants obtained through *in vitro* cultivation can be grouped into three categories based on their survival rates. The highest survival rate was observed during the fifth week using a mixture of sphagnum moss and rice husk combined with vermiculite. Conversely, only one-third of the plants survived the same period when a coconut fiber and vermiculite mixture was used. Lastly, nearly 90% of the plants died when Plantmax® was used as the substrate.

Seedlings of *D. schwackei* subjected to acclimatization exhibited a survival rate of 50% after 60 days in biochemical oxygen demand (BOD) chambers, which declined to 25% by 90 days. This low survival rate highlights the fragility of *D. schwackei* tissues, which likely impedes their successful establishment *in vitro*. Additionally, plants experience substantial stress during acclimatization as they transition from controlled *in vitro* conditions to the external environ

The first instance of plant death was recorded with Plantmax®, where complete mortality occurred within 35 days of transferring the seedlings to the substrate. Plantmax® is a commercial substrate composed of various materials, including pine bark, peat, expanded vermiculite, and ground coal, characterized by its specific granulometry due to varying particle sizes. For *D. schwackei*, however, it proved inefficient, failing to support seedling survival.

The first signs of damage in non-surviving plants included dry and withered leaves. While the substrate's impact on plant height was less pronounced than its effect on survival, a gradient in plant height was observed. Seedlings grown in sphagnum moss developed larger rosettes compared to those grown in rice husk and vermiculite mixtures.

Choosing the right substrate is crucial for ensuring high survival rates during plant acclimatization (Schafer and Lerner, 2022). Substrates should retain adequate water while also allowing proper drainage and root aeration. Additionally, they should be free from saprophytic substances and infectious agents that could harm the plants. Sphagnum moss meets these requirements effectively, with its high water retention capacity and acidic pH, making it an ideal substrate and contributing to the highest survival rates observed.

The results obtained are consistent with those of Sender et al. (2022), who report that *Drosera* species often grow in substrates composed of *Sphagnum* moss, a dehydrated product derived from plants of the genus *Sphagnum* harvested from wetlands, characterized by its lightness, porosity, and high water retention capacity, ranging from 10 to 20 times its original weight, low mineral content, and a pH ranging from 3.5 to 4.0. These substrate characteristics may favor the growth of *Drosera*, given that these plants prefer moderately warm climates, acidic and nutrient-poor soils rich in organic matter, with a pH below 6 (Zarzycki and Szeląg, 2006). Furthermore, the favorable conditions provided by *Sphagnum* moss, combined with continuous light exposure, may have facilitated the acclimatization of the seedlings, as *Drosera* species are capable of growing under variable light intensities, adapting to prolonged sun exposure, maximizing light capture, and promoting photoprotection.

Conclusions

The disinfestation of *D. schwackei* seeds using a sodium hypochlorite solution with 2% active chlorine for 10 minutes of immersion is effective and does not harm germination. The cultivation of *D. schwackei* in MS1/3 and MS1/2 media promotes the *in vitro* germination of seeds and seedling growth. Additionally, low concentrations of BAP stimulate optimal plant development *in vitro*. *Drosera schwackei* seeds can be cryopreserved in liquid nitrogen (-196 ºC) without compromising their physiological quality for up to 120 hours of storage. The use of *Sphagnum* moss as a substrate is an effective alternative for the acclimatization of *D. schwackei* seedlings, promoting their healthy establishment under *ex vitro* conditions. Future research is essential to enhance *ex vitro* cultivation techniques for this species.

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Author contribution

KAA: Investigation, Methodology, Formal Analysis, Data Curation, Visualization, Writing - Original Draft, Writing - Review & Editing. **DAC:** Investigation, Writing - Review & Editing. **MT:** Investigation, Writing - Review & Editing. **FCN:** Writing - Review & Editing. **PMG:** Investigation, Writing - Review & Editing. **TOS:** Writing - Review & Editing. **MCN:** Investigation, Conceptualization, Methodology, Formal Analysis, Supervision, Resources, Validation, Writing - Original Draft, Writing - Review & Editing.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

Data will be made available upon request to the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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