GERMINAÇÃO E SOBREVIVÊNCIA DE PLÂNTULAS DE *RHIPSALIS* GAERTN. (CACTACEAE) EM DIFERENTES TEMPERATURAS E POTENCIAIS HÍDRICOS

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SUMÁRIO

INTRODUÇÃO GERAL

Semente é a unidade de dispersão comum das plantas conhecidas como espermatófitas, caracterizada por um embrião e tecidos de diferentes origens, com funções de reserva e revestimento (BEWLEY *et al.*, 2013). A semente representa uma das mais importantes inovações na história evolutiva das plantas, pois contribuiu para um grande aumento na diversidade desses organismos e consequente expansão de sua presença no ambiente terrestre (SCHULZE *et al.*, 2019). A germinação da semente, nome dado ao conjunto de processos fisiológicos que culminam na expansão e protrusão do eixo embrionário através dos tecidos envoltórios, depende principalmente de fatores abióticos que variam no tempo e no espaço, como a presença de água, O_2, luz e temperatura. Uma vez dispersas, as sementes podem permanecer no ambiente em estado quiescente, ou seja, não dormentes, até que requisitos específicos sejam atendidos (BASKIN & BASKIN, 2014). As condições ambientais bióticas e abióticas necessárias para que haja sucesso na germinação, estabelecimento e posterior maturação de um novo indivíduo definem o nicho de regeneração da planta, essencial para a compreensão da diversidade, evolução e manutenção das comunidades vegetais (GRUBB, 1977).

A disponibilidade hídrica é o fator ambiental mais importante para a germinação, pois ela afeta a entrada de água na semente, passo que dá início a uma série de modificações estruturais e metabólicas que caracterizam o processo germinativo (BEWLEY *et al*., 2013). A embebição se dá por conta da diferença entre os potenciais hídricos do meio e da semente, forças que governam o movimento da água, o aumento do potencial do meio externo leva a entrada de água na semente; à medida que o déficit hídrico se intensifica no ambiente, a porcentagem de sementes germinadas é reduzida, enquanto que o tempo para que a germinação ocorra é aumentado (BEWLEY *et al*., 2013). Outro fator crucial para a germinação é a temperatura, responsável por regular o metabolismo da semente; as sementes respondem à temperatura e a disponibilidade de água dentro de um determinado gradiente marcado por limites extremos e uma faixa ótima, onde geralmente se observam maiores valores de germinabilidade, sincronia e velocidade de germinação (BEWLEY *et al*., 2013). Os gradientes de potencial hídrico e temperatura dentro dos quais a germinação pode ocorrer indicam uma relação entre a variabilidade ambiental e a capacidade de adaptação da planta ao seu meio, dessa forma servem como pista para que a germinação ocorra no momento mais favorável ao desenvolvimento e crescimento (BASKIN & BASKIN, 2014). Por conta da susceptibilidade dos indivíduos quando recém-germinados, os primeiros estágios do

desenvolvimento vegetal são reconhecidamente importantes para a compreensão da composição e distribuição das comunidades vegetais (DONOHUE *et al*., 2010).

Em ambientes marcadamente heterogêneos, como é o caso dos ocupados por plantas epífitas, a diversidade de respostas germinativas das espécies tem ajudado a explicar sua ocorrência em campo (PEREIRA *et al*., 2009; WAGNER *et al*., 2013; MARQUES *et al*., 2014; DUARTE *et al*., 2017). Epífitas são aquelas que se fixam sobre outras plantas, utilizando-as apenas como suporte mecânico, sem estabelecer conexões para absorção de água ou nutrientes (ZOTZ, 2016); devido a essa forma de crescimento, estão relativamente mais expostas às flutuações de temperatura e umidade da atmosfera (MURAKAMI *et al*., 2023), não apenas por apresentarem grandes porções de suas raízes em contato com o ar, mas também pela enorme diversidade topográfica e de composição dos substratos disponíveis para ancoragem (TAY *et al*., 2023). Nestes, em geral, a água pode estar abundantemente disponível após chuvas intensas ou praticamente indisponível em períodos sem precipitação, pois são pouco capazes de retê-la por longos períodos (VENEKLAAS & VAN EK, 1990; CALLAWAY *et al*., 2002; SUN *et al*., 2018, 2022; TAY *et al*., 2023).

As plantas epífitas têm recebido pouca atenção em estudos de germinação e desenvolvimento pós-seminal, sendo que a maioria das espécies avaliada até o momento pertence à família Bromeliaceae (BASKIN & BASKIN, 2014; MONDRAGON & VALVERDE, 2015). Frequentemente, as espécies respondem às variações dos diferentes fatores ambientais de acordo com as condições em que podem ser observadas na natureza (PINHEIRO & BORGHETTI, 2003; BADER *et al*., 2009; MARÍN *et al*., 2008; CORREA & ZOTZ, 2014; EINZMANN & ZOTZ, 2014). De forma geral, a germinação de epífitas ocorre em um amplo gradiente de temperaturas (15 - 35°C), atingindo níveis ótimos entre 20 e 25°C (TSUTSUMI *et al*., 2011; MÜLLER *et al*., 2017). A disponibilidade de água tem sido considerada como o fator abiótico mais limitante para a ocorrência de plantas epífitas no ambiente natural (GENTRY & DODSON, 1987; DE LA ROSA MANZANO & BRIONES, 2010; GOODE & ALLEN, 2009; TSAI *et al*., 2023); embora possuam sementes relativamente tolerantes à seca, a sobrevivência de plântulas é afetada pela deficiência hídrica e o aumento das temperaturas pode ter papel importante na modulação dessas respostas (BADER *et al*. 2009; PEREIRA *et al*. 2009; MONTES-RECINAS *et al*., 2012; WAGNER *et al*., 2013; CORREA & ZOTZ, 2014; MARQUES *et al*., 2014; DUARTE *et al*., 2017).

Dentre os grupos com representantes epífitos que tiveram suas respostas germinativas menos exploradas, está à família Cactaceae (DE LA ROSA MANZANO & BRIONES, 2010; SIMÃO *et al*. 2010; LONE *et al*., 2016). Os cactos são reconhecidos por possuírem

características morfoanatômicas marcantes, como a transformação de folhas em espinhos, desenvolvimento de caules suculentos com parênquima fotossintetizante, e presença de meristemas axilares encurtados e especializados chamados de aréolas, de onde se originam estruturas vegetativas e reprodutivas (MAUSETH, 2006). Nativos das Américas, os cactos ocorrem em uma ampla diversidade de habitats, que vão do Canadá até a Patagônia (Mauseth 2016); principalmente em ambientes áridos e semiáridos, mas também em vegetações florestais, savânicas e campestres (TAYLOR & ZAPPI 2004; ZAPPI & TAYLOR 2020).

Os centros de diversidade das cactáceas localizam-se, em ordem crescente de representatividade, no México, sudoeste dos Estados Unidos, região central dos Andes e no leste do Brasil (TAYLOR, 1997), este último abriga endemismo em 74% de suas espécies (TAYLOR & ZAPPI 2004). Apenas uma espécie da família ocorre naturalmente fora do continente americano, a epífita *Rhipsalis baccifera* (J.M. Muell.) Stearn, que pode ser encontrada nas florestas tropicais do leste da África, Madagascar e Sri Lanka (BARTHLOTT, 1983). No Brasil está confirmada a ocorrência de 272 espécies e 37 gêneros de cactos, sendo que 14 gêneros e 188 espécies são endêmicos do país (ZAPPI & TAYLOR 2020). Cerca de 10% da diversidade da família Cactaceae é representada por espécies epífitas, distribuídas em duas subfamílias, Hylocereeae e Rhipsalideae (KOROTKOVA *et al*., 2021), mais comumente encontradas em vegetações florestais úmidas (ZAPPI & TAYLOR, 2020).

As respostas germinativas da família são frequentemente espécie-específicas, o que pode explicar sua ocorrência e distribuição, entretanto a maioria dos estudos tem focado em espécies terrestres (RAMÍREZ-PADILLA & VALVERDE, 2005; MARTINS *et al*., 2012; CONTRERAS-QUIROZ *et al*., 2016; GUERRERO *et al*., 2016; MEIADO *et al*., 2016; BAUK et al. 2017; LIMA & MEIADO, 2017). Geralmente os cactos germinam entre 20 e 30°C (Barrios *et al*., 2020), atingindo níveis ótimos a 25°C em média, com redução na germinabilidade próximo aos extremos de 15 e 35°C (MEIADO *et al*., 2016, SEAL *et al*., 2017). Quanto à disponibilidade hídrica, suas sementes germinam em potenciais hídricos maiores ou iguais a -0,6 MPa (MEIADO *et al*., 2010, GURVICH *et al*., 2017, FLORES *et al*., 2017, NASCIMENTO *et al*., 2018).

Apesar da germinação de algumas espécies de cactos ter sido observada sob temperaturas e secas muito mais intensas, a maioria das espécies estudadas é significativamente afetada ou mesmo inibida fora das condições consideradas ótimas para a família (RAMÍREZ-PADILLA & VALVERDE 2005; MEIADO *et al*., 2010, 2016; KIN *et al*., 2015; ORDÓÑEZ-SALANUEVA *et al*., 2015; GURVICH *et al*., 2017, FLORES *et al*., 2017, NASCIMENTO *et al*., 2018; LINDOW-LÓPEZ *et al*., 2018). Adicionalmente, após

passarem por estresse térmico e/ou hídrico, sementes não germinadas podem permanecer viáveis e possivelmente dormentes (KIN *et al*., 2015; MARTINS *et al*., 2012; BARRIOS *et al*., 2021; CRUZ *et al*., 2021). Espécies capazes de espalhar sua germinação ao longo do tempo podem aumentar as chances de que o estabelecimento ocorra sob condições mais favoráveis (MEIADO *et al*., 2010, 2016; MARTINS *et al*., 2012; KIN *et al*., 2015; ARAGÓN-GASTÉLUM *et al*., 2018), já que a exposição a extremos de temperatura e seca também tem influência sobre a sobrevivência de plântulas (VENABLE & LAWLOR, 1980; FLORES & BRIONES, 2001; KIN *et al*., 2015; GURVICH *et al*., 2017).

O efeito de diferentes fatores abióticos sobre as sementes de cactos epífitos foi, até o momento, pouco testado. Dentre as espécies estudadas, *Rhipsalis floccosa* Salm-Dyck ex Pfeiff., *R. pilocarpa* Loefgr. e *R. teres* (Vell.) Steud. apresentam altas porcentagens de germinação entre 15 e 25°C, entretanto as maiores velocidades de germinação dessas espécies foram observadas a 20 e 25°C (LONE *et al*., 2016). De forma semelhante, *Schlumbergera truncata* (Haw.) Moran também apresenta temperatura ótima de germinação a 20°C (LONE *et al*., 2010). Para *Hylocereus setaceus* (Salm-Dyck) R.Bauer a temperatura ótima está entre 25 e 30°C (SIMÃO *et al*., 2007). A única espécie epífita que teve sua germinação testada sobre baixa disponibilidade hídrica, *R. baccifera* sofreu redução na germinação em potenciais acima de -0.16 MPa (DE LA ROSA MANZANO & BRIONES, 2010). Tais respostas parecem estar de acordo com o observado na natureza, uma vez que as espécies estudadas são comuns em ambientes florestais mais úmidos e amenos, entretanto o baixo número de espécies restringe generalizações. Neste contexto, suas respostas germinativas podem fornecer importantes subsídios para estudos fisiológicos e ecológicos, sendo úteis também para melhor prever as consequências de possíveis alterações climáticas sobre as espécies e seu ambiente (COCHRANE *et al*., 2011; SOUDZILOVSKAIA *et al*., 2013; DÜR *et al*., 2015; GARDNER *et al*., 2019). As mudanças climáticas se relacionam com o desenvolvimento inicial das plantas ao modificar pistas ambientais essenciais para a germinação (e.g. temperatura e disponibilidade hídrica) podendo, por exemplo, deslocar a distribuição de espécies no espaço (BELLARD *et al*., 2012; GREMER *et al*., 2020).

Modelos climáticos propostos para as próximas décadas mostram que extensas áreas de vegetação tropical no hemisfério sul podem se tornar proporcionalmente mais quentes e secas (IPCC 2022), portanto, com potencial de alterar a distribuição e estrutura de populações de plantas epífitas (HSU & WOLF, 2013; MURAKAMI *et al*., 2023; TSAI *et al*., 2023). Ainda, estima-se que 60% das espécies de cactos correm grave risco de desaparecerem caso as previsões menos conservadoras para o clima se confirmem (PILLET *et al*., 2022). O Brasil

abriga grande diversidade de cactos epífitos, principalmente pertencentes à tribo Rhipsalideae, destacando-se o gênero *Rhipsalis* Gaertn., das 37 espécies que ocorrem no país, 33 são endêmicas (KOROTKOVA *et al*., 2021). Apesar de distribuídas por todo o território nacional, nas mais diversas fitofisionomias, muitas espécies do gênero possuem ocorrência associada ou restrita à Mata Atlântica (ZAPPI & TAYLOR 2020). Essa situação é agravada pela constante perda de habitats, como consequência da expansão da atividade humana, colocando as espécies de *Rhipsalis* entre os cactos mais ameaçados do país (CALVENTE *et al*., 2005; MAUSETH 2016; MEIADO & ALMEIDA, 2022).

Diante do exposto, a fim de contribuir para a construção de conhecimento acerca de um grupo de plantas ainda negligenciado em estudos ecofisiológicos, o presente trabalho objetivou avaliar o efeito da variação de temperatura e disponibilidade hídrica sobre parâmetros germinativos de oito espécies de cactos epífitos pertencentes ao gênero *Rhipsalis*, avaliar a manutenção de germinabilidade de sementes após exposição a esses fatores, verificar o efeito das condições testadas sobre a viabilidade de sementes não germinadas, bem como a sobrevivência das plântulas emergidas durante os tratamentos. Para tal, foram conduzidos separadamente tratamentos com diferentes temperaturas (15, 20, 25, 30 e 35°C) e potenciais hídricos (0; -0,2; -0,4; -0,6; -0,8 e -1,0 MPa), seguidos de tratamentos de recuperação térmica (a 25°C) e hídrica (água destilada), respectivamente, bem como teste de viabilidade com as sementes não germinadas.

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CAPÍTULO 1. Germination and seedling survival of *Rhipsalis* Gaertn. (Cactaceae) species in different temperatures and water potentials

Os resultados referentes ao presente estudo são doravante apresentados na íntegra em forma de artigo científico a ser submetido em periódico internacional relacionado aos temas específicos nele investigados.

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ABSTRACT

Water availability and temperature are the most important environmental factors regulating germination and seedling survival. Seed and seedling responses to these variables provide crucial information on plant species ecophysiology and their ability to adapt under the changing climate. We investigated seed germination, germination recovery capacity, seed viability, and seedling survival of eight species of *Rhipsalis* Gaertn., a widely distributed but threatened genus of epiphytic cacti in Brazil. Tests were performed in five temperature treatments (15, 20, 25, 30 and 35ºC) and six water availability treatments (0, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa). For most species, optimal germination was obtained between 20-25°C, and water potential and temperature variation resulted in low, unsynchronized germination and longer germination time. High temperatures and pronounced water deficit inhibited germination and promoted seedling mortality. However, all species showed some recovery capacity once put to germinate in the control conditions and at the end of all experiments seed viability was not affected. Our results indicate that *Rhipsalis* seeds can tolerate unfavorable conditions in their environment by spreading or arresting germination, nevertheless, the extension of heat and drought periods predicted for tropical environments in the next decades could alter germination cues and increase seedling mortality, suggesting that recruitment and establishment of new individuals might be impaired if such climate changes are confirmed in the future.
Keywords: cacti, epiphytes, seeds, stress, viability.

1. Introduction

The Cactaceae family is a distinctive group of plants native to the American continent, generally found in arid and semi-arid habitats subjected to extended periods of heat and drought (Mauseth, 2016). Most species of cacti are terrestrial, but epiphytes represent a large and widely distributed group in the Cactaceae family, comprising 10% of known species, which occur from the Caribbean and Central America, through all of South America (Mauseth, 2016; Korotkova *et al*., 2021). *Rhipsalis baccifera* (J.M. Muell.) Stearn is the only cacti species not endemic to the Americas and also occurs in East African and Southeast Asian islands (Barthlott & Taylor, 1995). Epiphytic cacti species are commonly found in humid forests but are also present in open physiognomies, such as savannas and rocky outcrops (Zappi & Taylor, 2020). Most species are placed within the tribes Hyloceereae and Rhipsalideae (Korotkova *et al*., 2017).

Rhipsalideae is composed of some of the most widely distributed epiphytic cacti species in South America, especially in Brazil, which holds one of the most important centers of diversity of this group of plants (Calvente *et al*., 2011); the genus *Rhipsalis* Gaertn. has 37 recognized native species, of which 33 are endemic (Korotkova *et al*., 2021). Adults present numerous pendent or decumbent stems, that can be cylindrical, angled or flattened, and generally spineless (Zappi & Taylor, 2020). In their natural environments, the Cactaceae family has fallen victim to increasing human activity expansion, especially those related to land use and climate change (Goettsch *et al*., 2015; Hultine *et al*., 2023; Pillet *et al*., 2022), which aggravates the situation for species occurring in isolated locations and unprotected areas, such as many *Rhipsalis* species (Calvente *et al*., 2005; Meiado & Almeida, 2022).

Water deficit is considered the most important factor limiting epiphyte species spatial distribution and growth (Gentry & Dodson, 1987; Goode & Allen, 2009; Tsai *et al*., 2023). Similarly to plants in arid and semi-arid habitats, water might be available to epiphytes only during short periods following precipitation (Zotz, 2016), due to the low water holding capacity of substrates used by epiphytes and lithophytes for mechanical support, such as tree bark, twigs and bare rock (Veneklaas & Van Ek, 1990; Callaway *et al*., 2002; Sun *et al*., 2018, 2022; Tay et al., 2023). Water and temperature are the most important environmental factors regulating seed germination, influencing the establishment of recently emerged seedlings in the field (Baskin & Baskin, 2014). Thus, the germinative and post-seminal responses to variation in environmental conditions provide crucial information related to

species distribution, while also indicating how species might perform under possible climate scenarios (Cochrane *et al*., 2011; Soudzilovskaia *et al*. 2013; Dür *et al*. 2015). Many tropical environments, including South America, are expected to become increasingly hotter and drier in the next few decades (IPCC 2022). In both natural and disturbed environments, plants are exposed to variations in water availability and temperature, conditions to which adult individuals might be considerably tolerant, however, during the earliest stages of development, plants can be especially vulnerable (Baskin & Baskin, 2022).

Generally, terrestrial cacti seeds germinate in water potentials between 0 and -0.6 MPa and reach optimal germination between 25°C and 30ºC. An increase in these thresholds progressively results in reduced, delayed, and unsynchronized germination or complete inhibition (Meiado *et al*., 2010; Flores *et al*., 2017; Barrios *et al*., 2020). Nonetheless, some cacti species may present optimal germination rates (e.g. higher and faster germination) at high temperatures, such as 35 and 40°C, and tolerate arid conditions (Guillén *et al*., 2011). On the other hand, in warm, forested ecosystems, cacti species germinate better between 20 and 25°C (Meiado *et al*., 2016). It has been reported that cacti seeds inhibited by temperature and moisture variation can recover once the stress is alleviated, reaching rates similar to those in optimal conditions (Kin *et al*., 2015; Barrios *et al*., 2021). Exposure to high temperatures and reduced water availability, even at conditions considered moderate, has been related to terrestrial cacti seedling mortality (Venable & Lawlor, 1980; Flores & Briones, 2001; Kin *et al*., 2015; Gurvich *et al*., 2017), therefore seeds that remain viable during unfavorable conditions and are capable of initiating or resuming germination after stress are at an advantageous position in resource restricted environments (Dubrovsky, 1996; Lima & Meiado, 2018; Nascimento *et al*., 2021).

The general trend in epiphytic cacti is to produce higher germination rates between 20 and 25°C without water restriction, while increasingly hotter and drier conditions affect species, lowering and inhibiting seed germination (Simão *et al*., 2007; De La Rosa Manzano & Briones, 2010; Lone *et al*., 2010, 2016). The germination of *Rhipsalis* species has remained relatively unexplored and studies focused on germinative responses to environmental variables are limited to a few species (De La Rosa Manzano & Briones, 2010; Lone *et al*., 2016). Additionally, studies investigating the effect of temperature variation and water restriction on *Rhipsalis* species seed viability and post-seminal development are absent.

Within this context, the present study aimed to evaluate the germination parameters of eight *Rhipsalis* species with natural occurrence in Brazil in constant temperatures and osmotic potential treatments in order to answer the following questions: (1) What is the optimal

germination temperature for the *Rhipsalis* species studied? (2) Are germination and seedling survival affected by high temperatures and low water availability? (3) Are seeds of *Rhipsalis* able to recover their germinability after being exposed to different osmotic potentials and different temperatures? We expected species to reach optimal germination between 20 and 25°C, with pronounced temperature and water stress reducing seed germination and seedling survival but not seed viability.

2. Materials and methods

2.1. Species

Seeds from eight *Rhipsalis* species were used, according to the description in Table 1. According to the International Union for Conservation of Nature, *R. cereoides* species is considered near threatened, *R. dissimilis* is considered endangered and *R. pilocarpa* is considered vulnerable; the remainder of species are considered of least concern (Taylor & Zappi, 2013; 2017). Some species of *Rhipsalis* can be found growing both as epiphytes or lithophytes, but most species have only been observed in either the former or latter life forms (Barthlott & Taylor, 1995; Figure 1).

2.2. Experimental design

Fruits were harvested from at least three different individuals. Seeds were manually removed from the fleshy fruits and washed in running tap water until complete removal of the mucilaginous pulp, then placed to dry on filter paper for at least 24 hours at laboratory room temperature (± 25°C). Seeds were then stored in glass vials in the dark at laboratory room temperature. All experiments were executed within 3 months maximum after seed collection.

To determine the optimal temperature for germination and address the effect of high temperatures on germination, seeds were subjected to five constant temperature treatments: (I) 15°C, (II) 20°C, (III) 25°C, (IV) 30°C, and (V) 35°C. To address the effect of low water availability, seeds were subjected to different water restriction treatments at 25°C, with six osmotic potentials: (I) 0 (distilled water), (II) -0.2, (III) -0.4, (IV) -0.6, (V) -0.8 and (VI) -1.0 MPa. The osmotic potentials are dependent on temperature. For the water restriction treatments, polyethylene glycol (PEG 6000) solutions were prepared to be used at 25°C, according to Villela *et al*. (1991). Dishes containing 5 ml of PEG solutions were sealed with transparent plastic film (polyvinyl chloride) and solutions were replaced every 15 days to avoid alteration in solute concentration. To address seed germinability maintenance or

germination recovery capacity after exposure to different temperature and water restriction treatments, at the end of the 60-day record period, ungerminated seeds from the following temperature treatments, (I) 15°C, (IV) 30°C and (V) 35°C, and from all osmotic potentials were transferred to control conditions (25°C + distilled water), and a second 60-day record period was conducted. The temperature of 25°C was selected to incubate the control, osmotic stress, and recovery treatments because it is considered optimal for the germination of most cacti species (Barrios *et al*., 2020).

All treatments consisted of four repetitions of 25 seeds each for most species; for *R. cereoides* four repetitions of 20 seeds were used, for *R. dissimilis* the control and the 25°C treatments consisted of three repetitions of 10 seeds each while the 30 and 35°C treatments consisted of four repetitions of 12 seeds each, due to low seed production during the period of the study. Seeds were sown in 90 mm diameter Petri dishes lined with double layer filter paper, moistened with distilled water. Dishes were placed in germination chambers (Eletrolab®) and incubated under a 12 hour photoperiod of white fluorescent light (60 $\mu mol.m^{-2}.s^{-1}$). Germination was recorded daily for 60 days for all species. Germination was considered as seed radicle protrusion (Figure 2).

Seeds that did not germinate at the end of all experiments were subjected to the tetrazolium test (1% 2,3,5-triphenyl-2H-tetrazolium) to address their viability. Incubation was done in the dark for 72 hours at 25°C, and then seeds were manually dissected using a scalp and a stereo microscope. The total percentages of germinated and ungerminated (viable and unviable) were calculated based on the total number of seeds put to germinate in each treatment (treatment + recovery). When turgid embryos colored in pink, they were counted as viable. Darkened, softened embryos were counted as unviable.

For each repetition, the following parameters were calculated according to Ranal & Santana (2006): (1) Germination percentage [(ΣniN-1)100] as the ratio between the total number of germinated seeds (Σni) and the total number of seeds placed to germinate (N). (2) Mean germination time (MGT), (t=Σni.ti/Σni in which ti is the time in days for the onset of germination to the nth observation and ni is the number of seeds germinated in the nth observation during time i. (3) Germination synchrony index ($\bar{E}$), ($\bar{E}$ = - Σfi.log2fi in which fi is the relative frequency of germinated seeds within a time interval.

At the end of each germination treatment followed by its corresponding recovery period, seedling survival was calculated as the ratio of the total number of live seedlings to the total number of germinated seeds. Bright green colored and turgid seedlings were considered as living (Figure 2). Seedlings that presented darkened, softened tissues were considered dead.

2.3. Statistical analysis

Germination percentage, MGT, Ē and seedling survival percentage were tested using ANOVA followed by Tukey test ($\alpha<0.05$). Shapiro-Wilk and Bartlett tests were employed to verify data normality and homogeneity, respectively. When variance assumptions could not be met, data was transformed in Log_{10} or square root. Seedling survival was tested using Kruskal-Wallis followed by Bonferroni test ($\alpha<0.05$). Treatments were used as fixed factors. All analyses were conducted using the software R, version 4.3.0 (R Development Core Team, 2023).

3. Results

3.1. Responses to temperature

The highest germination percentages occurred at 20 and 25°C, except for *R. cereoides*, which germinated more at 20°C (Table 2). Incubation at 15°C affected germination for most species, except for *R. dissimilis* and *R. pilocarpa*, to which germination at 15, 20 and 25°C did not differ (Table 2). High-temperature treatments (30 and 35°C) negatively and progressively impacted all species, but *R. grandiflora* and *pilocarpa* germination reached more than 50% at 30°C (Table 2). Germination of *R. floccosa* subsp. *floccosa* was completely inhibited at 30 and 35°C, while *R. baccifera* subsp. *baccifera* and *R. lindbergiana* were inhibited only at 35°C.

Temperature treatments also affected the mean germination time (MGT) of species. Faster germination was observed at both 20 and 25°C in most study species, except for *R. cereoides* and *R. floccosa* subsp. *floccosa* which germinated faster at 20°C (Table 2). With the exception of *R. dissimilis*, for which germination was faster at 30°C in relation to 15 and 35°C, the lowest and highest temperatures delayed germination for all species and differences in the MGT between the 15, 30 and 35°C treatments were not observed (Table 2).

Both 20 and 25°C promoted more synchronized germination for most species, except for *R. cereoides*, which presented more synchrony at 25°C. Incubation at 15°C increased the germination synchrony for *R. baccifera* subsp. *baccifera* and decreased germination synchrony for *R. dissimilis* and *R. pilocarpa*. Incubation at 30 and 35°C did not affect germination synchrony for most species, with the exceptions of *R. dissimilis* (at 30 and 35°C) and *R. neves-armondii* (at 30°C), which germinated more synchronically in relation to 25°C, and *R. pilocarpa* for which 30°C reduced the germination synchrony (Table 2).

Most species displayed some germination recovery capacity after being exposed to the lowest and highest temperatures (Table 2). Germination recovery from 15°C was low in most

species, with the exception of *R. grandiflora* and *R. neves-armondii* which were able to germinate as much in the recovery as the seeds that were incubated in the control condition (Table 2). *Rhipsalis cereoides* and *R. lindbergiana* did not show germination recovery capacity after incubation at 15°C. Germination recovery from 30 and 35°C was also low for most species, with the exception of *R. floccosa* subsp. *floccosa* which was able to germinate as much in both recovery treatments as the seeds in the control, and *R. pilocarpa* which germinated as much as the control only after the 35°C treatment (Table 2). *R. baccifera* subsp. *baccifera* germination recovery was higher from 35°C than from 30°C; *R. cereoides* and *R. lindbergiana* only recovered germination from either 35 or 30°C, respectively (Table 2).

For most species, germination during recovery was delayed when compared to the control condition (Table 2). For *R. floccosa* subsp. *floccosa*, the MGT was not affected in the recovery treatments from 30 and 35°C, and for *R. grandiflora* and *R. lindbergiana*, the MGT was not affected in the recoveries from 15 and 30°C (Table 2). The germination synchrony of most species was affected during recovery. *Rhipsalis floccosa* subsp. *floccosa* and *R. neves-armondii* recovering from 15°C germinated more synchronically than the control; recovery from 30°C reached similar germination synchrony to the control for most species, while recovery from 35°C increased germination synchrony (Table 2). The synchrony of *R. pilocarpa* increased during the 30°C recovery and decreased during the 35°C recovery (Table 2).

3.2. Responses to water potentials (Ψ)

Water deficit negatively affected all study species, and as the concentration of PEG increased, germination was gradually reduced (Table 3). Seeds of *R. dissimilis*, *R. floccosa* subsp. *floccosa*, *R. grandiflora* and *R. pilocarpa* tolerated water deficiency at -0.2 MPa and germination did not decrease in relation to the control, whereas, for *R. baccifera* subsp. *baccifera*, *R. cereoides*, *R. lindbergiana* and *R. neves-armondii* germination was reduced at -0.2 MPa (Table 3). From -0.4 MPa onwards germination decreased sharply for *R. cereoides*, *R. floccosa* subsp. *floccosa*, and *R. lindbergiana*, but for *R. baccifera* subsp. *baccifera*, *R. dissimilis*, *R. neves-armondii*, and *R. pilocarpa* the decrease in germination was moderate (Table 3). *Rhipsalis grandiflora* was the only to present high germination at -0.4 MPa (>90%) and to germinate moderately at -0.6 MPa (Table 3). In turn, -0.6 MPa drastically reduced the germination of *R. baccifera* subsp. *baccifera*, *R. cereoides*, *R. dissimilis*, and *R. lindbergiana*, and inhibited the germination of *R. neves-armondii*, *R. floccosa* subsp. *Floccose*, and *R. pilocarpa*. More pronounced water deficits (-0.8 and -1.0 MPa) inhibited germination in all

species.

All PEG concentrations delayed germination for all species tested but means did not differ between treatments in most cases. For *R. dissimilis* and *R. pilocarpa*, MGT gradually increased as PEG concentrations increased (Table 3). The majority of species did not show alterations in germination synchrony during osmotic stress (Table 3). Incubation in the -0.2 MPa potential decreased germination synchrony for *R. dissimilis* and *R. lindbergiana*, and increased for *R. neves-armondii*. For *R. lindbergiana* and *R. neves-armondii*, -0.4 MPa produced more synchronized germination (Table 3).

During recovery, germination decreased equally between treatments for most species (Table 3). *Rhipsalis floccosa* subsp. *floccosa* germinated as much as the control in the -0.6, -0.8 and -1.0 MPa recoveries, *R. neves-armondii* germinated as much as the control only in the 0.8 MPa recovery, and *R. pilocarpa* recoveries from both -0.8 and -1.0 MPa did not differ from the control (Table 3). R. cereoides was the only species unable to recover from the highest PEG concentrations (Table 3). Most species during the recovery from different PEG concentrations showed MGT similar to the control, but for *R. baccifera* subsp. *baccifera, R. cereoides*, and *R. dissimilis* MGT increased. R. *floccosa* subsp. *floccosa* germinated as fast as the control in all recovery treatments (Table 3). Germination synchronization during recovery treatments was also not altered in most cases; *R. dissimilis, R. floccosa* subsp. *floccosa* and *R. neves-armondii* germination synchrony increased during -0.4 MPa recovery. For *R. grandiflora*, recoveries decreased germination synchrony (Table 3).

3.3. Seedling responses to temperature and water potentials ***(Ψ)***

Temperature treatments did not affect seedling survival percentage for most of the species. High seedling survival was observed in all temperature treatments (Table 2). Temperature variation negatively impacted *R. floccosa* subsp. *floccosa* only at 15°C; at 30°C, seedling survival was low for *R. lindbergiana*, and at 35ºC, *R. neves-armondii* showed complete mortality (Table 2). Seedlings that emerged during the recovery treatments also did not show a decrease in survival for most species. Only *R. neves-armondii* showed complete mortality for the 30°C recovery (Table 2).

Different PEG solutions did not affect seedling survival for the majority of study species. At the first osmotic potential tested (-0.2 MPa), seedling survival of *R. dissimilis* and *R. neves-armondii* decreased and was absent for *R. cereoides*. At -0.4 MPa, seedling survival decreased for *R. lindbergiana* (Table 3). All seedlings from *R. floccosa* subsp. *floccosa, R. dissimilis* and *R. neves-armondii* emerged during the -0.4 MPa treatment failed to survive, all

seedlings from *R. cereoides* and *R. dissimilis* emerged during the -0.6 MPa treatment also did not survive (Table 3). Recovery from different PEG solutions did not affect seedling survival for the majority of species; the only exception was *R. cereoides*, which showed complete mortality for the -0.2, -0.4 and -0.6 MPa recovery treatments (Table 3).

3.4. Seed viability

A high proportion of ungerminated seeds from all temperature and PEG concentrations treatments were viable by the tetrazolium test (Table 4).

4. Discussion

4.1. Responses to temperature

Germinability, mean time of germination, and synchrony are important parameters to describe the influence of environmental factors on the germinative process (Santana & Ranal, 2006). Our results showed the majority of *Rhipsalis* species performed better when temperatures were cool (20-25°C), reaching not only higher germinability, but also faster and more synchronized germination. Nonetheless, *R. cereoides* and *R. floccosa* subsp. *floccosa* germinated faster at 20°C, this confirms our hypothesis that species would reach optimal germination between 20 and 25°C. Although *R. dissimilis* and *R. pilocarpa* maintained high germination at 15°C and *R. dissimilis*, *R. grandiflora* and *R. pilocarpa* showed tolerance to higher temperatures (30 and 35°C), treatments gradually lead to lower, slower and unsynchronized germination. In some cases, higher temperatures produced more germination synchrony; however, this increase may be due to the reduced number of seeds germinated at the same time, which can influence the germination synchrony index (Meiado *et al.*, 2010). Epiphytic bromeliads and terrestrial cacti species that establish in warm, moist forests have been reported to display a similar pattern of optimum germination at cooler conditions and a tendency towards germination inhibition between 30 and 35°C (Pinheiro & Borghetti, 2003; Marques *et al.*, 2014; Meiado *et al.*, 2016; Seal *et al.*, 2017), similar to what was observed for the *Rhipsalis* species studied.

A given species' cardinal temperatures comprise a range of minimum (T_b), optimal (T_o), and maximum temperatures (T_c) beyond which germination of a plant is not possible (Bewley *et al.*, 2013). Seeds adapted to respond to the range of temperatures present in their environment can tune their germination to more adequate conditions in time and space, which is crucial for the establishment of new individuals (Montes-Recinas *et al.*, 2012; Lima &

Meiado, 2018). By doing so, plants avoid exposing recently emerged and vulnerable seedlings to conditions that could be potentially fatal (Rojas-Aréchiga, 1998; Ortega-Baes & Rojas-Aréchiga, 2007). Epiphyte species richness and abundance are higher in wet forests, such as the Atlantic Forest domain, which in turn offer relatively less seasonality and higher precipitation rates than open vegetation (Leitman *et al*., 2015; Ramos *et al*., 2021; Murakami *et al*., 2023); additionally, the majority of *Rhipsalis* species described occur exclusively in the Atlantic Forest (Zappi & Taylor, 2020). Our results suggest that seed germination of the study species will be more successful if such milder temperatures and humid conditions are met.

After the 60-day recording period, due to low germination related to the impact of temperature variations, many seeds of the study species did not germinate. The species *R. baccifera* subsp *baccifera*; *R. floccosa* subsp. *floccosa*, and *R. lindbergiana* even exhibited complete inhibition of germination at high temperatures. Incubation beyond the optimum range of a determinate species can induce a state known as thermo-inhibition, in which seeds remain viable under potential stress until optimal conditions are available (Hills & Van Staden, 2003). Viability maintenance after thermic stress has been observed in terrestrial cacti (Ramírez-Padilla & Valverde, 2005; Kin *et al*., 2015; Aragón-Gastélum *et al*., 2018), however, very little is known about this process, since cacti seed recovery from thermo-inhibition has rarely been accessed (Barrios *et al*., 2021). All *Rhipsalis* species were able to germinate in at least one of the recoveries from the 15, 30 and 35°C treatments. During recovery, germination was delayed and synchrony was not affected. The ability to in fact recover germinability was not the same between temperatures and most species responded to them differently. *R. pilocarpa*, for example, recovered more than 90% of germination after 35°C, but for species like *R. baccifera* subsp. *baccifera*, *R. cereoides* and *R. lindbergiana* germination was very low (<10%) or absent.

During recovery treatments, it was observed that the negative impacts on the germination were not followed by loss in seed viability. Once subjected to the tetrazolium test, cut embryos colored in pink, indicating that most seeds were viable, suggesting that temperatures below and above optimal might have induced some type of dormancy state. Dormancy can be defined by a state in which a seed will not germinate even if all essential requirements of temperature, water, light and O_2 of a given seed are present (Baskin & Baskin, 2014). Exposure to fluctuating temperatures and other factors such as light have been known to influence dormancy induction and release patterns in terrestrial cacti, allowing species to form seed banks (Rojas-Aréchiga & Mandujano-Sánchez, 2017; Ordóñez-Salanueva *et al*., 2017). If seeds are able to retain viability in response to abiotic factors, they

may persist longer in the environment, which may have strong implications on species distribution and regeneration performances in their natural habitats and in future climate scenarios (Grubb, 1977; Baskin & Baskin, 2022).

4.2. Responses to water potentials (Ψ)

Water availability is considered the most important factor regulating epiphyte presence and establishment in the field (Zotz, 2016), and as expected, increased PEG concentrations significantly impacted the germination of *Rhipsalis* species. *R. dissimilis*, *R. floccosa* subsp. *floccosa*, *R. grandiflora* and *R. pilocarpa* were not negatively affected at the first osmotic potential tested (-0.2 MPa) and were able to maintain moderate germination (>30%) at -0.4 MPa, however, as treatments progressed, germination became slower and unsynchronized. Only one species, *R. grandiflora*, displayed tolerance to lower osmotic potentials and was able to germinate moderately at -0.6 MPa. Nevertheless, MGT and Ē results showed that even minimal water deficiency during imbibition produced slower and, unsynchronized germination in *Rhipsalis* species. The increase in dryness conditions induced by the lowest osmotic potentials (-0.8 and -1.0) completely inhibited seeds. Studies have shown that in arid habitats, species considered tolerant to drought can germinate in relatively drier conditions, however, even in such habitats, their germination progressively decreases during water restriction (Flores & Briones, 2001; Bader *et al*., 2009; Pereira *et al*., 2009; Guillén *et al*., 2011; Flores *et al*., 2017). Most cacti and epiphytes cannot germinate at potentials below -0.6 MPa, and reach the highest germination when water is abundant (Montes-Recinas *et al*., 2012; Meiado *et al*., 2010; Nascimento *et al*., 2018; Cruz *et al*., 2021). Similarly to the temperature treatments, very few seeds germinating at the same time can influence the germination synchrony index to show that water deficiency treatments increased germination synchrony (Meiado *et al*., 2010). Germination depends on the amount of water the substrate offers and how fast it can enter the seed; this relationship will determine how long water must be available in the substrate for germination of a given species to be completed (Bewley *et al*., 2013). Delaying or arresting germination during drier periods may allow epiphytic cacti like *Rhipsalis* to better match their environment, avoiding the risk of exposing seedlings to fatal desiccation (Montes-Recinas *et al*., 2012).

The effect of alleviation of osmotic stress on cacti seeds has received some attention from seed physiologists (Barrios *et al*., 2020). Species that fail to germinate when exposed to limited quantities of water will commonly display high germination rates, sometimes

surpassing the control condition once seeds have been put to recover (Kin *et al*., 2015; Santini *et al*., 2017). *Rhipsalis* species showed a similar ability and germinated after 60 days of exposure to different levels of water deficit. As would be expected, when species were able to germinate in low water potentials, respective recovery treatments did not germinate much probably because most of the fittest seeds from the lot had already germinated. Nonetheless, the majority of the study species reached 50% or higher germination during recovery, with no significant MGT or synchrony alterations. In arid habitats, terrestrial cacti and other epiphytic plants, like atmospheric bromeliads, are commonly subjected to cycles of intense precipitation followed by droughts (Meiado *et al*., 2012; Correa & Zotz, 2014). These hydration-dehydration cycles may trigger imbibition and alter the functioning of the seeds; however, if water is no longer available before germination can be completed, seeds may retain structural and metabolic changes (Allen *et al*., 1993; López-Urrutia *et al*., 2014). Known as seed hydration memory, this adaptation allows seeds to remain viable in the field and homogenizes the physiological state of a given lot; consequently, seeds germinate more and faster once water is abundant and puts recently emerged seedlings at an advantageous standpoint in relation to species which might take longer to germinate (Dubrovsky, 1996). PEG acts by binding itself to water molecules in order to create resistance against their movement towards the interior of the seed (Bradford, 1986). Exposing seeds to water deficiency through different PEG concentrations mimics the process of discontinuous hydration to which plants might be naturally subjected (Lima & Meiado, 2017). Our results suggest that despite their presence in more mesic environments, epiphytic cacti like *Rhipsalis* are adapted to take advantage of the intermittent water supply that might be available on tree bark, rocks and other canopy substrates, extending their viability through time. Only *R. cereoides*, a rare lithophyte species restricted to inselbergs produced little to no germination during recovery; in such unique habitats, harsh conditions tend to select highly specialized seed traits (Hunter, 2003). The recovery responses observed for the *Rhipsalis* species studied is the first evidence of hydration memory in epiphytic Cactaceae. More research is required to confirm if these species can exhibit such a memory response to hydration. Ungerminated seeds of all species were considered viable as most embryos tested positive in the tetrazolium test. Similar to high and low temperatures, long exposure to PEG concentrations induced a dormancy state in *Rhipsalis* seeds. It is not known how long epiphytic cacti seeds may be able to retain viability or how abiotical factors like temperature and water are related to dormancy induction and possible release in species from this group of plants. This field of investigation could provide important answers regarding the functional diversity of epiphytic cacti as well as how species

will deal with the impending climate.

4.3. Seedling responses to temperature and water potentials ***(Ψ)***

Due to their small size and slow growth, epiphytic cacti seedlings may pose a challenge for ecophysiological investigations. At below and above optimal temperature conditions, seedling survival of the majority of study species was not altered. For cacti in arid habitats, the successful establishment has been linked to seedlings' ability to benefit from transient resources, such as water availability, to reach a considerable size before possible stressful conditions are installed on the environment (Dubrovsky, 1997; Kin, *et al*., 2015). Even when few seeds germinate, recently emerged and tolerant seedlings of *Rhipsalis* species may survive in their epiphytic habitats during adverse periods, however, low water availability affected a higher number of species than temperature and lead to complete mortality in some cases. Water restriction is considered the most important factor related to the low establishment and high mortality rates of epiphytic seedlings in the field (Bader *et al*., 2009; Olaya-Arenas *et al*., 2011; Montes-Recinas *et al*., 2012); the seedling mortality observed during more severe conditions indicate that this stage could be as sensitive as seed germination for *Rhipsalis* species, compromising species regeneration (Pérez-Noyola *et al*., 2020). In arid habitats, cacti and other xeric plants seedling survival and establishment are commonly associated to the presence of nurse plants or rocks that provide relatively milder temperature and humidity conditions under their shades (Godínez-Álvarez *et al*., 2003; Munguía-Rosas & Sosa, 2008). Our results suggest that *Rhipsalis* seedlings may require consistent periods of hydration after they emerge to prevent fatal dehydration. Also, curiously, seedlings that emerged during the recovery from both temperature and osmotic potential treatments were not negatively affected; these results indicate that the study species' ability to maintain viability and germinability during stress is not compromised after periods of exposure to stressful conditions. Plant species from distinct taxa that present seed hydration memory have been shown to produce stress-tolerant seedlings after hydration-dehydration cycles, enhancing the seedling successful establishment (Lima & Meiado, 2018; Freitas & Silva, 2023; Oliveira *et al*., 2024). Future studies are needed to better access those impacts on *Rhipsalis* as well as other epiphytic cacti species seedlings.

4.4. Conclusions

Our results showed that species germinate abundantly and faster in mild environments while increasing temperatures and low water availability were detrimental to their germination. Mean annual temperature elevations and decreasing water availability are key factors for epiphyte community composition and diversity alterations (Rapp & Silman, 2014; Vale *et al*., 2021; Tsai *et al*., 2023). The Atlantic Forest, home to the highest diversity of epiphytic cacti in South America (Mauseth, 2016) is one of such vast areas of vegetation where climatic change is predicted to affect biodiversity (Murakami *et al*., 2023). Epiphytic cacti are considered one of the plant groups most threatened by climate change. This is due to the expanding geographic range of rising temperatures and decreasing rainfall, which overlaps with their natural habitats. In addition, heat and drought periods are becoming more prolonged (Pillet *et al*., 2022). Retaining viable seeds can be an advantage for plants in such scenarios, but if the environment remains inadequate for extended periods or natural climatic patterns are modified, seeds can miss germination cues and lose their viability (Aragón-Gastélum *et al*., 2018). If milder temperatures and abundant water are not present, rare and slow-growing epiphytic plants, such as *Rhipsalis* species, may fail to recruit and establish new individuals.

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7. Tables and figures

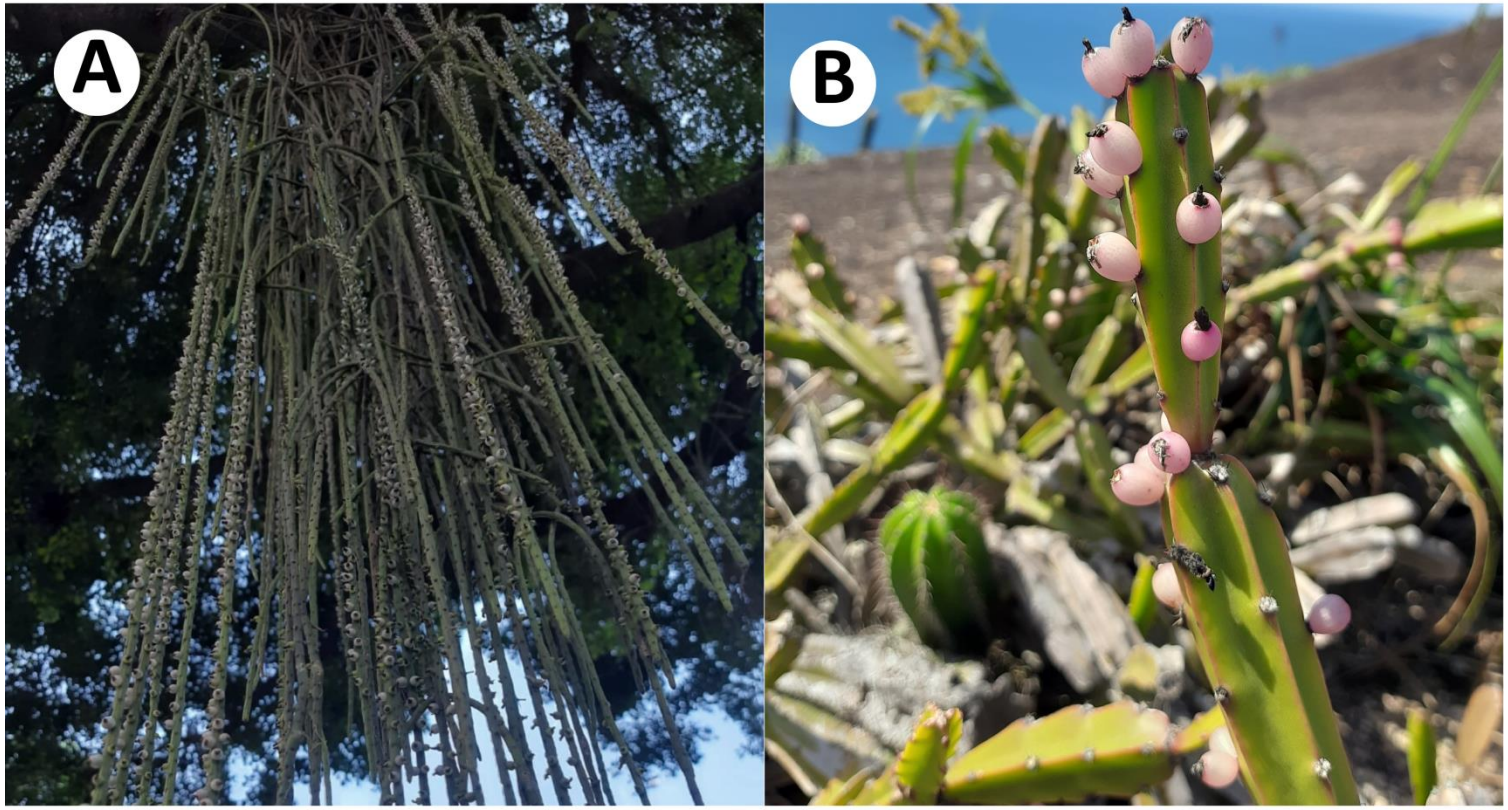

Figure 1. *Rhipsalis* species with different life forms. **(A)** Fruit-bearing individual of *R. lindbergiana* growing on tree bark in the Arboretum of the Rio de Janeiro Botanical Garden, Rio de Janeiro municipality, Rio de Janeiro, Brazil. **(B)** Fruit-bearing individual of *R. cereoides*, a lithophyte species growing on inselberg vegetation in Serra da Tiririca State Park, Niterói municipality, Rio de Janeiro, Brazil. Photographs taken by Diego R. Gonzaga.

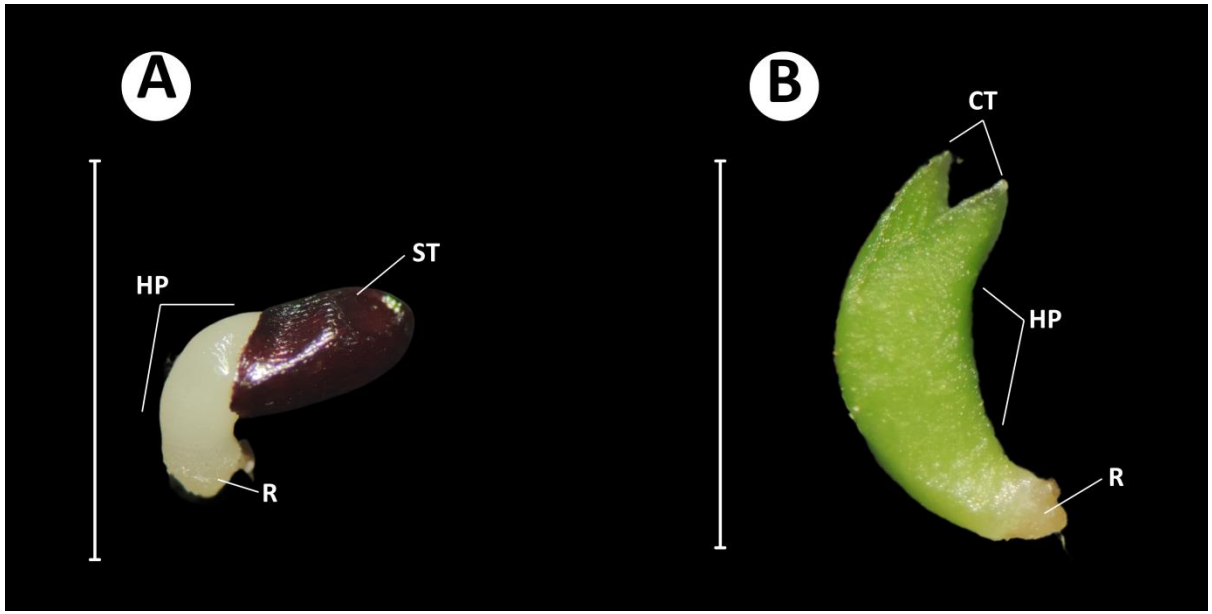

Figure 2. Seed approximately 24 hours after germination (A) and one-week old seedling (B) of *R. lindbergiana*, showing the general aspect of *Rhipsalis* species during initial development. CT: cotyledons. HP: hypocotyl. R: radicle. ST: seed testa. Bars: 2mm. Photographs taken by Rafael R. Gonçalo.

Table 1. Data on *Rhipsalis* species and seed collection. Life form of individuals whose seeds were collected. Vegetation type according to Zappi & Taylor (2020).

Species	Vegetation type	Life form	Collection period	Collection site	Herbarium voucher
R. baccifera (J.M.Muell.) Stearn subsp. *baccifera*	Coastal Forest (Restinga); Forest	Epiphyte	Nov/2023	Belém (Pará)	HF #5044
R. cereoides (Backeb. & Voll) Backeb.	Coastal Forest (Restinga); Forest; Inselberg	Lithophyte	Jan/2024	Niterói (Rio de Janeiro)	RB #626860
R. dissimilis (G.Lindb.) K.Schum.	Grassland; Rock outcrop	Epiphyte	Nov/2022	Salto (São Paulo)	VIES#60388
R. floccosa Salm-Dyck ex Pfeiff. subsp. *floccosa*	Coastal Forest (Restinga); Forest; Rock outcrop	Epiphyte	Nov/2022	Salto (São Paulo)	R#248699
R. grandiflora Haw.	Coastal Forest (Restinga); Forest; Rock outcrop	Epiphyte	Dez/2022	Ubatuba (São Paulo)	HASSI #processing
R. lindbergiana K.Schum.	Coastal Forest (Restinga);	Epiphyte	Jan/2024	Rio de Janeiro (Rio de Janeiro)	RB #829638

Species	Vegetation type	Life form	Collection period	Collection site	Herbarium voucher
R. neves-armondii K.Schum.	Forest Coastal Forest (Restinga); Forest	Epiphyte	Nov/2022	Casimiro de Abreu (Rio de Janeiro)	R#248694
R. pilocarpa Loefgr.	Forest	Epiphyte	Nov/2023	Maracaí (São Paulo)	HASSI #processing

HASSI: Herbário Assisense. HF: Herbarium of the Universidade Federal do Pará. R: Herbarium of the National Museum of Brazil. RB: Instituto de Pesquisas do Jardim Botânico do Rio de Janeiro. VIES: Herbarium of the Universidade Federal do Espírito Santo.

Table 2. Mean (± standard error) of seed germination percentage, mean germination time, germination synchronization index and seedling survival percentage of *Rhipsalis* species in different temperatures, and respective recovery treatments. Percentage of recovery was calculated based on the number of ungerminated seeds coming from each previous treatment. Different upper case letters indicate significant differences between the control (25°C) and recovery germination treatments by ANOVA followed by Tukey test (α = 5%). Different lower case letters indicate significant differences between germination treatments of different temperatures by ANOVA followed by Tukey test (α = 5%). Different upper case letters indicate significant differences between seedling survival of the control (25°C) and recovery treatments by Kruskal-Wallis followed by Bonferroni test (α = 5%). Different lower case letters indicate significant differences between seedling survival percentages in different temperatures by Kruskal-Wallis followed by Bonferroni test (α = 5%).Ē: germination synchronization index, G: germination, MGT: mean germination time. n/g: no germination. n/c: not calculated. rec: recovery. *: not included in the analyses. **: mean for one repetition not included in the analyses.

Species	Treatment (°C)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
R. baccifera subsp. *baccifera*	15	12 ± 5b	36 ± 8b	0.8 ± 0.3b	100a
	15rec	19 ± 9C	15 ± 7AB	0.1 ± 0.1B	100A

Species	Treatment (°C)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
	20	98 ± 2a	7 ± 0.2a	1.7 ± 0.2a	100a
	25	96 ± 4Aa	6 ± 0,1Aa	1.6 ± 0.3ABa	99 ± 2Aa
	30	3 ± 2b	n/c	n/c	67 ± 58a
	30rec	5 ± 2C	14 ± 0**	0.4**	100A
	35	n/g	n/g	n/g	n/g
	35rec	50 ± 10B	17 ± 1B	2.3 ± 0.3A	100A
R. cereoides	15	13 ± 10c	44 ± 6*	1 ± 0.5*	100a
	15rec	n/g	n/g	n/g	n/g
	20	70 ± 14a	11 ± 0.6a	2.4 ± 0.5a	89 ± 13a
	25	39 ± 10b	8 ± 1b	1 ± 0.2b	95 ± 8a
	30	11 ± 0**	15 ± 0**	0.4 ± 0**	100**
	30rec	n/g	n/g	n/g	n/g
	35	12 ± 11*	16 ± 0**	0.8 ± 0**	12 ± 16**
	35rec	8 ± 2*	47 ± 0**	0.6 ± 0**	50 ± 70*
R. dissimilis	15	83 ± 11a	22 ± 2b	3.8 ± 0.8a	74 ± 31a
	15rec	n/c	n/c	n/c	n/c
	20	99 ± 2a	7 ± 0.4a	2.6 ± 0.4b	100a
	25	90 ± 7a	8 ± 0.3a	2.4 ± 0.1b	93 ± 11a
	30	36 ± 18b	14 ± 2c	0.8 ± 0.4c	50 ± 58a
	30rec	9 ± 10**	6 ± 5**	n/c	100*
	35	31 ± 11b	37 ± 12b	0.5 ± 0.3c	50 ± 41a
	35rec	4**	4**	n/c	0**

Species	Treatment (°C)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
R. floccosa subsp. *floccosa*	15	54 ± 10b	42 ± 5c	2.8 ± 0.6a	43 ± 14b
	15rec	32 ± 10B	11 ± 3A	0.8 ± 0.4C	74 ± 21A
	20	84 ± 13a	10 ± 1b	2.6 ± 0.5a	97 ± 3a
	25	82 ± 17Aa	17 ± 5Aa	3 ± 0.2Aa	100Aa
	30	n/g	n/g	n/g	n/g
	30rec	64 ± 25AB	13 ± 2A	2.8 ± 0.5AB	94 ± 5A
	35	n/g	n/g	n/g	n/g
	35rec	67 ± 25AB	25 ± 14A	2.2 ± 0.6B	98 ± 4A
R. grandiflora	15	46 ± 21b	23 ± 6b	2 ± 0.6a	91 ± 13a
	15rec	62 ± 35A	8 ± 2A	1.2 ± 0.3AB	94 ± 12A
	20	98 ± 2a	6 ± 0.1a	2 ± 0.2a	84 ± 7a
	25	100 ± 0Aa	6 ± 0.5Aa	1.7 ± 0.4Aa	96 ± 8Aa
	30	55 ± 7b	16 ± 4b	2.8 ± 0.6a	83 ± 12a
	30rec	8 ± 6B	3 ± 0**	0.4**	50 ± 50A
	35	2 ± 2*	n/c	n/c	100*
	35rec	18 ± 4B	12 ± 2B	1 ± 0.3B	95 ± 10a
R. lindbergiana	15	48 ± 13b	19 ± 1b	2 ± 0.3a	81 ± 16ab
	15rec	n/g	n/g	n/g	n/g
	20	99 ± 2a	9 ± 0.2a	2 ± 0.1a	98 ± 4a
	25	98 ± 2Aa	9 ± 0.5Aa	2 ± 1Aa	99 ± 2a

Species	Treatment (°C)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
	30	25 ± 13b	29 ± 12b	1.3 ± 0.6a	37 ± 34b
	30rec	29 ± 10B	9 ± 5A	1 ± 0.3A	73 ± 23A
	35	n/g	n/g	n/g	n/g
	35rec	n/g	n/g	n/g	n/g
R. neves-armondii	15	64 ± 13b	29 ± 8b	2.9 ± 0.5a	84 ± 14a
	15rec	57 ± 32A	21 ± 15A	1 ± 0.3B	87 ± 25A
	20	90 ± 3a	9 ± 0.6a	2.7 ± 0.2a	98 ± 5a
	25	85 ± 9Aab	9 ± 2Aa	3 ± 0.3Aa	95 ± 9a
	30	12 ± 13c	29 ± 25ab	0.7 ± 0.4b	90 ± 16a
	30rec	3 ± 3*	n/c	n/c	0*
	35	6 ± 8*	20 ± 7*	0.6 ± 0.3*	0
	35rec	4 ± 4B	6 ± 0**	0.3**	33 ± 58A
R. pilocarpa	15	95 ± 5a	23 ± 1b	3.5 ± 0.5b	100a
	15rec	n/c	n/c	n/c	n/c
	20	99 ± 2a	11 ± 1a	2.3 ± 0a	100a
	25	100 ± 0Aa	9 ± 2Aa	2.4 ± 0.2Aac	99 ± 2a
	30	60 ± 10b	21 ± 2b	2.8 ± 0.2bc	95 ± 10a
	30rec	60 ± 21B	14 ± 3B	1.4 ± 0.5B	100A
	35	4 ± 3c	43 ± 0**	0.1 ± 0.2**	50 ± 50a
	35rec	99 ± 2A	12 ± 1AB	3.7 ± 0.6C	98 ± 2A

Species	Treatment (°C)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
					[1]

When there was absence of germination or seedling survival in less than three repetitions, means were not included in the analyses. For treatments that had at least two germinated seeds in only one repetition, Ē and MGT were calculated for that one repetition but not included in the analyses. When there was only one germinated seed per replica, MGT and Ē could not be calculated. Also, in some cases when germination was high (± 80%), the number of ungerminated seeds was insufficient to calculate the means of corresponding recovery treatments.

Table 3. Mean (± standard error) of seed germination percentage, mean germination time, germination synchronization index and seedling survival percentage of *Rhipsalis* species in different polyethylene glycol concentrations, and respective recovery treatments. Percentage of recovery was calculated based on the number of ungerminated seeds coming from each previous treatment. Different upper case letters indicate significant differences between the control (0 MPa) and recovery treatments by ANOVA followed by Tukey test (α = 5%). Different lower case letters indicate significant differences between treatments of different polyethylene-glycol concentrations by ANOVA followed by Tukey test (α = 5%). Different upper case letters indicate significant differences between the seedling survival of the control (0 MPa) and recovery treatments by Kruskal-Wallis followed by Bonferroni test (α = 5%). Different lower case letters indicate significant differences between the seedling survival percentages in different polyethylene-glycol concentrations by Kruskal-Wallis followed by Bonferroni test (α = 5%). Ē: germination synchronization index, G: germination, MGT: mean germination time. n/g: no germination. n/c: not calculated. rec: recovery. *: not included in the analyses. **mean for one repetition not included in the analyses.

Species	Treatment (MPa)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
R. baccifera subsp. *baccifera*	0	96 ± 4Aa	6 ± 0.1Aa	1.6 ± 0.3Aa	99 ± 2Aa
	-0.2	50 ± 20b	13 ± 4b	1.9 ± 0.6a	100a

[1]

Species	Treatment (MPa)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
	-0.2rec	23 ± 6B	14 ± 5B	0.7 ± 0.3B	100A
	-0.4	39 ± 8b	15 ± 4b	1.5 ± 0.4a	95 ± 6a
	-0.4rec	24 ± 12B	14 ± 3B	0.8 ± 0.4B	95 ± 10A
	-0.6	1 ± 2**	n/c	n/c	100**
	-0.6rec	21 ± 10B	22 ± 8B	1 ± 0.4AB	84 ± 24A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	28 ± 6B	13 ± 9AB	1.5 ± 0.5AB	100A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	13 ± 3B	11 ± 5AB	0.8 ± 0.3B	100A
R. cereoides	0	39 ± 10a	8 ± 1a	1 ± 0.2a	95 ± 8a
	-0.2	1 ± 0**	9 ± 0**	0.3 ± 0**	0**
	-0.2rec	1 ± 0**	21 ± 0**	0.2 ± 0**	0**
	-0.4	14 ± 2b	26 ± 3b	0.5 ± 0.1b	71 ± 21a
	-0.4rec	1 ± 0**	20 ± 0**	0.2 ± 0**	0**
	-0.6	5 ± 4c	31 ± 0**	0.6 ± 0**	0
	-0.6rec	3 ± 3*	n/c	n/c	0*
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	n/g	n/g	n/g	n/g
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	n/g	n/g	n/g	n/g
R. dissimilis	0	90 ± 7Aa	8 ± 0.3Aa	2.4 ± 0.1Aa	67 ± 11Aa
	-0.2	82 ± 6a	12 ± 1b	3.4 ± 0.7b	31 ± 18b

Species	Treatment (MPa)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
	-0.2rec	25 ± 32*	12 ± 0**	0.2**	100*
	-0.4	50 ± 13b	31 ± 2c	2 ± 0.3a	0
	-0.4rec	31 ± 27B	17 ± 1C	1 ± 0.6B	100A
	-0.6	6 ± 9*	34 ± 1**	0.9**	0*
	-0.6rec	53 ± 7B	6 ± 1A	2.5 ± 0.7A	91 ± 18A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	61 ± 12B	9 ± 2AB	2.8 ± 0.4A	98 ± 4A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	65 ± 8B	12 ± 2B	3 ± 0.8A	96 ± 7A
R. floccosa subsp. *floccosa*	0	82 ± 17Aa	17 ± 5Aa	3 ± 0.2Aa	82 ± 17a
	-0.2	75 ± 8a	29 ± 1b	2.5 ± 0.6a	94 ± 8a
	-0.2rec	33 ± 31BC	9 ± 4*	0.5 ± 0.2*	83 ± 29A
	-0.4	13 ± 12b	21 ± 5ab	1 ± 0.6b	0
	-0.4rec	21 ± 16C	6 ± 2B	1 ± 0.8B	97 ± 5A
	-0.6	n/g	n/g	n/g	n/g
	-0.6rec	69 ± 8AB	8 ± 2B	3 ± 0.5A	82 ± 12A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	77 ± 12A	6 ± 1B	2.7 ± 0.4A	93 ± 10A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	78 ± 9A	4 ± 2B	2.2 ± 0.4A	85 ±7A
R. grandiflora	0	100 ± 0Aa	6 ± 0.5Aa	1.7 ± 0.4Aa	96 ± 8Aa
	-0.2	96 ± 4a	18 ± 3b	2.9 ± 1ab	93 ± 6a

Species	Treatment (MPa)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
	-0.2rec	n/c	n/c	n/c	n/c
	-0.4	95 ± 4a	24 ± 3b	3.3 ± 0.5b	72 ± 16a
	-0.4rec	n/c	n/c	n/c	n/c
	-0.6	35 ± 18b	9 ± 3a	1.6 ± 0.7a	64 ± 28a
	-0.6rec	56 ± 16B	4 ± 2A	0.9 ± 0.2A	96 ± 8A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	75 ± 16B	7 ± 1A	3 ± 0.7B	84 ± 16A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	67 ± 16B	9 ± 4A	3 ± 0.6B	94 ± 8A
R. lindbergiana	0	98 ± 2Aa	9 ± 0.5Aa	2 ± 1Aab	97 ± 2Aa
	-0.2	78 ± 5b	18 ± 0.4b	3 ± 0.2b	84 ± 12a
	-0.2rec	44 ± 30B	9 ± 7A	0.6 ± 0.2A	89 ± 19A
	-0.4	34 ± 11c	22 ± 3b	1 ± 0.4c	22 ± 21b
	-0.4rec	15 ± 7B	13 ± 7A	0.6 ± 0.4A	79 ± 25A
	-0.6	4 ± 3d	37**	0.6**	100a
	-0.6rec	18 ± 8B	7 ± 2A	1 ± 0.5A	100A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	46 ± 24B	7 ± 2A	2 ± 1A	82 ± 24A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	15 ± 11B	13 ± 10A	0.8 ± 0.5A	58 ± 28A
R. neves-armondii	0	85 ± 9Aa	9 ± 2Aa	3 ± 0.3Aa	81 ± 9a
	-0.2	45 ± 20b	25 ± 10b	1.8 ± 0.7b	45 ± 32a

Species	Treatment (MPa)	G (%)	MGT (days)	Ē(bits)	Seedling survival (%)
	-0.2rec	27 ± 3C	14 ± 7A	0.8 ± 0.2B	64 ± 33A
	-0.4	24 ± 11b	33 ± 2b	1.1 ± 0.2b	0
	-0.4rec	31 ± 17BC	10 ± 2A	1.3 ± 0.7B	97 ± 5A
	-0.6	5 ± 4c	29 ± 1*	0.4 ± 0.1*	0
	-0.6rec	47 ± 10B	13 ± 4A	2.3 ± 0.4AB	77 ± 35A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	57 ± 33ABC	13 ± 7A	2.7 ± 1.4A	100A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	67 ± 24B	7 ± 1A	2.6 ± 0.1A	78 ± 29A
R. pilocarpa	0	100 ± 0Aa	9 ± 2Aa	2.4 ± 0.2Aa	99 ± 2a
	-0.2	91 ± 9ab	20 ± 2b	2.6 ± 0.7a	99 ± 1a
	-0.2rec	n/c	n/c	n/c	100*
	-0.4	65 ± 30b	35 ± 6c	2 ± 0.7a	99 ± 2a
	-0.4rec	65 ± 33B	10 ± 3A	1.5 ± 1.2A	77 ± 33A
	-0.6	n/g	n/g	n/g	n/g
	-0.6rec	68 ± 18B	12 ± 2A	3.3 ± 0.7A	75 ± 29A
	-0.8	n/g	n/g	n/g	n/g
	-0.8rec	93 ± 5AB	8 ± 2A	3.6 ± 0.9A	73 ± 18A
	-1.0	n/g	n/g	n/g	n/g
	-1.0rec	92 ± 6ABb	8 ± 1A	3.1 ± 0.1A	73 ± 15A[2]

When there was absence of germination or seedling survival in less than three repetitions, means were not included in the analyses. For treatments that had at

[2]

least two germinated seeds in only one repetition, Ē and MGT were calculated for that one repetition but not included in the analyses. When there was only one germinated seed per replica, MGT and Ē could not be calculated. Also, in some cases when germination was high (± 80%), the number of ungerminated seeds was insufficient to calculate the means of corresponding recovery treatments.

Table 4. Total percentage of germinated and ungerminated (viable or unviable) seeds of *Rhipsalis* species evaluated at the end of all different temperature and osmotic potential treatments accessed by the tetrazolium test. Percentages calculated based on the total number of seeds put to germinate at the beginning of each experiment (treatment + recovery). rec: recovery.

Species	Treatment	Seeds (%)		
		Ungerminated		Germinated
		Viable	Unviable	
	Temperature (°C)			
R. baccifera subsp. *baccifera*	15 + rec	70	1	29
	20	8	0	92
	25	4	0	96
	30 + rec	91	1	8
	35 + rec	50	0	50
	Osmotic potential (MPa)			
	-0.2 + rec	39	0	61
	-0.4 + rec	47	0	53
	-0.6 + rec	79	0	21
	-0.8 + rec	71	1	28
	-1.0 + rec	84	3	13
	Temperature (°C)			
R. cereoides	15 + rec	86	1	13
	20	28	1	71
	25	71	0	29
	30 + rec	96	1	3
	35 + rec	75	15	10
	Osmotic potential (MPa)			
	-0.2 + rec	94	4	2
	-0.4 + rec	74	10	16

Species	Treatment	Seeds (%)		
		Ungerminated		Germinated
		Viable	Unviable	
	-0.6 + rec	81	14	5
	-0.8 + rec	86	14	0
	-1.0 + rec	86	14	0
	Temperature (°C)			
	15 + rec	17	0	83
	20	1	0	99
	25	9	1	90
	30 + rec	52	3	45
R. dissimilis	35 + rec	63	3	35
	Osmotic potential (MPa)			
	-0.2 + rec	13	2	85
	-0.4 + rec	33	1	66
	-0.6 + rec	32	1	57
	-0.8 + rec	38	1	61
	-1.0 + rec	34	2	64
	Temperature (°C)			
	15 + rec	49	0	54
	20	9	7	84
	25	17	2	81
	30 + rec	33	4	63
R. floccosa subsp. *floccosa*	35 + rec	25	8	67
	Osmotic potential (MPa)			
	-0.2 + rec	19	1	80
	-0.4 + rec	22	47	31
	-0.6 + rec	23	8	69
	-0.8 + rec	24	0	76
	-1.0 + rec	21	1	78
	Temperature (°C)			
	15 + rec	54	0	46
	20	1	1	98
	25	0	0	100

Species	Treatment	Seeds (%)		
		Ungerminated		Germinated
		Viable	Unviable	
R. grandiflora	30 + rec	38	4	58
	35 + rec	76	3	21
	Osmotic potential (MPa)			
	-0.2 + rec	3	0	97
	-0.4 + rec	5	0	95
	-0.6 + rec	28	1	71
	-0.8 + rec	26	0	74
	-1.0 + rec	33	0	67
	Temperature (°C)			
R. lindbergiana	15 + rec	52	0	48
	20	1	0	99
	25	2	0	98
	30 + rec	53	1	46
	35 + rec	99	1	0
	Osmotic potential (MPa)			
	-0.2 + rec	2	3	86
	-0.4 + rec	49	7	44
	-0.6 + rec	75	5	21
	-0.8 + rec	46	11	43
	-1.0 + rec	70	15	15
	Temperature (°C)			
R. neves-armondii	15 + rec	33	3	64
	20	10	0	90
	25	15	0	85
	30 + rec	86	0	16
	35 + rec	89	0	11
	Osmotic potential (MPa)			
	-0.2 + rec	31	9	60
	-0.4 + rec	30	23	47
	-0.6 + rec	39	11	50

Species	Treatment	Seeds (%)		
		Ungerminated		Germinated
		Viable	Unviable	
	-0.8 + rec	38	5	57
	-1.0 + rec	30	3	67
	Temperature (°C)			
	15 + rec	5	0	95
	20	1	0	99
	25	0	0	100
	30 + rec	13	1	86
R. pilocarpa	35 + rec	0	1	99
	Osmotic potential (MPa)			
	-0.2 + rec	4	1	95
	-0.4 + rec	7	8	85
	-0.6 + rec	29	1	70
	-0.8 + rec	7	0	93
	-1.0 + rec	5	2	92