

Germination dynamics of *Neoglaziovia variegata* (Arruda) Mez (Bromeliaceae): impact of thermal and saline thresholds on the ecology of the species

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ABSTRACT: Global warming significantly impacts the environment, directly affecting plant development. *Neoglaziovia variegata* (Arruda) Mez is a Bromeliaceae endemic to the Caatinga, has highly resistant fibers used for income generation and has landscaping and medicinal potential. The study aimed to identify the thermal and saline thresholds that influence the germination of this species. In different experiments, the seeds were placed to germinate at constant temperatures between 15 and 40 °C and in substrate soaked in NaCl solution from 0 to 14 dS.m⁻¹. The combination of thermal and salt stresses and the recovery of seeds after stress were also evaluated. All experiments followed a completely randomized design, with four replications of 25 seeds and lasted 28 days. The data were analyzed by thermal time and halotime models, establishing the thresholds for germination. The minimum and maximum temperatures for germination were 14.3 and 41.7 °C, respectively, with the ideal temperature of 31.1 °C. The germination threshold in saline medium was 16.2 dS.m⁻¹. The seeds showed viability after being removed from the stress condition. The wide temperature range and high saline threshold show that the species can survive the most pessimistic climate change scenarios.

Index terms: abiotic factors, Caatinga, caroá, climate change.

RESUMO: O aquecimento global impacta significativamente o meio ambiente, afetando diretamente o desenvolvimento das plantas. *Neoglaziovia variegata* é uma Bromeliaceae endêmica da Caatinga, possui fibras de alta resistência usadas para geração de renda e apresenta potencial paisagístico e medicinal. O estudo teve como objetivo identificar os limites térmicos e salinos que influenciam a germinação dessa espécie. As sementes foram colocadas para germinar em temperaturas constantes entre 15 e 40 °C e em outro experimento em substrato embebido em solução de NaCl de 0 a 14 dS.m⁻¹. Foi também avaliada a combinação de estresses térmicos e salinos e a recuperação das sementes a condições estressantes. Os experimentos seguiram um delineamento inteiramente casualizado, com quatro repetições de 25 sementes durante 28 dias. Os dados foram analisados pelos modelos de tempo térmico e salino, estabelecendo-se os limites para germinação. As temperaturas mínimas e máximas para germinação são 14,3 e 41,7 °C, respectivamente, com a temperatura ideal de 31,1 °C. O limite de germinação em meio salino é 16,2 dS.m⁻¹. As sementes demonstraram viabilidade após serem removidas do estresse. A ampla faixa de temperatura e o alto limite salino mostram que a espécie pode sobreviver aos cenários mais pessimistas de mudanças climáticas.

Termos para indexação: Fatores abióticos, Caatinga, caroá, mudanças climáticas.

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INTRODUCTION

Germination can be defined as the resumption of embryo growth, culminating in the formation of a normal and healthy seedling (Brasil, 2009). For the germination process to start, the seed needs to leave its dormant or quiescent state under favorable environmental conditions for its growth and development (Soltani et al., 2022). Temperature and water availability are the most important abiotic factors during germination (Silva et al., 2020). Temperature is related to the chemical reactions that occur in cells, interfering with the speed, percentage, and uniformity of germination, while the water absorbed by the seed signals the resumption of metabolic activities, such as the activation of enzymes linked to reserves degradation and mobilization (Marcos-Filho, 2015; Silva et al., 2018).

The presence of salts in the soil solution decreases its osmotic potential, reducing the ability for water absorption and resulting in water deficit (Sheldon et al., 2017). In addition, the excessive amount of salts induces ionic toxicity in the plant and impairs the absorption of essential nutrients such as potassium and nitrogen (Parihar et al., 2015).

For the germination of each species, there is a base temperature (below which germination does not occur), an optimal temperature (at which the greatest germination occurs in a shorter time), and a ceiling temperature (above which germination does not occur). In addition, seed germination has suboptimal and supraoptimal ranges, in which the speed decreases or increases according to temperature, respectively (Garcia-Huidobro et al., 1982; Probert, 1993).

Regarding the osmotic potential (ψ) of the imbibition solution, the availability of pure water ($\psi=0$) is the ideal condition, and the more negative the solution is, the more constrained germination will be, until reaching the base osmotic potential, below which seeds fail to germinate (Soltani et al., 2022).

The effects of temperature, osmotic potential and the interaction between them on seed germination are described by thermal time, hydrotime and hydrothermal time models, respectively (Ellis et al., 1986; Bradford, 2002). Models can be used both to predict germination behavior (Bakhshandeh et al., 2017; Saeed et al., 2022) and to estimate the behavior of species according to the predicted climate change scenarios (Gareca et al., 2012; Dantas et al., 2020).

The Caatinga domain, which is part of the Seasonally Dry Tropical Forests biome, is exclusive to Brazil and is distributed throughout the northeastern semiarid region (Prado, 2003; Pennington et al., 2006; Queiroz et al., 2017). The climate is seasonal, with an average annual rainfall of 773 mm (which occurs from December to March), high solar radiation, high temperatures, water deficit and large areas with shallow and salinized soils (Junior and Silva, 2010; Andrade et al., 2017). The high evapotranspiration and low rainfall expected in the climate change scenario will cause an increase in temperature, intense droughts, and a large water deficit (IPCC, 2023). Although its vegetation is highly resilient, the northeastern region is one of the most vulnerable regions to the climate change scenario in Brazil, which can lead to its desertification (Marengo, 2014). In the most pessimistic scenario of climate change, with a temperature increase of up to 5.4 °C, this semi-arid region could turn into an arid area, characterized by lower rainfall during the rainy season and greater soil moisture deficits throughout the year, culminating in desertification (Marengo et al., 2017).

Neoglaziovia variegata (Arruda) Mez is a species of the Bromeliaceae family, Bromelioideae subfamily endemic to the Caatinga (Tardivo, 2020). Popularly known as caroá/caruá, it is a xerophytic herbaceous plant, with ornamental potential (Beckmann-Cavalcante et al., 2017), with economic and cultural importance due to the use of its fiber in handicrafts as well as in the production of indigenous clothing (Silveira et al., 2011; Dario, 2018), in addition to the health benefits of its extract such as antioxidant, antibacterial and gastroprotective effects (Oliveira-Junior et al., 2015; Gomes et al., 2021; Lira et al., 2021).

Information on the germination of *N. variegata* under different temperature and salinity conditions is useful to understand its growth and development in different ecological contexts. Thus, the objective of this study was to identify the thermal and saline thresholds and evaluate their impact on the germination of *N. variegata* seeds.

MATERIAL AND METHODS

Seed collection: *N. variegata* (caroá) fruits were collected in April 2023 in the municipality of Petrolina, PE (09°04'16.4"S, 40°19'5.37"W). According to Köppen's classification, the climate in the city is BSh - Dry semi-arid climate, with high temperatures, prolonged droughts and an average annual rainfall of 435 mm (Teixeira, 2010; Alvares et al., 2013).

N. variegata seeds were extracted from the fleshy fruits, washed with running water to remove the mucilage and dried in the laboratory at room temperature (25 ± 4 °C) for one week, to obtain the initial lot. The seeds were packed in cloth bags and kept in a cold chamber during the tests ($T = 10$ °C; $RH = 60\%$).

The evaluation of the water content of these seeds was carried out by the greenhouse method at 105 °C for 24 hours with two replications of 25 seeds each (Brasil, 2009).

Seed germination under environmental stresses: before carrying out the experiments, *N. variegata* seeds were immersed in a solution with distilled water and neutral detergent ($10 \text{ drops} \cdot \text{L}^{-1}$) for 7 minutes, for superficial disinfection. After this period, the seeds were washed under running water and placed to germinate. The experiments were set up in a completely randomized design, using four replications of 25 seeds in each trial. *N. variegata* seeds were sown in Gerbox boxes, distributed on two sheets of blotting paper moistened with an osmotic solution or distilled water used in the proportion of 2.5 times the weight of the paper. The boxes were incubated in B.O.D (Biochemical Oxygen Demand) germination chambers with a 24-h photoperiod. To simulate thermal stress, *N. variegata* seeds were germinated at the following constant temperatures: 15, 20, 25, 30, 35 and 40 °C. Salt stress was evaluated using the NaCl solution at different electrical conductivities: 0 (distilled water), 2, 4, 8, 10, 12, 14 and 16 $\text{dS} \cdot \text{m}^{-1}$ at a temperature of 30 °C (Richards, 1980). All experiments were maintained for 28 days, with daily evaluations and germination was considered as the emergence of 2 mm of the main root. After the 28 days of the experiment, the seeds of the treatments that showed germination $\leq 50\%$ were placed in distilled water at 30 °C to evaluate their recovery after stress.

Thermal time and halotime modeling: for each temperature, the accumulated germination was plotted as a function of time and fitted to a Boltzmann sigmoidal curve, from which the time to reach 50% of germination (t_{50}) was estimated. Germination rate (GR) was calculated as a reciprocal function of the time required to reach 50% germination ($1/t_{50}$) (Covell et al., 1986). With the GR of each temperature, linear regression analyses were performed to calculate the interception on the x-axis in the suboptimal and supraoptimal temperature ranges, generating estimates of the base temperature (T_b) and the ceiling temperature (T_c) for germination, below and above which, respectively, GR is equal to zero (Covell et al., 1986; Ellis et al., 1986). The interception between the two linear regression lines at suboptimal and supraoptimal temperatures was used to estimate the optimal temperature (T_o) (Covell et al., 1986).

To establish the halotime, the germination in saline solution of NaCl was accumulated and plotted as a function of time, with the fit to a Boltzmann sigmoidal curve and using it to estimate the t_{50} , which was plotted as a function of the osmotic potential to estimate the interception on the x-axis and the slope of the linear regression. Interception on the x-axis indicated the base potential, below which germination does not occur (Seal et al., 2018).

To simulate thermal stress combined with salt stress, an experiment was carried out in a completely randomized design in a double factorial scheme (2×3 , solutions \times temperatures). In this experiment, the seeds were sown on blotting paper with NaCl solutions with the ψ that caused a 50% reduction in the germination rate and distilled water in combination with the optimal temperature for germination and with the one that caused a 50% reduction in the germination rate of the suboptimal and supraoptimal range, based on the experiment previously described.

Statistical analysis: modeling of the data for thermal time and halotime was performed in Origin software. The data from the experiment to simulate combined stress were tested for normality and homogeneity using SPSS software (SPSS, 2021). Analysis of variance (ANOVA) and Scott-Knott's means test at 5% probability level were performed using AgroStat software (Barbosa and Maldonado, 2012).

RESULTS

N. variegata seeds showed initial water content of 13.43% and germination of 76% at 30 °C. A high germination (>60%) was observed in the thermal range between 25 and 35 °C (Figure 1), and the optimal temperature for the seeds according to the thermal model was estimated at 31.1 °C (Figure 1). It was observed that the temperatures that cause a 50% reduction in the germination rate in the suboptimal and supraoptimal ranges are 22 and 37 °C, respectively.

The thermal time obtained for supraoptimal temperatures ($\theta_{\text{supra}} = 99.1$ hours) was lower than that obtained for suboptimal temperatures ($\theta_{\text{sub}} = 157.1$ hours). The germination of *N. variegata* did not occur at 15°C, or at 40 °C in the laboratory experiments; however, estimates obtained with the thermal model, based on the germination rate, indicated that the germination temperature range is above 14 ± 1 °C and below 41 ± 1 °C (Figure 1). It was found that salt stress negatively impacted the germination of *N. variegata* seeds. Germination and germination rate showed the highest values when the seeds were kept in distilled water, decreasing up to 12 dS.m⁻¹ (Figure 2). The seeds did not germinate at electrical conductivities as of 14 dS.m⁻¹. However, the estimates obtained through the halotime modeling indicated that the base electrical conductivity was 16.2 dS.m⁻¹, and the reduction of 50% in germination rate occurred at 8 dS.m⁻¹. The halotime (θ_H), the time required by the seed to germinate under salinity conditions, was 169.2 hours (Figure 2). The germination rate decreased as the electrical conductivity increased (Figure 2).

The combined stress data showed normality by the Shapiro-Wilk test at 5% probability level for germination ($p = 0.238$). ANOVA showed that there was no interaction between the studied factors (salinity and temperature), with $p = 0.737$ (Table 1).

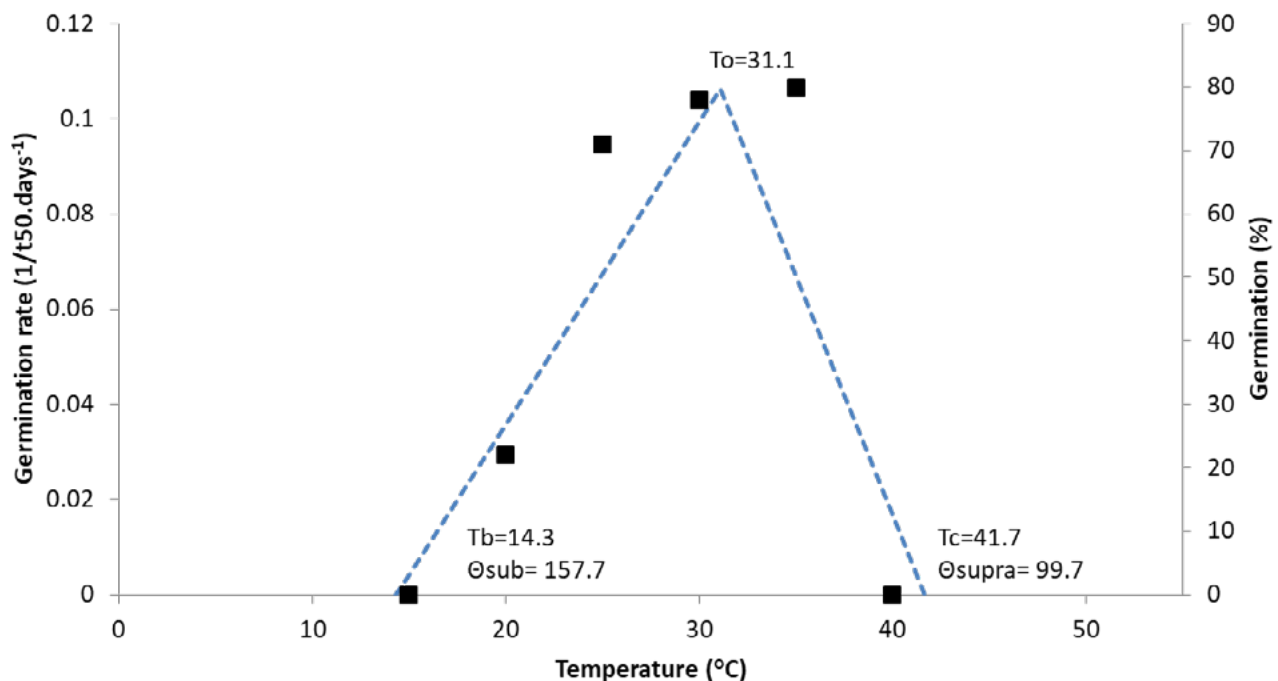


Figure 1. Germination rate (GR), germination (G), thermal thresholds and requirements of *Neoglaziovia variegata* subjected to different temperatures. T_b and T_c correspond to the base and ceiling temperatures of germination, respectively (point at which the regression curves intersect the x-axis); T_o is the optimal temperature; θ_{sub} and θ_{supra} correspond, respectively, to the thermal time of the suboptimal and supraoptimal temperature ranges, obtained by the reciprocal function of the angle of the regression curve.

The results of the recovery of *N. variegata* seeds showed that, in general, the seeds showed viability when they were taken out of the stress condition and placed under ideal germination conditions (30 °C in distilled water) (Figure 3). Those seeds did not germinate under stress conditions (15 °C, 40 °C and 14 dS.m⁻¹) and showed germination higher than 55% after recovery under ideal conditions (Figure 3).

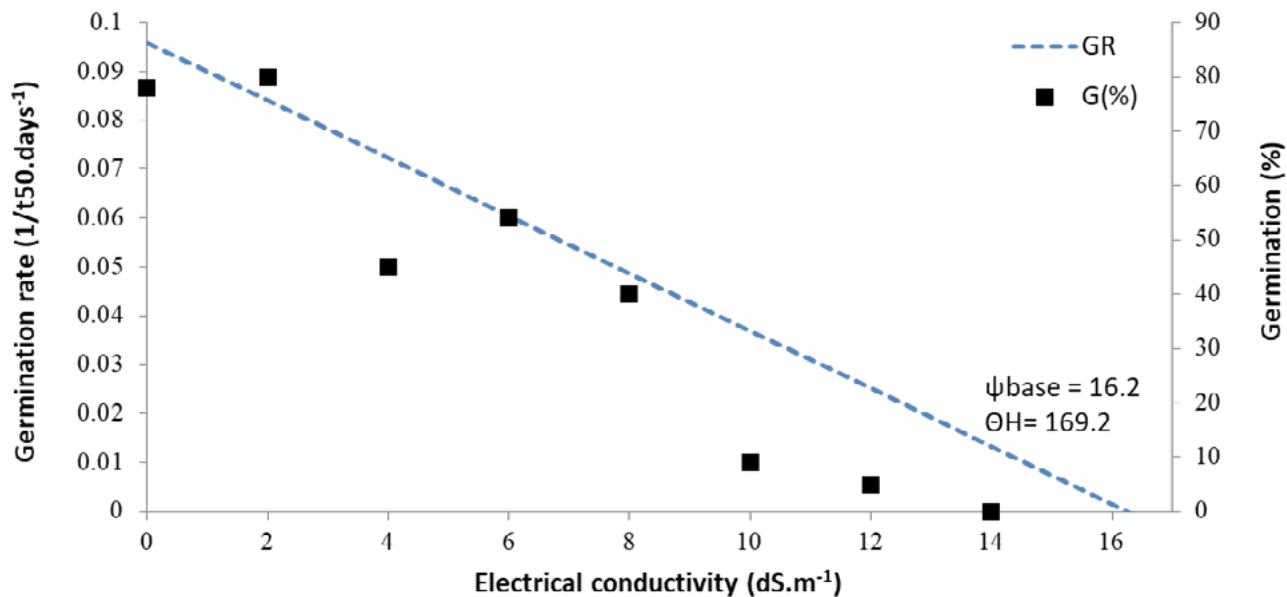


Figure 2. Germination rate (GR), germination (G), halotime and requirements of *Neoglaziovia variegata* subjected to different NaCl solutions. ψ_{base} is the base solution for germination; ΘH corresponds to the halotime obtained by the reciprocal function of the angle of the regression curve.

Table 1. Germination percentage of *Neoglaziovia variegata* seeds as a function of temperature and NaCl solution.

Temperature (°C)	NaCl solution (dS.m ⁻¹)	
	0	8
22	58 aA	10 aA
30	76 aA	36 aA
37	64 aA	24aA
CV (%)	26	

Means followed by the same lowercase letters in the columns and uppercase letters in the rows do not differ from each other by the Scott-Knott test at 5% probability level.

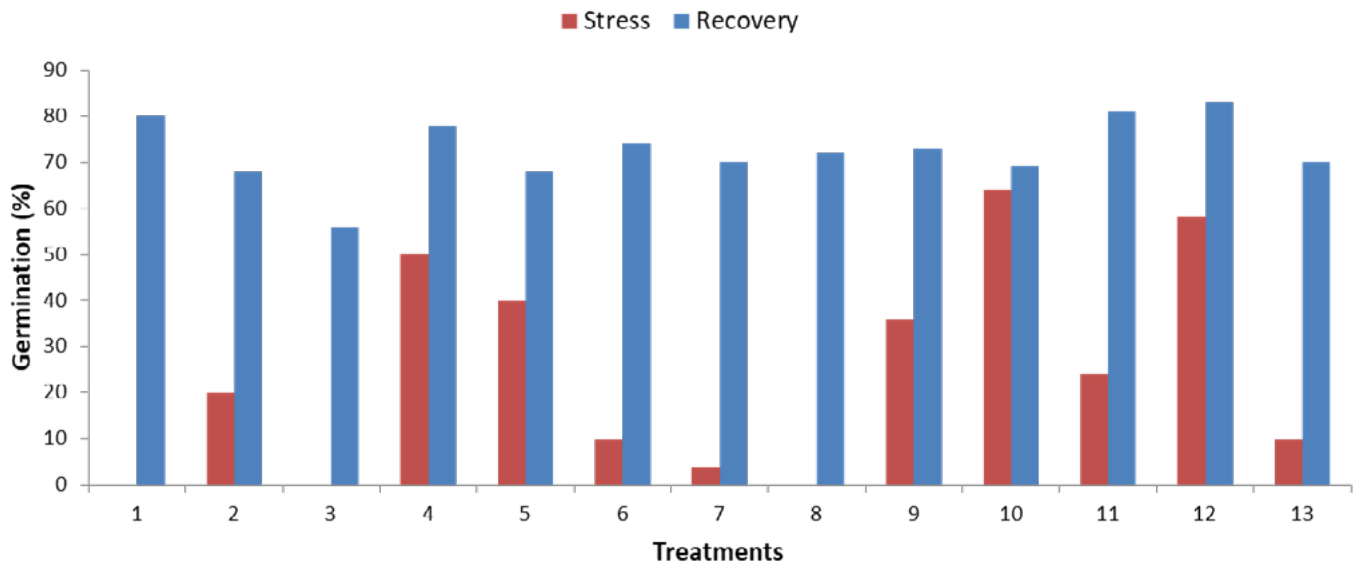


Figure 3. Germination of *Neoglaziovia variegata* seeds placed to recover after germination test under stress conditions. Treatment 1= 15 °C, treatment 2= 20 °C, treatment 3= 40 °C, treatment 4= 6 dS.m⁻¹, treatment 5= 8 dS.m⁻¹, treatment 6= 10 dS.m⁻¹, treatment 7= 12 dS.m⁻¹, treatment 8= 14 dS.m⁻¹, treatment 9= 30 °C x 8 dS.m⁻¹, treatment 10= 37 °C x H₂O, treatment 11= 37 °C x 8 dS.m⁻¹, treatment 12 = 22 °C x H₂O, treatment 13 = 22 °C x 8 dS.m⁻¹.

DISCUSSION

Different species have germination processes with varying optimal temperature ranges and thermal, water, and saline thresholds, which directly influence their viability, as well as germination percentages and rates (Dürr et al., 2015). Studies reveal distinct patterns of thermal tolerance among various Bromeliaceae species, especially those found in tropical and rupestrian grassland habitats (Marques et al., 2014; Duarte et al., 2018). For example, for *Dyckia encholirioides* (Gaudichaud) Mez, a bromeliad endemic to the Atlantic Forest, alternating temperature between 20 and 30 °C is considered optimal for germination (Pompelli et al., 2006), while for some species of the genus *Tillandsia* the optimal temperatures range from 15 to 35°C (Marques et al., 2014).

Species with wide temperature ranges for germination, such as *N. variegata*, which has a variation of 22 °C between the base and ceiling temperatures, tend to have wider ecological niches, which can provide greater adaptability to different environmental conditions (Donohue et al., 2010). In addition to the seeds of *N. variegata*, seeds of other species of the same subfamily Bromelioideae can germinate in wide temperature ranges and at high thresholds. Seeds of *Aechmea floribunda* Mart. ex Schult. & Schult. F. maintain high germination between 15 and 30 °C, which a drastic decrease from 35 °C, with a maximum thermal threshold of 52 °C (Pinheiro and Borghetti, 2003). In addition, seeds of *Aechmea nudicaulis* (L.) Griesebach show a high percentage of germination at temperatures between 15 and 40 °C, with a significant decrease only at 45 °C, with the optimal range between 20 and 30 °C (Pinheiro and Borghetti, 2003).

The most pessimistic scenario of climate change indicates an increase in the frequency and intensity of extreme events, such as prolonged droughts and torrential rains, besides an increase of 3.2 to 5.4 °C in the global average temperature (IPCC, 2023). The identification of cardinal temperatures (T_b, T_o, T_c) provides information that contributes to understanding how species will respond according to climate change scenarios (Dantas et al., 2020). From the identification of the cardinal temperatures of 41 species of bromeliads, it was possible to assess that, for 85% of them, the average annual temperature of their environments is below the optimal temperature. Moreover, for 93% of these species, a 3 °C increase in temperature does not exceed their germination amplitude; on the contrary, future

temperatures will be closer to their optimal temperatures (Muller et al., 2018). In a comparison of the two subfamilies Tillandsioideae and Bromelioideae, an increase of 3 °C may limit the germination of species for Tillandsioideae, while there will be no significant effect for Bromelioideae (Muller et al., 2017).

Regionalized models for the Sertão region of Pernambuco indicate for the pessimistic scenario an average annual temperature of 30.25 °C in 2055 (Dantas et al., 2020). When relating these data to the thermal model of germination of *N. variegata*, the species follows the same response pattern as other bromeliad species, with this increase being closer to the optimal temperature and not exceeding its germination thresholds.

The classification of water for irrigation is a system developed to evaluate the adequacy of water used in agriculture, based on its salinity, measured by electrical conductivity (EC), and sodicity, measured by the sodium adsorption ratio (SAR) (Zaman et al., 2018). The classification is formed by four classes, both for salinity (C1 to C4) and for sodicity (S1 to S4). Class C1 corresponds to low salinity, with EC of up to 0.25 dS.m⁻¹. Class C2 indicates moderate salinity, with EC between 0.25 and 0.75 dS.m⁻¹. Class C3 represents high salinity, with EC ranging from 0.75 to 2.25 dS.m⁻¹. Class C4, on the other hand, is characterized by very high salinity, with EC higher than 2.25 dS.m⁻¹ (USSS Staff, 1954).

Regarding salinity tolerance, *N. variegata* seeds can germinate in solutions with salinity of up to 16 dS.m⁻¹ (class C4) (-0.5 MPa). When comparing these results with those reported in the study conducted by Silveira et al. (2011), in which *N. variegata* seeds were subjected to different osmotic potentials using polyethylene glycol 6000 (PEG 6000) to simulate water stress, it was observed that the seeds germinated up to -0.6 MPa, with no germination at -0.8 MPa. This comparison suggests that *N. variegata* seeds are more affected by salinity than by drought.

The increase in the concentration of salts in the substrate determines the reduction in water potential, resulting in a lower capacity for water absorption by the seeds, which generally influences the germination capacity and the development of seedlings (Zhang and Dai, 2019; Cipriano et al., 2023). Salinity also causes a significant accumulation of ions (Na⁺ and Cl⁻) and hinders the absorption of K⁺ and Ca²⁺, resulting in ionic imbalance (Ibrahimova et al., 2021). In addition, it raises the level of reactive oxygen species (ROS) in plant cells, causing oxidative stress. Toxic ROS can lead to lipid peroxidation, deterioration of cell membranes, and DNA and protein damage (AbdElgawad et al., 2016).

Arid and semi-arid areas face a high accumulation of salts in soil and water (Arif et al., 2020). Caatinga species show tolerance to salinity, with *Manihot* germinating up to 8 dS.m⁻¹ (-0.2 MPa) (Alencar et al., 2022), *Astronium urundeuva* (M.Allemão) Engl. and *Bauhinia cheilantha* (Bong) Stend. germinating up to 12 dS.m⁻¹ (-0.4 MPa) (Oliveira et al., 2014), *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschu germinating up to 16.6 dS.m⁻¹ (-0.5 MPa) (Gomes et al., 2023), and *Cenostigma pyramidale* (Tul.) Gagnon & G.P.Lewis germinating up to 27 dS.m⁻¹ (-1 MPa) (Dantas et al., 2020). *N. variegata*, as an endemic species of the Caatinga, follows the same response pattern, showing high tolerance to substrate salinity, with >50% germination up to 6 dS.m⁻¹.

For the Brazilian semi-arid region, the most critical climate change scenario predicts a reduction of up to 30% in rainfall and an increase of up to 5 °C in the average temperature, which will aggravate the water deficit (Dantas et al., 2020). This increase in water deficit will result in a greater accumulation of salts on the surface and in the upper layers of the soil, intensifying salinity (Stavi et al., 2021). Based on the halotime modeling, the germination of *N. variegata* will not be inhibited up to 14 dS.m⁻¹, so it is able to germinate even in the worst scenario.

The loss of viability in seeds after being kept at higher temperatures can be attributed to their deterioration through the denaturation of essential proteins (Marcos-Filho, 2015). This type of response was observed in the epiphytic bromeliad *Aechmea costantinii* (Mez) LB Sm., whose seeds lost the ability to germinate when subjected to a temperature of 45 °C (Rios et al., 2016). However, for *N. variegata*, the higher temperature inhibited its germination, but did not cause the seeds to lose their ability to germinate once better environmental conditions are reestablished. The recovery observed in *N. variegata* seeds in all treatments tested (thermal stress, salt stress and combined stress) shows resilience to abiotic stresses.

CONCLUSIONS

N. variegata seeds were tolerant to temperature and salinity, indicating that their germination will not be inhibited by current or future environmental conditions. The viability of *N. variegata* seeds after recovery from stressful conditions suggests a high resilience of the seeds. The recovery capacity of *N. variegata*, maintaining viability after exposure to thermal and salt stress, indicates a robust adaptation to adverse environmental conditions, essential for its survival in the Caatinga domain.

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