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Germination performance of *Syagrus romanzoffiana* seeds subjected to water and salt stress

Desempenho germinativo de sementes de *Syagrus romanzoffiana* submetidas aos estresses hídrico e salino

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Highlights

Osmotic potential influences the germination and vigor of *Syagrus romanzoffiana* seeds.
Water stress prolongs and delays *Syagrus romanzoffiana* seed germination.
Abiotic stresses impact the germination and vigor of *Syagrus romanzoffiana* seeds.

Abstract

Queen palm (*Syagrus romanzoffiana*) reproduces through seeds, which typically germinate slowly and unevenly. These seeds face various abiotic stresses, including water and salt stress, which hinder water uptake and germination. This study aimed to assess the germination of queen palm seeds under water and salt stress conditions. We employed a completely randomized experimental design with four replications, using a factorial arrangement (2 × 5) involving two osmoconditioning agents (NaCl and PEG 6000) and five osmotic potentials (0.0 - control, -0.3, -0.6, -0.9, and -1.2 MPa). Key outcomes measured

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were germination percentage, germination speed index, and mean germination time. Results showed that NaCl significantly affected germination percentage, which decreased with lowering osmotic potential. Significant declines in seed germination were observed starting from -0.6 MPa. Both germination speed index and mean germination time were adversely affected by the osmotic potentials, regardless of the osmoconditioning agent used. Under water stress induced by PEG 6000, seeds germinated later and over an extended period compared to those under NaCl stress. The study concluded that queen palm seeds exhibit tolerance to both water and salt stress, managing to germinate even at the lowest tested osmotic potential (-1.2 MPa).
Key words: Arecaceae. Abiotic stress. NaCl. PEG 6000. Osmotic potential. Stress tolerance.

Resumo

A propagação da palmeira jerivá (*Syagrus romanzoffiana*) ocorre por meio de sementes, cuja germinação se dá de forma desuniforme e lenta. Assim, as sementes encontram-se sujeitas a múltiplos estresses, como o hídrico e o salino, que limitam a embebição e suas chances de germinação. Objetivou-se avaliar a germinação de sementes da palmeira jerivá submetidas aos estresses hídrico e salino. O delineamento utilizado foi o inteiramente casualizado, com quatro repetições, em esquema fatorial (2 × 5), sendo dois agentes osmocondicionantes (NaCl e PEG 6000) e cinco potenciais osmóticos (0,0 - controle, -0,3, -0,6, -0,9 e -1,2 MPa). Ao final do experimento, calculou-se: porcentagem de germinação, índice de velocidade de germinação e tempo médio de germinação. Com relação aos potenciais osmóticos, para porcentagem de germinação, houve efeito significativo de maneira isolada somente para NaCl, com diminuição na porcentagem de acordo com a redução do potencial osmótico da solução. A partir do potencial -0,6 MPa, houve redução significativa na germinação das sementes. O índice de velocidade e o tempo médio de germinação das sementes foram afetados negativamente pelos potenciais osmóticos das soluções, independente do agente osmocondicionante. No estresse hídrico simulado pelo PEG 6000, as sementes começaram a germinar mais tarde e por um período mais prolongado, quando comparado àquelas submetidas ao estresse de NaCl. Concluiu-se que as sementes da palmeira jerivá apresentam tolerância aos estresses hídrico e salino, pois conseguiram germinar até mesmo no potencial osmótico mais negativo (-1,2 MPa).
Palavras-chave: Arecaceae. Estresse abiótico. NaCl. PEG 6000. Potencial osmótico. Tolerância.

Introduction

Syagrus romanzoffiana (Cham.) Glassman, belonging to the Arecaceae family, is a native South American palm found naturally in Brazilian biomes such as Cerrado, Atlantic Forest, and Pampa. Commonly known as the queen palm, this species is utilized for a variety of purposes including oil and fiber production, heart of palm extraction, and as an ornamental plant due to its aesthetic appeal and minimal maintenance requirements. It plays a crucial ecological role as well, offering a vital food source for various frugivorous mammals and birds during food shortages, thanks to the sweet taste of its fruits (Noblick, 2017; Bruno et al., 2019; Weirich et al., 2020).

This species is propagated by seeds (Meerow & Broschat, 2015), which are known for their tolerance to partial drying enhancing their longevity and vigor (Goudel et al., 2013). Like many palm species, germination is slow and uneven, potentially due to factors such as integument hardness causing physical dormancy, embryo immaturity leading to physiological dormancy, and natural chemical inhibitors complicating seedling production. Environmental conditions such as temperature and humidity significantly impact germination and must be carefully managed to optimize outcomes (Meerow & Broschat, 2015; Nascimento et al., 2019). Factors like low rainfall, soil salinization, and elevated temperatures may further hinder germination and plant development, even for species adapted to arid conditions (Marcos, 2015; G. M. Oliveira et al., 2019).

Seed germination is fundamentally driven by water movement through embryonic tissues (Bewley et al., 2013). For successful germination, seeds require water to initiate metabolic chemical reactions that allow the embryo to develop (Inocente & Barbedo, 2019). Water facilitates several key processes, including tissue hydration, enhanced respiratory activity, and protein hydrolysis, providing essential nutrients and energy for growth (Duarte et al., 2018). However, limited water availability can impede the diffusion of solutes necessary for metabolic development, where intensive enzymatic activities take place (Marcos, 2015).

Salinized soil poses another significant stress factor, potentially inhibiting water uptake by seeds or damaging their structure, thus altering germination dynamics (Almeida et al., 2020). Salinity can disrupt seed cell structure, metabolism, and hormonal balance, adversely affecting seedling growth and reducing overall crop productivity (Akram et al., 2019). Understanding seed tolerance and germination capacity in saline environments is critical for identifying species capable of thriving under such conditions (D. C. Silva et al., 2019a).

Analyzing the germination response of seeds under artificial stress conditions serves as a valuable method for understanding a species' resilience and adaptability to natural stressors (T. F. Oliveira et al., 2021). To simulate these stresses in a laboratory setting, various compounds are used to alter the osmotic potential during seed germination. For drought conditions, mannitol, and polyethylene glycol 6000 (PEG 6000) are employed, while potassium chloride (KCl) and sodium chloride (NaCl) are used to simulate saline environments (Marcos, 2015; Lucchese et al., 2018; M. F. Silva et al., 2019b; Sarmento et al., 2020; Bousba et al., 2021).

Previous studies have demonstrated that certain palm species, such as *Carpentaria acuminata* (H. Wendl. & Drude) Becc. and *Ptychosperma elegans* (R. Br.) Blume, exhibit salt tolerance during seed germination (Batista et al., 2016). Similar tolerance has been observed in *Phoenix dactylifera* 'Mabroom' (Al-Qurainy et al., 2020) and *Dypsis decaryi* (Jum.) Beentje & J. Dransf. (Vieira et al., 2023).

Given this context, our research aims to assess the germination performance of queen palm (*Syagrus romanzoffiana*) seeds when subjected to water and salt stress, conditions mimicked by the introduction of PEG 6000 and NaCl.

Materials and Methods

Fruits of *Syagrus romanzoffiana* were harvested in July 2021 from four mother plants on a private farm in Indianópolis, in the microregion of Uberlândia, southwestern Minas Gerais State, Brazil (18°52'14.5" S, 47°58'12.9" W, 849 m altitude). The plants were spaced 3 meters apart. The fruits were collected after they began to detach from the bunches, indicating ripeness through their orange epicarp (T. G. S. Oliveira et al., 2015). They were then transported to the Laboratory of Horticultural Plant Seeds at the Department of Agricultural Production Sciences, Faculty of Agricultural and Veterinary Sciences (UNESP/FCAV), Jaboticabal Campus, São Paulo state, Brazil.

Before germination testing, fruits were manually pulped by rubbing against a steel mesh sieve (6 mm) to remove epicarp and mesocarp. Diaspores (seeds with adhered endocarp) were aseptically treated in a 3% sodium hypochlorite solution for 10 minutes using a commercial solution with 2.5% active chlorine to disinfect seeds, which were subsequently rinsed with running tap water (Nascimento et al., 2019). Malformed or insect-damaged seeds were discarded.

Seeds were air-dried on paper towels for one hour to remove surface moisture. Seed water content was then determined using the oven drying method at 105 ± 3 ºC for 24 hours (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009), with results expressed as a percentage. The number of samples and seeds used was based on the study by Ferreira et al. (2021) for the same species. Initial seed water content was measured at 21.54%. The interval between harvesting and the initiation of testing was two days. The test batch was created by thoroughly mixing seeds from all the mother plants.

The study utilized a completely randomized design with four replications, each containing 25 seeds. Treatments followed a 2 × 5 factorial design involving two osmoconditioning agents (NaCl and PEG 6000) and five osmotic potentials (0.0 - control, -0.3, -0.6, -0.9, and -1.2 MPa).

To simulate water and salt stress, solutions of polyethylene glycol 6000 (PEG 6000) and sodium chloride (NaCl) were prepared to varying osmotic potentials using the Van't Hoff equation, $\Psi\pi = -RTC$; wherein: $\Psi\pi$ = osmotic potential (atm), R = general constant of perfect gases (0.082 $atm.L.mol^{-1} K^{-1}$), T = temperature (K), and C = concentration ($mol\ L^{-1}$), following that described by Santos and Silva (2020). For the control (0.0 MPa), only distilled water was used. Concentrations were adjusted to create the desired osmotic potentials: 11.15, 22.90, 33.40, and 44.58 g/500 mL for PEG 6000, and 2.10, 4.20, 6.30, and 8.40 g/500 mL for NaCl.

Seeds underwent pre-soaking in either distilled water (control) or PEG 6000 solutions (physiological conditioning) within transparent Gerbox-type plastic boxes (11 × 11 × 3 cm), each containing 50 seeds and 50 mL of each solution. These boxes were closed and placed in a B.O.D. (Biochemical Oxygen Demand) germination chamber (ELETROLAB®, model EL202/4) for 24 hours at 25 °C with an 8-hour light photoperiod, as proposed by Santos and Silva (2020) for the tree species *Samanea tubulosa* (Benth.) Barneby & J. W. Grimes. After pre-soaking the seeds, the excess solution was rinsed off using distilled water. The seeds were then left to air dry on a bench to reduce excess moisture back to their initial water content, or close to it, before sowing (Sher et al., 2019).

Seeds were sown in transparent Gerbox-type plastic boxes filled with medium vermiculite, and moistened with the appropriate NaCl solutions for salt stress or distilled water for water stress. The vermiculite was chosen for its common use in germination tests for Arecaceae species (Vieira et al., 2023) and kept at 100% water retention capacity. These boxes were wrapped in transparent plastic bags to prevent evaporation and placed in B.O.D. germination set to alternate between 25-35 °C with a 16-hour light/8-hour dark photoperiod (Ferreira et al., 2021).

Germination was monitored daily until stabilization, which lasted 50 days, using the onset of germination buds as the germination criterion (Vieira et al., 2023). Germination percentage (%G) (MAPA, 2009), Germination Speed Index (GSI) (Maguire, 1962), and Mean Germination Time (MGT $days^{-1}$) (Labouriau, 1983) were calculated.

Analysis of variance (ANOVA) was performed using the F-test at a significance level of 5% (α = 0.05). When significant differences were observed for the osmoconditioning agents, the Tukey test at a 5% probability level was employed to compare means. If significant differences among concentrations were found, polynomial regression analysis was utilized to examine the behavior of the variables relative to the osmotic potentials of the solutions. Data for germination percentage were transformed into arc sine (x/100)1/2 for statistical analysis. The statistical analysis was carried out using AgroEstat® software version 1.1.0.711 (Barbosa & Maldonado, 2015). To visualize the daily germination behavior, graphs depicting the distribution of germination over time were generated.

Results and Discussion

Germination percentage (%) of *Syagrus romanzoffiana* seeds was significantly affected by the osmoconditioning agents used (Figure 1A). However, the germination speed index (GSI) did not show a significant interaction between the agents (Figure 1B). Mean germination time (MGT) differed significantly based on the interaction between the osmoconditioning agents, with seeds exposed to the PEG 6000 solution taking longer to germinate (Figure 1C). Several factors could explain these outcomes, including genetic factors, the origin of the mother plants where fruits were harvested, and the temperature fluctuations during the experiment (25-35 ºC), all of which may account for the observed differences in tolerance to water and salt stress.

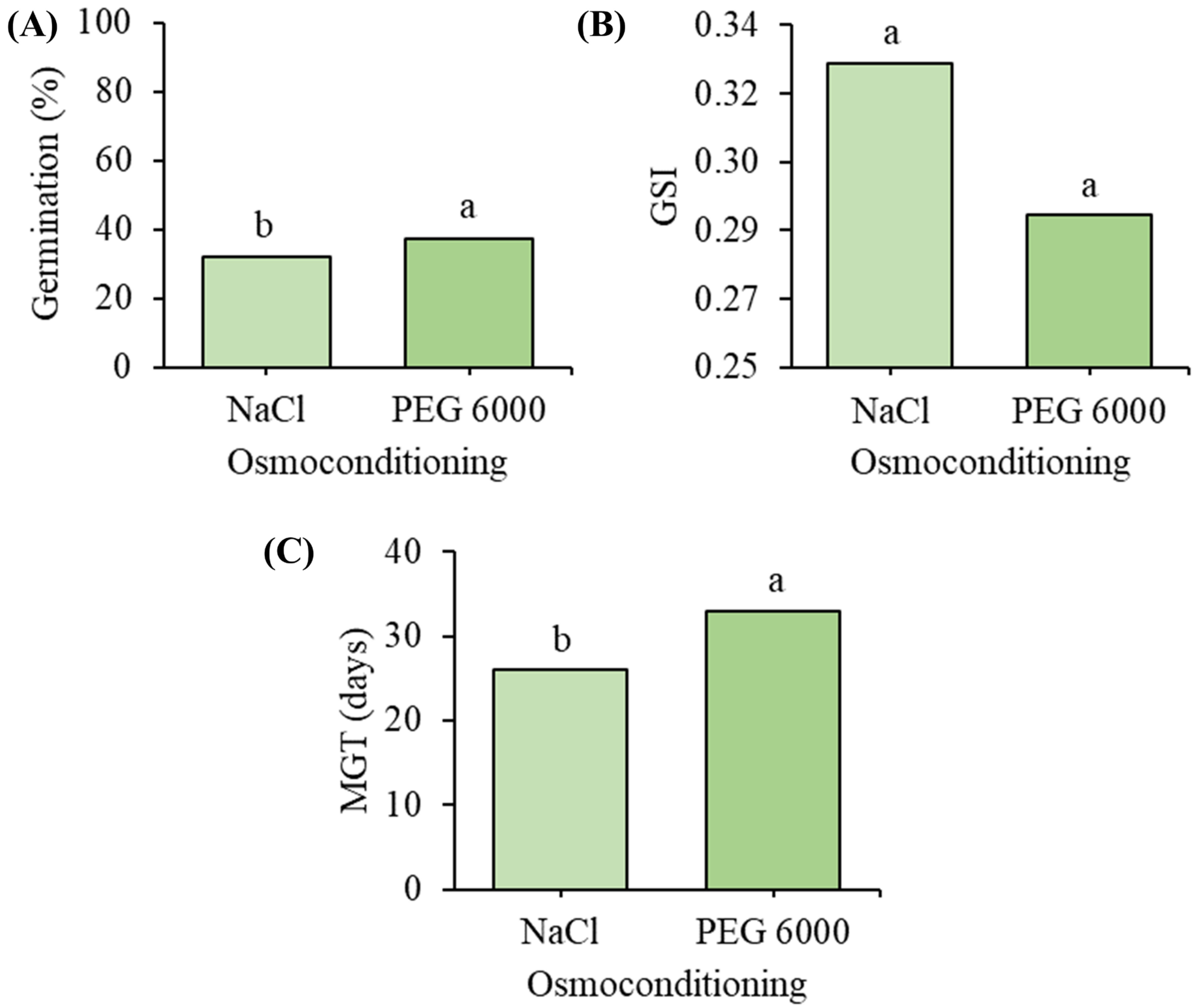

Figure 1. Germination (%) (A), germination speed index (GSI) (B), and mean germination time (MGT - days) (C) of *Syagrus romanzoffiana* seeds as a function of the osmoconditioning agents NaCl and PEG 6000 used. Jaboticabal, São Paulo State, Brazil, 2022.
Means followed by the same letter within the column do not differ from each other by Tukey's test (5%).

The more pronounced decrease in germination caused by NaCl compared to PEG 6000 can be attributed to the toxicity of sodium in the chemical composition of NaCl. Sodium (Na^+) and chloride (Cl-) ions can cause protoplasmic swelling, impacting enzymatic activity and leading to disrupted energy production by interfering with the respiratory chain, and inhibiting germination (E. C. Silva et al., 2021). Additionally, high salt concentrations may elevate reactive oxygen species levels, resulting in lipid peroxidation of cell membranes, loss of cellular function, cell death, and ultimately seed death (Liang et al., 2018). However, these effects vary among species, underscoring the importance of such studies to determine tolerance levels and the survival capacity of species in salt-affected environments (D. C. Silva et al., 2019a).

Some palm species exhibit salinity tolerance during germination, including *Carpentaria acuminata* (H. Wendl. & Drude) Becc. and *Ptychosperma elegans* (R. Br.) Blume (Batista et al., 2016), *Phoenix dactylifera* 'Mabroom' (Al-Qurainy et al., 2020), and Dypsis decaryi (Jum.) Beentje & J. Dransf. (Vieira et al., 2023). *Cocos nucifera* L. also possesses physiological mechanisms that confer partial tolerance to water and/or salt stress, allowing it to establish in salinized areas, provided that its water needs are at least partially met (A. R. A. Silva et al., 2017). These findings align with observations by Chen et al. (2018), who reported various morphological, anatomical, cellular, biochemical, and molecular changes that species undergo in stress response, which varies by the type, duration, and intensity of the stress encountered.

Conversely, PEG is a high molecular weight, non-toxic, and chemically inert polymer that does not penetrate seed tissues due to its size; its molecules do not cross cell membranes, allowing seeds to absorb only water (Marcos, 2015). However, highly negative osmotic potentials induced by PEG can reduce water absorption, potentially hindering the sequence of events necessary for germination. This results in a delay and reduction in both the germination percentage and the germination speed index, as PEG affects the viscosity of water and the solubility of oxygen, both crucial for germination (Sarmento et al., 2020).

In terms of osmotic potentials affecting germination percentage, there was a significant isolated effect observed only for NaCl, with germination percentages decreasing as the solution's osmotic potential decreased (Figure 2A). From an osmotic potential of -0.6 MPa onwards, a marked reduction in seed germination was noted. There was a 15.87% decrease in germination from 0.0 to -1.2 MPa. However, germination still occurred even at the most negative osmotic potential. A similar phenomenon was reported for the *Phoenix dactylifera* palm, cultivar 'Mabroom', where increasing salt concentrations, simulated by NaCl, decreased the total percentage of germinated seeds (Al-Qurainy et al., 2020).

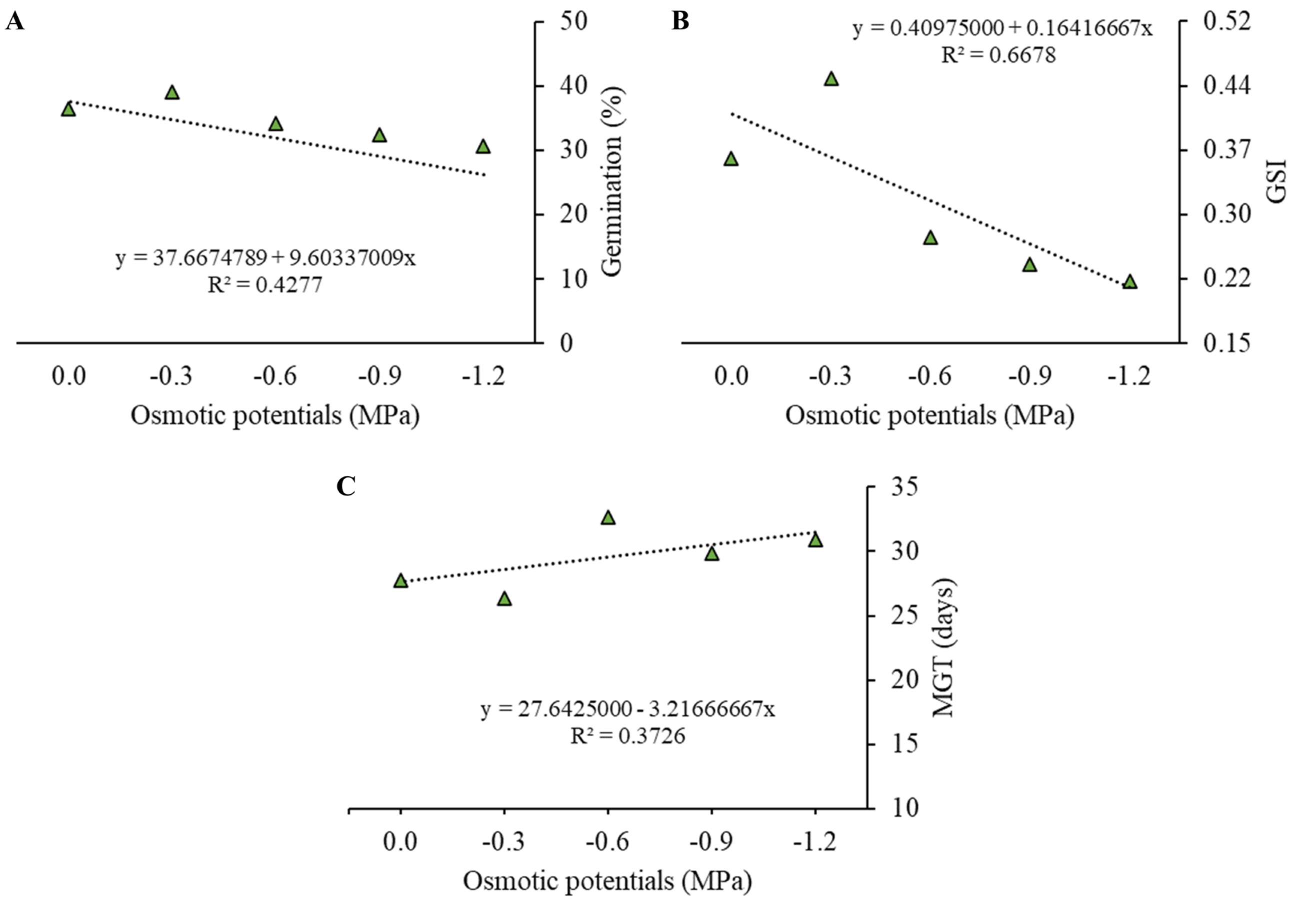

Figure 2. Germination (%) (A), germination speed index (GSI) (B), and mean germination time (MGT - days) (C) of *Syagrus romanzoffiana* seeds as a function of different osmotic potential levels (MPa) used. Jaboticabal, São Paulo State, Brazil, 2022.
Significant at 5% probability.

The GSI was adversely impacted by the osmotic potentials of the solutions, irrespective of the osmoconditioning agent used, showing a 51.11% reduction at an osmotic potential of -0.3 MPa compared to the lowest potential of -1.2 MPa (Figure 2B). For MGT, decreasing osmotic potentials resulted in delayed maximum seed germination (Figure 2C). Thus, under water stress conditions, seed MGT is extended.

Water potential reduction, induced by both water and salt stress, has similarly led to a decrease in germination percentage and speed for various species, including *Anadenanthera colubrina* (Vell.) (Duarte et al., 2018), *Toona ciliata* M. Roem. var. *australis* (Lucchese et al., 2018), *Clitoria fairchildiana* Howard (Silva et al., 2019a), *Samanea tubulosa* (Benth.) Barneby & J. W. Grimes (Santos & Silva, 2020), *Combretum leprosum* Mart. (Leal et al., 2020), as well as *Zea mays* L. and *Phaseolus lunatus* L. (Almeida et al., 2020). These findings underscore that the rate of seed water absorption not only varies significantly among distinct species but also depends on the specific environmental conditions to which the seeds are exposed, as detailed by M. F. Silva et al. (2019b).

The observed delay in seed germination can be attributed to a postponement in phase III of germination, which is the stage requiring substantial water intake for radicle protrusion (Leal et al., 2020). Hydration cycles significantly influence the embryo's growth potential, which is linked to enhanced water absorption capacity, cell division, embryo expansion, and mobilization of endosperm reserves (Soares et al., 2021). When water is scarce, it limits the diffusion of solutes essential for developmental metabolism where intense enzymatic activity is required (Marcos, 2015). Additionally, Bhanuprakash and Yogeesha (2016) highlighted that the physiological responses to water restriction and salinity are intertwined, suggesting that both stress types are perceived by plant cells as events of water deprivation.

Seeds exposed to the lowest osmotic potentials exhibited delayed germination (Figures 2B, C), suggesting their unsuitability for areas with high salinity soils or those irrigated with saline water (Almeida et al., 2020). This is because seed vigor could be compromised under such conditions. Our findings suggest that both salt stress and water scarcity can negatively impact plant establishment. Increased mean germination times could affect the uniformity of seedling emergence, potentially leading to unevenly developed seedlings. Additionally, seeds that germinate more rapidly are typically more likely to survive in adverse field conditions (Lucchese et al., 2018). However, it is important to recognize that the spread of germination over time is a natural strategy employed by plants to enhance survival chances under unfavorable environmental conditions, such as periods of drought, fires, and floods (Bewley et al., 2013; Ferreira et al., 2021; Soares et al., 2021).

Results of distribution and peak timing of seed germination showed noticeable variability between the osmoconditioning agents in terms of the onset, peak, and total number of germinated seeds (Figure 3). While peaks in seed germination were recorded for both agents, the process was irregular, supporting observations by Meerow and Broschat (2015) that palm seed germination is typically slow and uneven.

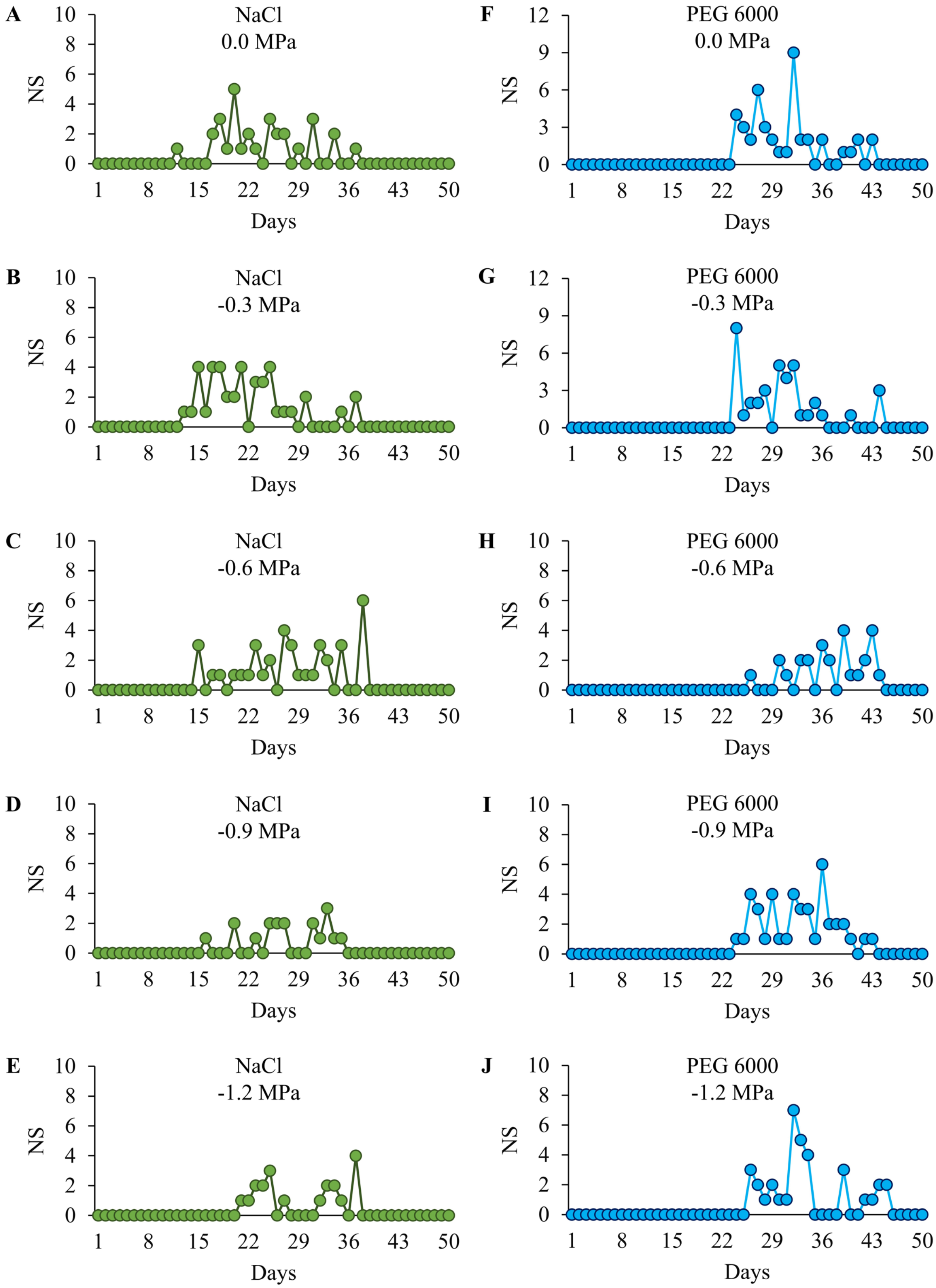

Figure 3. Distribution of seed germination (NS - number of seeds germinated a day) over 50 days from a batch of 100 *Syagrus romanzoffiana* seeds under MPa concentrations of NaCl (A, B, C, D, and E) and PEG 6000 (F, G, H, I, and J). Jaboticabal, São Paulo State, Brazil, 2022.

Seeds subjected to salt stress induced by NaCl began germinating between the 12th and 21st days, with a progressive delay as the osmotic potential of the solution decreased. The germination process then stabilized between the 35th and 38th days (Figures 3A, B, C, D, and E). The more negative the water potential in the NaCl solution, the longer it took for seeds to initiate germination, leading to a dispersed germination timeline. This pattern is also observed in the *Phoenix dactylifera* palm, cultivar 'Mabroom' (Al-Qurainy et al., 2020), and other botanical families (Lucchese et al., 2018; Almeida et al., 2020; Santos & Silva, 2020).

In terms of salinity tolerance, plants are categorized into glycophiles (mildly tolerant) and halophiles (highly tolerant). The primary distinction lies in the ability of halophiles to prevent excessive salt absorption and tolerate the toxic effects of high salt concentrations (Soundararajan et al., 2019). Our findings indicate that *Syagrus romanzoffiana* seeds are capable of germinating even at the most negative osmotic potential of -1.2 MPa, demonstrating characteristics typical of halophytic plants, which suggest a robust tolerance to stress induced by NaCl in the solutions.

Regarding water stress simulated by PEG 6000, seed germination commenced between the 24th and 26th days and stabilized between the 43rd and 45th days (Figures 3F, G, H, I, and J). This indicates that seeds exposed to PEG 6000 began to germinate later and over a more extended period compared to those subjected to NaCl stress. This delayed germination onset and extended distribution over time as the water potential decreased with PEG 6000 has also been noted by Inocente and Barbedo (2019) and Bousba et al. (2021).

The variability in the onset of seed germination (Figure 2) might also be influenced by the physiological conditioning before sowing in treatments with PEG 6000 solutions. Immersing seeds in water can be detrimental, as rapid water entry may impede seed aeration and lead to deterioration (Marcos, 2015). Ferreira et al. (2021) made similar observations for other species within the Arecaceae family, where the seed rehydration process (soaking) negatively affected the germination speed index and mean germination time.

Despite reductions in the variables analyzed due to the established treatments, seeds were able to germinate at even the most extreme osmotic potentials. This resilience may be linked to the broad geographical distribution of the species (Noblick, 2017; Bruno et al., 2019) and the tolerance of its seeds to partial drying, which enhances their longevity and vigor (Goudel et al., 2013). However, it is crucial to recognize that under natural conditions, sporadic water availability resulting from quick and irregular rainfall combined with high soil evaporation directly impacts seed germination through both hydration and dehydration cycles. These conditions may trigger lipid peroxidation and initiate the seed deterioration process, thus potentially preventing the completion of germination (Lima & Meiado, 2017; Bewley et al., 2013).

Conclusions

Syagrus romanzoffiana seeds demonstrate tolerance to water and salt stress, simulated by the presence of PEG 6000 and NaCl, as they were capable of germinating even at the most adverse osmotic potential of -1.2 MPa. However, osmotic potentials below -0.6 MPa significantly impair seed germination by adversely affecting the germination percentage, germination speed index, and mean germination time.

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