ARTICLE

Germination niche of a neotropical dry forest species: seed osmotic stress and recovery

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ABSTRACT: *Erythrina velutina* Willd is a native Fabaceae with wide occurrence, mainly in the Caatinga Domain with multiple uses. This study aimed to compare the vulnerability/ tolerance of seeds of four accessions of *E. velutina* to osmotic stresses. Four replications were conducted with 25 seeds, germinated in osmotic solutions of NaCl or PEG up to -1.73 MPa. Seeds that did not germinate after 14 days were recovered in distilled water. Obtained data were fitted in Boltzmann curves and t_{50} and synchrony were evaluated. A probit analysis was performed, and hydrotime and halotime models were constructed. In distilled water, the germination curve was similar for all accessions. When the concentration of salt or PEG in the substrate increased, the accessions differentiated into two groups. Two accessions - Jutaí 2012 and Jutaí 2015 - maintained high germination up to -0.86 MPa NaCl, while Caboclo 2008 and Caiçara 2011 accessions presented a lower germination speed. The hydrotime and halotime analyses separated the germination response of accessions according to their physiological quality and tolerance to osmotic and saline conditions. Seeds viability was maintained at both stresses, since germination was reestablished at distilled water, thus constituting a crucial response for this species' survival and conservation.

Index terms: dry forest, Mulungu, seed stress, semi-arid.

RESUMO: *Erythrina velutina* Willd é uma Fabaceae nativa com ampla ocorrência, principalmente no Domínio Caatinga com múltiplos usos. O objetivo deste estudo foi comparar a vulnerabilidade/tolerância de sementes de acesos de *E. velutina* a estresses osmóticos. Foram semeadas quatro repetições com 25 sementes em soluções osmóticas de NaCl ou PEG até -1,73 MPa. As sementes não germinadas após 14 dias foram recuperadas em água destilada. Os dados obtidos foram ajustados em curvas de Boltzmann e t50 e sincronia foram avaliados. Uma análise probit foi realizada e modelos de hidrotempo e halotempo foram construídos. Em água destilada, a curva de germinação foi semelhante para os acesos. Quando a concentração de sal ou PEG no substrato aumentou, os acesos se diferenciaram em dois grupos. Dois acesos - Jutaí 2012 e Jutaí 2015 - mantiveram alta germinação até -0,86 MPa NaCl, enquanto os acesos Caboclo 2008 e Caiçara 2011 apresentaram menor velocidade de germinação. As análises de hidrotempo e halotempo separaram a resposta germinativa dos acesos de acordo com sua qualidade fisiológica e tolerância às condições osmóticas e salinas. A viabilidade das sementes foi mantida em ambos os estresses, pois a germinação foi restabelecida em água destilada, sendo uma resposta crucial para a sobrevivência e conservação desta espécie.

Termos para indexação: floresta seca, Mulungu, estresse em sementes, semiárido.

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INTRODUCTION

Seasonally Dry Tropical Forests (SDTFs) are one of the least studied biomes and one of the most endangered in the world with 60% of its area destroyed (Dexter et al., 2018). In northeastern Brazil, the Caatinga Domain contains the largest continuous stretch of a SDTF and holds the greatest species richness, in comparison to other SDTF remnants worldwide, due to the flora's adaptations to the local soil and climatic conditions (Silva et al., 2017). Plants native to SDTFs, such as in the Brazilian Caatinga, have morphological and physiological adaptations to endure harsh conditions (high temperature and drought) in which they grow, such as shedding leaves to decrease water loss, efficient water absorption and storage by roots, protective metabolic adaptations to cope with oxidative stress (Souza et al., 2010; Peguero-Pina et al., 2020).

The seeds of these species have developed adaptations that confer resistance to adverse conditions in the soil, in seed banks or even during storage, such as tolerance to water deficit, to stochastic small rainfall events and to soil salinity (Gomes et al., 2019; Dantas et al., 2020). These adaptations, such as the presence of an impermeable testa, physical and/or physiological dormancy and desiccation tolerance of seeds and seedlings, promote the maintenance of viability and vigor in the seeds, as the seed can recover and germinate when the stress is eliminated (Kranner et al., 2010).

Given the environmental adversities encountered in these tropical arid environments, it is important to study physiological and biochemical aspects of seed germination processes under abiotic stresses (Ribeiro-Reis et al., 2012). Information on the tolerance/vulnerability of seeds to stress is necessary to understand how this may affect the survival of such species in future climate scenarios (Dantas et al., 2020). When environmental stresses impact seeds, they can affect all plant production and reproduction and result in the loss of biodiversity (Kranner et al., 2010).

Erythrina velutina Willd. (Fabaceae) is a widely occurring tree species, found in the Caatinga Domain, Cerrado and Atlantic Forest, widely used in ecological restoration and urban forestry. Moreover, its wood and seeds are used in craftwork and pharmaceuticals, thereby serving as a source of income (Souza et al., 2016).

Climate projections predict vulnerability of forest ecosystems to deforestation and degradation and also predict consequent disappearance of tree populations. In tropical dry forests, adverse conditions such as high temperatures, water scarcity, increased aridity, high evaporation rates, and soil salinization are likely to be aggravated by climate change (IPCC, 2023). In addition, the climate conditions of the growing season and the microclimate of the areas where these seeds are collected may influence their quality and tolerance to environmental stresses (Oliveira et al., 2019; 2020). Thus, research on seed stresses can allow the knowledge of seeds performance in different environmental and climatic scenarios, contributing with important information about ecological adaptations, biodiversity and survival potential.

In this study, we hypothesize that *E. velutina* seeds, which are tolerant to some of these stresses (Ribeiro-Reis et al., 2012; Ribeiro et al., 2014), show varied responses depending on the growing season and provenance. Thus, this study aimed to compare the vulnerability/tolerance of seeds of four accessions of *E. velutina* to osmotic stresses and their recovery upon reestablishment of optimum germination and growth conditions. Still, we also aimed to predict the germination niche and the survival potential among different populations and accessions, and the regeneration of this species in current and future climate scenarios.

MATERIAL AND METHODS

Plant Materials and Provenances: seeds of *E. velutina* were collected from natural populations in different years and occurring in three municipalities in the west of the state of Pernambuco (PE), Brazil.

Climate data were collected at the Bebedouro Automatic Agrometeorological Station of Embrapa Semi-Arid (40° 22' W 09° 09' S), which is no more than 50 km from seed harvest sites, for the harvest years (2008, 2011, 2012, 2015). Temperatures and precipitation during these years are summarized in Table 1. The rainy season in the collection sites

Table 1.   Location of acessions of *Erythrina velutina* Willd. and weather characteristics in harvest years.

Acession	Geographic coordinates/Altitude	Precipitation (mm)	Average temperature (°C)	Maximum temperature (°C)	Mínimum temperature (°C)
Caboclo 2008	08° 28' 29" S; 40°55'45" W; 570 m	191.03	26.26	32.51	20.85
Caiçara 2011	09° 07' 473" S; 40°23'34" W; 379 m	464.31	25.76	31.98	20.16
Jutaí 2012	08° 33' 54" S; 40°09'47" W; 396 m	122.68	26.35	32.46	20.49
Jutaí 2015	08° 33' 54" S; 40°09'47" W; 396 m	333.25	25.79	31.50	20.74

occurs from December to April, when the minimum temperature can reach 20 °C and the average 30 °C. The dry season occurs from May to November, with little rainfall (2 to 20 mm in the month) or no rainfall, the temperature in this season can reach over to 35 °C. The dispersion and harvesting of *E. velutina* seeds occurs from July to September.

Seeds from ripe and dry fruits were removed manually and only intact seeds were stored in sealed polyethylene bags (0.15 mm thick) at 30±5 °C, 56±6% UR until the experiments began (Ribeiro et al., 2018). The seeds are orthodox, remain viable after desiccation and maintain high germination after several years of storage at low or room temperature.

We followed the same procedure for seed collection and processing in all the periods. Prior to the experiment, scarification of the testa of the seeds was done mechanically with a mini rotary grinder (Western R-50), following which, the seeds were immersed in Captan fungicide solution (3 mg.L^{-1}) for 2 min (Ribeiro-Reis et al., 2012). The seeds presented 100% germination and more than 75% produced vigorous seedlings.

Seed Germination at Different Osmotic Potentials: the seeds of each accession were sowed on sheets of germitest paper moistened with distilled water or the respective osmotic solutions of sodium chloride (NaCl) or polyethylene glycol 6000 (PEG) up to -1.73 MPa to a volume of 2.5 times the weight of the dry paper (Richards, 1980; Vilela et al., 1991; Brasil, 2009). Osmotic solutions and semi-sterilized paper were renewed every 2 days since the beginning of seeding to keep the osmotic potentials of each treatment stabilized (Ribeiro et al., 2014). Four replications with 25 seeds were kept in rolls and stored in sealed polyethylene containers and kept in 25 °C and a photoperiod of 12 h for a period of 14 days (Ribeiro et al., 2014).

Germinated seeds (2 mm root protrusion) were initially assessed at every 2 h until 48 hours of imbibition and then at every 24 h until germination stabilization (Ribeiro-Reis et al., 2012). After 14 days of germination in the above mentioned osmotic solutions, the seeds' tolerance germination (TG) was determined. The seeds' capacity to recover the germination process was evaluated after transferring the non-germinated seeds (no visible radicle) of each treatment to semi-sterilized Germitest paper moistened with distilled water. The seeds were arranged on paper rolls and maintained under the same conditions for an additional period of 14 days. The percentage of seeds that recovery germination (RG) was determined (Li et al., 2010) and added to TG to obtain the result for the final germination (FG).

Obtaining the Tolerance Thresholds: based on the results of germination for each osmotic potential, Boltzmann sigmoidal curves were fitted (cumulative germination × time) to estimate the time required for germination of 50% of the seed population (t_{50}). The germination rate (GR) was calculated as a reciprocal function of this time ($1/t_{50}$) (Covell et al., 1986).

For each seed lot, the hydrotime (θH_{PEG}; MPa.d^{-1}) and halotime (θH_{NaCl}; MPa.d^{-1}) to germination at suboptimal osmotic potentials were calculated (Covell et al., 1986; Seal et al., 2018; Seal et al., 2021) and a repeated probit analysis was performed in Genstat (v. 14.2.6967; VSN International) varying Ψ_b value until best fit was obtained (Bradford, 1990).

Data Analysis: The seeds' physiological quality was determined by the daily germination count and final germination percentage (G%) after 14 days, synchrony (Z) (Ranal and Santana, 2006), and the time for 50% germination (t_{50}) of the seeds (Dantas et al., 2020). We first tested for normality of the residuals for the variables using the Shapiro-Wilk test. Levene's test was used to determine homogeneity of the variances. Subsequently, generalized linear models (GLM) were applied to the data.

Deviances were calculated based on different accession and treatment factors and their interactions. Significant differences among the variables were analyzed within each accession and treatment. We performed pairwise comparisons of means using post-hoc Tukey HSD test at a significance level of 0.05. Means were adjusted by the Šidák method. We used R Software (R Core Team, 2020) to perform the analyses.

RESULTS

The cumulative seed germination for the four accessions presented a Boltzmann fit for all salinities (Figure 1) except at -1.73 MPa; therefore, these data were not shown. The cumulative germination curves for osmotic solutions of polyethylene glycol 6000 (PEG) up to -0.8 MPa were fitted to the Boltzmann equation for all the accessions (Figure 2). The germination curve for distilled water was similar for all accessions (Figures 1a, 2a). However, increased concentration of salt or PEG in the substrate (reduced osmotic potential) divided the accessions into two groups. The Jutaí 2012 and Jutaí 2015 accessions maintained high and fast germination (~100%) up to -0.86 MPa of NaCl, while the Caboclo 2008 and Caiçara 2011 accessions presented a similar response with a lower speed of cumulative germination at a lower concentration of salt (Figures 1b–f). In PEG solutions, germination began later and was reduced for all accessions at -0.6 MPa. At -0.9 MPa (PEG), the Caiçara 2011 seeds did not germinate (Figure 2e).

We observed a reduced germination speed of the seeds treated in both osmotic solutions when we estimated the time for germination of 50% of the population in each treatment (t_{50}). The t_{50} of Jutaí 2012 and Jutaí 2015 accessions were lower than the others in all the treatments (Table 2). These accessions also demonstrated greater synchrony of germination when subjected to higher salt concentrations. In general, the seed germination in all the accessions was highly synchronized when seeds were germinated at -0.43 MPa of NaCl. Conversely, synchrony was lower in more concentrated PEG solutions (Table 2).
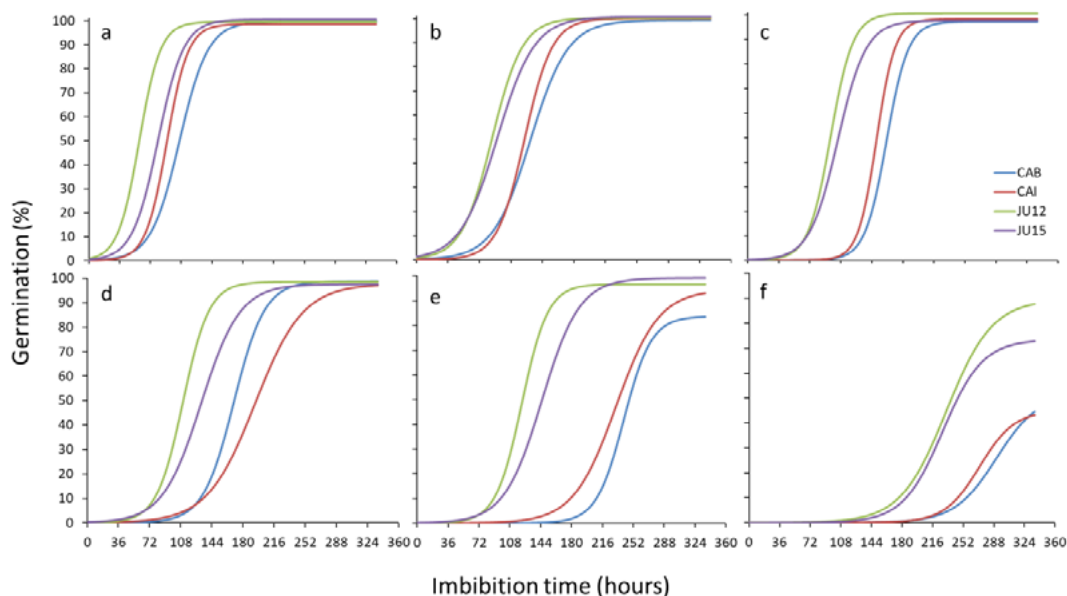

Figure 1. Accumulated germination in different osmostic potentials of *Erythrina velutina* seed lots harvested at in three municipalities of Pernambuco State, Brazil in 2008, 2011, 2012 and 2015. Data was fitted to Boltzmann sigmoidal model, as a function of time. Seeds germinated in distilled water 0 MPa (A) and in NaCl solutions at -0.21 MPa (B); -0.43 MPa (C); -0.65 MPa (D); -0.86 MPa (E) and -1.29 MPa (F). Seed lots: Caboclo 2008 (CAB); Caiçara 2011 (CAI); Jutaí 2012 (JUT12) and Jutaí 2015 (JUT15).
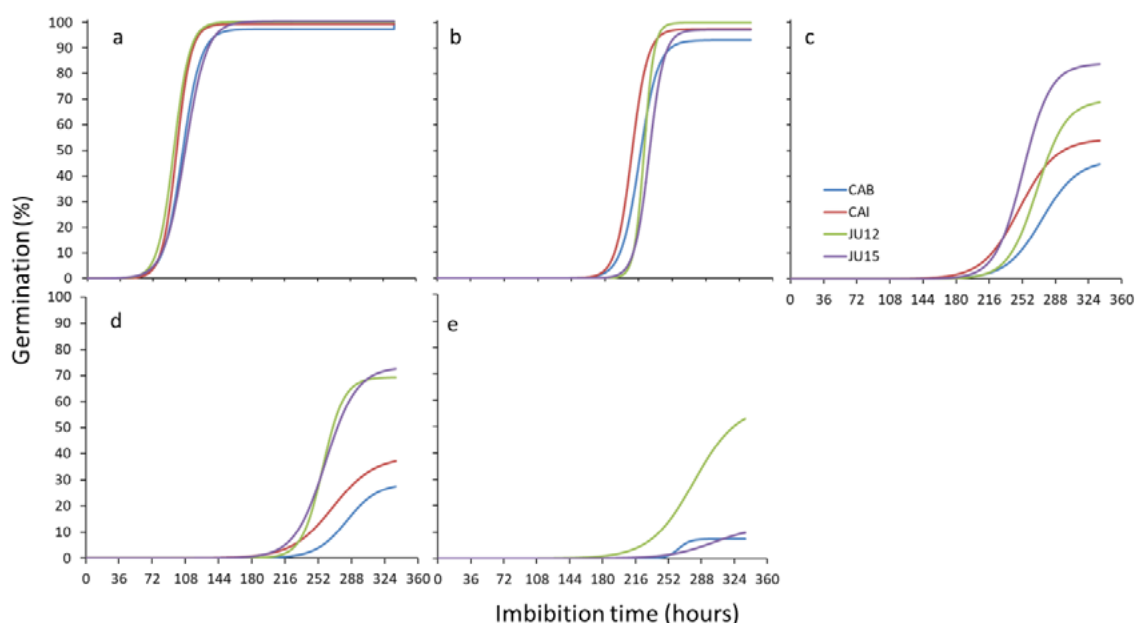
Figure 2. Accumulated germination in different osmostic potentials of *Erythrina velutina* seed lots harvested at in three municipalities of Pernambuco State, Brazil in 2008, 2011, 2012 and 2015. Data was fitted to Boltzmann sigmoidal model, as a function of time. Seeds germinated in distilled water 0 MPa (A) and in PEG solutions at -0.3 MPa (B); -0.6 MPa (C); -0.7 MPa (D) and -0.8 MPa (E). Seed lots: Caboclo 2008 (CAB); Caiçara 2011 (CAI); Jutaí 2012 (JUT12) and Jutaí 2015 (JUT15).

Table 2. Germination kinetic traits of seeds of *Erythrina velutina* seed lots harvested at three municipalities of Pernambuco State, Brazil in 2008, 2011, 2012 and 2015. Seeds were germinated in distilled water and in NaCl or PEG osmotic solutions. t_{50} = time for germination of 50% of seed population; Z= germination syncronism.

	NaCl (MPa)					PEG 6000 (MPa)			
Salinity (MPa)	Caboclo 2008	Caiçara 2011	Jutaí 2012	Jutaí 2015	Osmotic (MPa)	Caboclo 2008	Caiçara 2011	Jutaí 2012	Jutaí 2015
					t50				
0	4.35 dA	3.78 dA	2.52 cB	2.55 dAB	0	4.12 cA	4.08 cA	3.92 cA	4.48 cA
-0.21	5.64 cA	5.22 cA	3.57 cB	3.84 cB	-0.3	9.05 bA	8.65 bA	8.34 bA	8.47 bA
-0.43	6.66 bA	6.27 cA	4.14 bB	3.33 cB	-0.6	11.34 aA	11.15 aA	10.29 aA	10.62 aA
-0.65	7.13 bA	8.32 bA	4.62 bB	4.47 bB	-0.7	8.75 bA	11.11 aA	9.79 aA	10.84 aA
-0.86	7.50 bAB	9.63 bA	5.08 bB	6.18 aAB	-0.8	12.29 aA	3.38 cC	8.63 bB	6.25 bB
-1.29	11.89 aA	11.33 aA	9.67 aB	7.05 aC	-0.9	12.75 aA	0.00 dB	9.63 aA	10.13 aA
-1.73	13.25 aA	13.00 aA	9.63 aB	6.25 aC					
					Z				
0	0.22 bB	0.28 bA	0.31 aA	0.28 aA	0	0.36 aB	0.43 aAB	0.52 aA	0.33 bB
-0.21	0.23 bA	0.23 bA	0.24 bA	0.24 bA	-0.3	0.36 aB	0.41 aAB	0.57 aA	0.47 aAB
-0.43	0.32 aA	0.34 aA	0.34 aA	0.21 bB	-0.6	0.28 bA	0.29 bA	0.26 bA	0.27 cA
-0.65	0.24 bA	0.18 cB	0.27 bA	0.25 bA	-0.7	0.32 bA	0.26 bA	0.29 bA	0.25 cA
-0.86	0.24 bA	0.17 cB	0.25 bA	0.22 bA	-0.8	0.17 cA	0.08 cB	0.18 cA	0.01 dB
-1.29	0.23 bA	0.28 bA	0.19 cB	0.15 cB	-0.9	0.00 dA	0.00 dA	0.00 dA	0.00 dA
-1.73	0.01 cB	0.01 dB	0.30 aA	0.30 aA					

Means followed by the same capital letters in the line and lowercase on the column do not differ by Tukey ranking values at 5% probability.

The tolerance germination (TG) was similar and close to 100% for all accessions in saline conditions at an osmotic potential of up to -0.65 MPa. At -0.85 MPa, TG for seeds from the Caboclo 2008 accession was lower than the others. At -1.29 MPa, the seeds exhibited a drastic reduction in TG (Table 3). Seeds germinated well when they were transferred to distilled water after 14 days in osmotic solutions of NaCl and PEG. This indicates a recovery of germination (RG) of over 70% for all accessions when treated with osmotic solutions. As an exception, seeds from Jutaí 2012 accession did not recover entirely from PEG solution at -0.7 MPa and presented low RG and final germination (FG) (Table 3). Since TG decreased at more negative osmotic potentials in PEG and NaCl solutions, higher amplitudes of RG were observed in Caiçara 2011 seeds (71% and 25%, respectively). The number of dead seeds was higher for this accession, as reflected by the lower percentages of RG and FG. For the other accessions, FG was high and approached the control at lower potentials and was significantly different (p<0.05) at more negative potentials regardless of the osmotic substance tested (Table 3).

The Probit analysis and hydrotime and halotime models also allowed us to separate the germination responses of the accessions according to their physiological quality and tolerance to osmotic and saline conditions. We observed that the seeds of the less vigorous Caboclo 2008 and Caiçara 2011 accessions exhibited less tolerance than seeds of

Table 3.   Germination of *Erythrina velutina* seeds in salt (NaCl) or polyethylene-glycol (PEG 6000) solutions and its recovery in distilled water.

Salinity (MPa)	Caboclo 2008	Caiçara 2011	Jutaí 2012	Jutaí 2015	Osmotic (MPa)	Caboclo 2008	Caiçara 2011	Jutaí 2012	Jutaí 2015
				TG (tolerance germination) (b/c) × 100					
0	100 aA	99 aA	100 aA	100 aA	0	97 aA	99 aA	100 aA	100 aA
-0.21	98 aA	100 aA	100 aA	100 aA	-0.3	95 aA	98 aA	100 aA	97 aA
-0.43	98 aA	99 aA	100 aA	98 aA	-0.6	45 bB	59 bB	71 bA	85 bA
-0.65	99 aA	98 aA	99 aA	97 aA	-0.7	27 cB	37 cB	72 bA	74 bA
-0.86	83 bB	94 aA	99 aA	100 aA	-0.8	8 dB	11 dB	54 cA	10 cB
-1.29	44 cC	44 bC	86 bA	73 bB	-0.9	1 dA	0 eB	3 dA	3 cA
-1.73	1 dB	1 cB	13 cA	12 cA					
				RG (recovery germination) [(a−b)/(c−b)] × 100					
0	0	0	0	0	0	0	0	0	0
-0.21	0	0	0	0	-0.3	100	100	0	0
-0.43	100	0	0	0	-0.6	100	73	83	93
-0.65	100	100	0	0	-0.7	99	78	29	100
-0.86	76	83	0	0	-0.8	98	79	85	97
-1.29	88	55	93	74	-0.9	98	71	92	95
-1.73	83	24	57	70					
				FG (final germination) (a/c) × 100					
0	100 aA	99 bB	100 aA	100 aA	0	97 dC	99 bB	100 aA	100 aA
-0.21	98 bB	100 aA	100 aA	100 aA	-0.3	100 aA	100 aA	100 aA	97 cB
-0.43	100 aA	99 bB	100 aA	98 bC	-0.6	100 aA	95 cC	95 bC	99 bB
-0.65	100 aA	100 aA	99 bB	97 cC	-0.7	99 bB	86 dC	80 eD	100 aA
-0.86	96 cC	99 bB	99 bB	100 aA	-0.8	98 cA	81 eD	93 cC	97 cB
-1.29	93 dB	75 cC	99 bA	93 aA	-0.9	98 cA	71 fD	92 dC	95 dB
-1.73	83 eA	25 dD	63 cC	74 eB					

Means followed by the same capital letters in the line and lowercase on the column do not differ by Tukey ranking values at 5% probability; a = is the sum of the number of seeds germination in solutions (salinity or osmotic) plus those that recovered to germination in distilled water; b = is the total number of seeds germinated in treatment solutions; c = is the total number of seeds tested. Where RG=zero, TG=FG or dead seeds.

Table 4.   Germination osmotic potential thresholds, hydrotime and halotime of accessions of *Erythrina velutina.*

Acession	Osmotic potential (PEG solutions)				Osmotic potential (NaCl solutions)			
	Ψ_{bPEG} (MPa)	σ	R^2	θ_{Hydro} (MPa d^{-1})	Ψ_{bNaCl} (MPa)	σ	R^2	θ_{Halo} (MPa d^{-1})
Caboclo 2008	-0.706	4.642	0.734**	2.92	-1.379	2.229	0.755**	5.00
Caiçara 2011	-0.772	4.810	0.755**	2.91	-1.573	3.429	0.854**	5.83
Jutaí 2012	-1.022	2.875	0.584*	4.17	-1.730	1.913	0.753*	3.75
Jutaí 2015	-1.001	2.192	0.635*	3.75	-1.712	1.738	0.796**	4.58

Ψ_b is base osmotic potential; σ is standard deviations and R^2 is square of the correlation coefficients for germination and different hydrotimes and halotimes (θ_{Hydro} and θ_{Halo}, respectively). ** $P < 0.01$; * $P < 0.05$.

Jutaí 2012 and 2015 accessions in both conditions. For all the accessions, the Ψb generated by PEG, which ranged from approximately -0.7 to -1 MPa, was higher than the Ψb generated by NaCl, which was under -1.3 MPa. Regarding hydrotime, the less vigorous accessions required approximately 2.9 MPa per day, while the most vigorous required approximately 4 MPa per day. This relationship was reversed for halotime. The less vigorous accessions required around 5 MPa per day and seeds of Jutai 2012 and 2015 accessions required approximately 4 MPa per day (Table 4).

DISCUSSION

Any impediment to the imbibition process seems to affect primarily the cellular hydration status and consequently causes delays in metabolic reactions. Therefore, studying the influence of stresses on seeds will provide insights into stress-tolerance of species. Water is a vital factor in the germination process. During water imbibition, seed's tissues are hydrated and metabolic activities that provide energy and nutrients for embryonic growth are triggered (Bradford, 1990).

Factors such as microclimate and location of a seed's origin have an important effect on their development of vigor and tolerance to desiccation and other stresses related to the seed's hydration status. Furthermore, genetic variation between individuals of different populations, between individuals within the same population, and even between different seeds of the same plant influences the response of seeds of native species to stressful conditions during germination (Pritchard et al., 2014; Ribeiro et al., 2018; Oliveira et al., 2020). When exposed to stresses, seed lots respond according to the physiological potential of each seed (Dutta, 2018). In this study, different accessions of *E. velutina* seeds of different provenances and with different storage times exhibited different responses of tolerance to osmotic stresses and different responses to each osmolyte, NaCl or PEG . Thus, seeds of Jutaí 2012 and 2015 accessions, which were less aged, had the best responses to the applied stresses in comparison to Caboclo 2008 and Caiçara 2011 (Figures 1–2; Tables 2–4).

The tolerance thresholds for salt stress were wider, with lower Ψb (below -1.3 MPa), than the thresholds for osmotic stress by PEG (Ψb greater than -1.0 MPa). Tolerance to osmotic and ionic stress induced by water restriction and salts constitutes complex and generally interconnected processes that involve the interaction of several metabolic pathways. From a molecular point of view, the signal transduction of stresses caused by drought and salts consist of signaling pathways linked to ionic and osmotic homeostasis, detoxification response pathways (damage control and repair), and growth regulation pathways and influence cell cycle activation and growth (Zhu, 2002).

To maintain homeostasis under conditions of salt or water stress, organisms use osmoregulation (or osmotic adjustment) to balance the intra- and extracellular space with osmolytes and electrolytes, such as salts, sugars, amino acids, and other osmotically active molecules. The stresses caused by NaCl and PEG led to a decrease in soluble sugars and proteins in *E. velutina* seeds, especially at higher concentrations (Ribeiro-Reis et al., 2012; Dantas et al., 2014).

However, the intracellular concentration of proline, a potent osmoprotectant, increased during seed germination in saline solutions (Dantas et al., 2014), not in PEG solutions (Ribeiro-Reis et al., 2012). As for the detoxification response,

E. velutina seeds were more sensitive to osmotic stress induced by PEG because water deficiency allows a greater accumulation of reactive oxygen species (ROS). Also, in dehydrated *E. velutina* seed tissues the systems for ROS removal, such as the enzymes catalase and ascorbate peroxidase are less efficient (Ribeiro et al., 2014).

Considering the previous and current results for *E. velutina* seeds, water restriction can be considered the main climate feature that prevents germination or that metabolically affects the process with a slower, non-uniform germination over time. However, when the optimum germination condition of the immediate environment was restored, viable seeds were able to resume rehydration of embryonic tissues and germinate, thereby reverting the effect of water restriction caused by the osmolytes (NaCl and PEG). In these studies, the seeds' physiological quality with respect to tolerance and post-stress germination should also be considered. For all the *E. velutina* accessions tested herein, the seeds, regardless of the harvest year, exhibited maximum initial vigor. In this experiment, no osmolyte caused toxic permanent damage to *E. velutina* seeds.

Aside from preventing the entry of water in the tissues, the 14-day period of exposure in PEG solutions (up to -0.9 MPa) did not cause more severe damage to the seeds or delays in radicle protrusion (Table 3).

On the other hand, the response after being transferred to distilled water was heterogeneous among the accessions, specifically those exposed to saline solutions at potentials below -0.86 MPa, which presented progressively lower results for recovery germination (RG). Comparatively, RG of the seeds of Caboclo 2008 and Jutaí 2015 accessions was higher than the others. The seeds' ability to recover germination has been considered an important adaptive trait for species in their natural environments. Water restriction in embryonic tissues has been considered a key point for rapid seed germination when stress is alleviated. For example, *Cakile maritima* Scop. (Brassicaceae), a halophytic species of the Mediterranean coast, demonstrated rapid germination when soil salinity was reduced by rainfall (Debez et al., 2018). In their study, the phenotypic plasticity of the plant during germination was best explained by proteomic data. Salt exposure triggered a slower degradation of seed storage reserves and a significant reduction in proteins involved in several biological processes (primary metabolism, energy, stress response, folding, and stability).

Regarding reduced germination under osmotic stress, as observed in our results for *E. velutina* seeds, the maintenance of viable seeds and a post-stress germination may be explained by gene control involving ABA-dependent and ABA-independent pathways in seeds, and the adverse effects of salinity on seeds can be attributed to the initial water restriction caused by salt and subsequent ion toxicity (Arif et al., 2020). Also, germination and ethylene production in an ABA-dependent pathway decreased and both responses to salt stress constitute a convergent regulation of primary energy metabolism and embryonic growth potential in *Stylosanthes humilis* (Kunth) (Fabaceae) seeds. Seeds' ABA concentration seems to increase only in response to NaCl osmotic stress leading to lower ethylene production and higher recovery ability, than seeds subjected to PEG osmotic stress (Silva et al., 2018).

Under conditions of osmotic stress during germination, concentrations of intracellular soluble proteins and amino acids in the embryonic axis of *E. velutina* seeds are lower due to the inhibition of proteases in the cotyledons and the translocation of their products (amino acids) to the embryonic axis (Ribeiro-Reis et al., 2012), wich can affect the mobilization of these storage proteins may be involved soon after relieving the stress (Zhu, 2002). In adverse situations for germination, seeds use escape mechanisms or tolerance (Blanvillain et al., 2011). The resistance of the testa, in some species, such as *E. velutina* seeds, is a characteristic that may be interpreted as an adaptive mechanism for arid conditions of SDTF (Kranner et al., 2010).

The different accessions evaluated herein responded according to the genetic variability of each genotype. In general, as part of the plant's strategy for survival, *E. velutina* seems to avoid germination and establishment under water restriction both caused by salinization and PEG. Under more severe restriction, it may generate an effect of osmotic conditioning and maintain seed viability and germinability under post-stress conditions, sustained by active metabolic machinery.

Due to the crystalline formation of Caatinga soils and the rise in irrigated areas, soil salinity has become a major problem, especially in places with high evapotranspiration and elevated temperatures (Silveira et al., 2011). Moreover,

climate projections indicate increased drought and soil salinization, which will lead to desertification (IPCC, 2023). Based on the pessimistic scenarios of the fifth IPCC (International Panel of Climate Change) report, a reduction of up to 40% in the volume of annual precipitation will result in a shorter period of water availability for seed germination and the initial development of seedlings of species of Caatinga (Gomes et al., 2019; Oliveira et al., 2019; Dantas et al., 2020). Despite the seeds' tolerance to osmotic (induced by NaCl) potentials under -1 MPa (> 15dS m^{-1}), the lack of rainfall could restrict seed germination and the regeneration of *E. velutina* populations in a pessimistic future climate. This study demonstrated the tolerance and capacity for recovery of the seeds in the Jutaí group to saline environments or with water deficits which can be useful in restoration/reforestation programs in degraded areas with harsh climate conditions.

CONCLUSIONS

Physiological quality, origin, and storage time influenced response to osmotic stress during germination and post-stress recovery germination.

Osmolytes (NaCl or PEG) influenced both seed germination and recovery germination after optimal conditions were restored.

The seeds' viability was maintained even under saline stress and germination was restored under ideal conditions, thus constituting an important response for the species' survival in adverse situations.

The wide tolerance thresholds associated with the impermeable testa of *E. velutina* seeds and its ability to recover will enable conservation of this species under harsh climatic conditions.

ACKNOWLEDGEMENTS

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