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Influence of Induced Salt Stress on Germination, Proline, Sugar, Total Soluble Proteins and Peroxidase Activity in *Parkia Pendula* (Willd.) Benth. Ex Walp Seeds

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Abstract

Parkia pendula is a tree species of great economic and ecological interest in the Amazon region. The aim of this work was to evaluate the effects of salt stress (NaCl) on germination and biochemistry of *Parkia pendula* seeds and seedlings. For this, salt solutions at concentrations of 0.0 (control); -0.2; -0.4 and -0.6 MPa were used. Evaluations of germination percentage and normal seedlings, Germination Speed Index (GSI), synchronization index, mean germination time (MGT) and relative germination frequency, Root Length (RL) and Shoot Length (SL), Collar Diameter (CD) and Seedling Dry Mass (SDM) were daily determined for 10 days. Peroxidase Activity (PA), Total Soluble Proteins (TSP) and Total Soluble Sugars (TSS) and Proline Content (PC) were evaluated at 0; 3; 6 and 9 days. Salt stress with NaCl negatively affected the germination process of *P. pendula* seeds, reducing the percentage values and GSI, increasing MGT, mainly at potential of -0.6 MPa. The increase in salt stress reduced growth variables (RL and SL, CD and SDM). Collection intervals and osmotic potentials altered TSP, TSS, proline and PA compared to control in *P. pendula* seeds and seedlings, indicating that TSS and TSP reserves are source of energy to promote germination and substrate for the formation of cell structures. TSS at longer collection intervals and at more negative osmotic potentials is a good indicator of salt stress in *Parkia pendula* seeds and seedlings.

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Introduction

Parkia pendula (Willd.) Benth. ex Walp. is a tree species found in the phytogeographic domains of the Atlantic Forest and Amazon [1], belonging to the Fabaceae family. It is considered a heliophyte and perennial species, reaching height between 20 and 50 meters, ecologically grouped as a secondary species [2] and popularly known as "angelim-saia". It stands out for being a species widely used in the recovery of degraded areas due to its rapid growth [3,4]. In addition, it has the ability to fix nitrogen in the soil and attract wild animals [1].

Under field conditions, forest species such as *P. pendula*, face adverse situations, for example water deficits, which tend to increase salt concentrations [5]. Thus, studies on the ability of the species to tolerate salt environments are necessary to elucidate its physiological and biochemical characteristics, considering the numerous negative impacts described in different stages of plant growth due to salt stress, in which there is low photosynthetic activity, changes in carbohydrate and protein metabolism and accumulation of organic acids and osmolytes [6,7].

Salt stress has negative impact on germination and plant growth, causing changes in physiological and metabolic functions, as well as anatomical changes in forest plants [8]. In addition to low photosynthetic activity, there are changes in carbohydrate and protein metabolism and accumulation of organic acids and osmolytes due to salt stress in plants [6,7]. However, there are some gaps in the ability of these forest seeds to produce osmoregulatory compounds such as proline.

The levels of total soluble sugars were mobilized in the plant, mainly in the germination of *P. pendula*, and reserve compounds (proteins) in the cotyledons tend to present higher levels in *P. pendula* compared to *P. multijuga* [9]. Considering seven-month-old *P. pendula* plants submitted to drought, reduction in protein levels in roots and leaves was observed [4]. However, knowledge about the effects of salt stress on *P. pendula* is incipient, whether in the germination or seedling stage, and it is important to determine the levels of biochemical variables (proline, proteins, total soluble sugars and peroxidase activity).

In this scenario, the study investigation is based on two hypotheses: i. *Parkia pendula* has no salt stress tolerance below -0.10 MPa and there are changes in germination and biochemical variables. ii. The longer the exposure time of *Parkia pendula* seeds to salt stress, the greater the changes in biochemical variables. Therefore, this study aims to evaluate the effects of salt stress on the germination and biochemistry of *Parkia pendula* seeds and seedlings.

Material and Methods

This work was carried out at the Laboratory of Ecophysiology and Plant Propagation of the UNEMAT Campus, Alta Floresta-MT.

Parkia pendula seeds were provided by Usina São Manoel, located at the municipality of Paranaíta-MT in October 2015, and were stored in refrigerator at 5°C for a period of 5 months.

Experiment I: Germination

A completely randomized design was used with four NaCl potentials (0 MPa control/distilled water; -0.2; -0.4 and -0.6 MPa) to simulate salt stress, according to the Vant'Hoff equation, cited by [10], with four replicates of 25 seeds each.

To overcome dormancy, seeds were previously immersed in sulfuric acid (H_2SO_4 98%) for 20 minutes, followed by washing in running water. Asepsis was performed in commercial sodium hypochlorite diluted at 2% in distilled water (v:v) for 5 minutes and washed in distilled water. Subsequently, seeds were treated with fungicide Captan® (Captan) in the proportion of 0.5% of the seed weight.

To conduct germination, seeds were placed in transparent plastic boxes (11x11x4 cm) on blotting paper with 12 mL of each solution and kept at temperature of 30°C and 12-hour photoperiod inside BOD chamber. About 3 mL of solution was replaced in boxes every two days to maintain hydration. Evaluations were daily performed for 10 days, with evaluation of the following variables:

Germination percentage: Seeds that presented root extension equal to or greater than 2 mm were considered germinated. Calculations were performed according to Laboriau and Valadares [11]:

$G\ (\%) = (N/A)x100$, where N = Number of germinated seeds and A = total number of seeds.

Germination speed index (GSI): Determined according to [12], together with germination:

$GSI = \Sigma(Gi/ni)$, where Gi = number of germinated seeds and ni = counting day.

Mean Germination Time (MGT) and Relative Germination Frequency (Fr): Obtained by equations proposed by Labouriau and Valadares:

$MGT = \Sigma ni*ti/\ \Sigma ni$, where ni = number of seeds germinated per day, ti = evaluation time after the beginning of the test.

$Fr = ni/\ \Sigma ni$, where ni = number of seeds germinated per day, and Σni = total number of germinated seeds.

Evaluation of the **Percentage of normal seedlings (PN)** was performed together with the germination test, considering normal seedlings those with root system, hypocotyl, epicotyl, cotyledonary leaves and plumule well developed at 10 days of the germination test.

Collar diameter (CD) was measured on the tenth day with the aid of digital caliper at the base of the collar at height of 1 cm from the root. The diameter values of each normal seedling were divided by the total number of seedlings evaluated per replicate, obtaining average values.

Shoot (SL) and root length (RL) were obtained using all normal seedlings of each replicate, measured with the aid of ruler graduated in millimeters. Lengths were divided by the total number of seedlings evaluated per replicate, obtaining average values.

To obtain the seedling dry mass (SDM), all seedlings used to evaluate length were used. Seedlings were dried in an oven at 65ºC for 48 hours, weighed on a precision scale of 0.001g, obtaining average data in g/seedling per replicate and treatment [13].

Treatment means were compared by the Tukey's test at 5% probability, using the ESTAT statistical package.

Experiment II: Biochemical analyses

The design was completely randomized in a 4x3 factorial scheme (collection intervals x salt potentials) + control (Time

"0", frozen seeds after dormancy overcoming), totaling 13 treatments with three replicates of 25 seeds (and/or seedlings) per treatment.

Salt solutions were prepared according to the Vant'Hoff equation, and seeds were previously scarified and underwent asepsis and fungicide treatment as described in experiment I. However, seed subsamples used in collection time "0" were only scarified and washed in distilled water before being frozen in freezer at -20°C. Then, seeds were placed to germinate as described in experiment I.

To proceed with biochemical analyses, the seed coats of all seeds were removed, including control subsamples, which were harder because they were not soaked. Subsamples of each replicate and treatment were packed in plastic bags and kept in freezer at -20°C until the analyses were performed.

Peroxidase activity (PA): For enzyme extraction, fresh samples composed of 100mg of whole seeds or 100mg of seedlings of each replicate and treatment were weighed. Then, they were macerated in porcelain mortar containing 5mL of 0.2 M phosphate buffer, pH 6.7 and centrifuged for 10 minutes at 10,000 Xg according to [14]. Absorbance reading was performed in spectrophotometer at 505 nm. The reaction rate was expressed in units (U), which corresponds to 1µmol of decomposed H_2O_2/ min^{-1} mg^{-1} of g^{-1} of fresh mass.

Total soluble proteins (TSP): Determination was carried out according to method of [15] using the extract obtained to determine the peroxidase activity. As a standard, casein was used. Reading was carried out in spectrophotometer at 595 nm, and the protein content was expressed in mg g^{-1} of fresh mass.

Total soluble sugars (TSS): Analyzed by the phenol-sulfuric method, according to [16]. As a standard, glucose was used. Reading was performed in spectrophotometer at 490 nm, with total soluble sugars expressed in mg glucose g^{-1} of dry mass.

Proline content (PC): Determined according to method of [17]. A 0.5 g portion of fresh material was used, homogenized in 10 ml of 3% aqueous sulfosalicylic acid and centrifuged at 2000 Xg for 5 minutes. Then, 0.2 mL aliquots of the extract were collected, adding 2.0 mL of acid ninhydrin and 2.0 mL of glacial acetic acid. Subsequently, samples were kept in water bath at 100ºC for 1 hour. After cooling in ice bath, the colored compound was measured at 520 nm. The absorbance obtained was compared with the proline standard curve and results were expressed in µg g^{-1} of fresh mass.

Data from experiment I were submitted to analysis of variance (ANOVA) using the F test and means were compared using the Tukey test ($p<0.01$). Principal component analysis (PCA) was used to correlate the biochemical response of seeds under salt stress. PCA allows maximizing total variance, with new components that elucidate most of the variability in initial data. Thus, X is considered a random vector and the dimensionality is established by: (p+q ×1), with covariance matrix by sample $S_{(p+q)x(p+q)}$. Where: $X^{(1)}$ (p×1) and $X^{(2)}$ (q×1) vectors defined as the partitions of the original vector X, described as a group with p variables (biochemical) and another with q variables (areas), respectively. [18] described that the random vector X, the covariance matrix, can be presented as follows (Eq. 1):

$$S_{(p+q)x(p+q)} = \begin{bmatrix} S_{(pxp)}^{(11)} & S_{(pxq)}^{(12)} \\ S_{(qxp)}^{(21)} & S_{(qxq)}^{(22)} \end{bmatrix} \quad \text{(Eq. 1)}$$

The PCA method provides for performing rigid rotation on the original coordinate axes system, so new axes establish a direction in the greater data variability, and their coefficients act as eigenvectors of a data sampling covariance matrix. All analyses were performed using the R statistical software, which is free and open-source code (R Development Core Team 2017).

Results

The germination percentage result of *P. pendula* **(Figure 1A)** showed maximum germination at 0 MPa (control, with 98%), not statistically differing from potential -0.2 MPa (90%), while at potentials - 0.4 and -0.6 MPa, reduction in germination was observed (< 20%).

The present study showed that 23 to 25% of seeds germinated up to -0.2 MPa formed normal seedlings **(Figure 1A)**. At -0.4MPa, reduction in germination (17.5%) and in the total number of normal seedlings (40% of germinated seeds) was observed. The results indicate that the germination test, although important, does not provide information on vigor and that the normal seedling count was efficient to determine seed vigor, revealing low vigor of seeds used, probably because they were stored seeds.

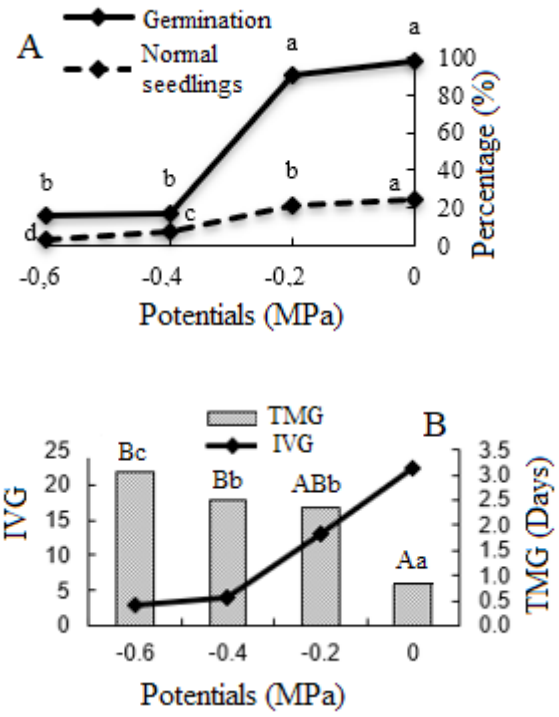

Figure 1: Percentage of germination and Normal seedlings **(A)** and Average time and germination speed index **(B)** of *Parkia pendula* seeds, subjected to saline stress with NaCl. The same capital letters for the slash and lowercase letters for the line do not differ from each other (P < 0,05), by Tukey test.

At -0.4 MPa, the effects of stress on germination were more intense (reduction from 90% to 17.5%, between -0.2 and -0.4 MPa), affecting the normal *P. pendula* development. Under more negative potential (-0.4MPa), it is common to observe smaller potential difference between seeds and solution, with consequent reduction in the volume of absorbed solution, which affects the germination process due to water and salt stress.

Regarding the GSI results (Figure 1B), the lowest values under more negative potencies indicate a delay in the physiological processes necessary to complete germination, especially

between -0.4 and -0.6 MPa. The MGT values of seeds, between 0 MPa and -0.2 MPa, showed increase from 1.2 to 2.4 days, respectively, while at potentials -0.4 and -0.6 MPa, the highest MGT values occurred (2.5 and 3.1 days, respectively), demonstrating that more negative salt potential slows down germination **(Figure 1B)**.

The graph of relative germination frequencies **(Figure 2)** illustrates the germination distribution pattern in salt potentials over the ten days of evaluation. Unimodal polygons are observed at all potentials, but with peaks varying as the potential becomes more negative. In the control treatment (0 MPa), the germination frequency was concentrated on the first day, coinciding with the average germination time (1.2 days) and the highest number of seeds germinated in the period (Nt= 24.5). For the other potentials, the germination peak occurred on the second day, but with increase in the average time to 2.4, 2.5 and 3.1 days at -0.2, -0.4 and -0.6 MPa, respectively, and lower number of germinated seeds (Nt = 22.5; 8.9 and 7.8, respectively), when compared to the control treatment (Nt = 24.5), showing that salt stress significantly compromises *P. pendula* propagation.

The longest shoot and root lengths were observed in the control treatment (0 MPa), statistically differing from the other potentials under study (Figure 3A). This effect confirms the pattern observed in the normal seedling percentage results (Figure 1A), and that seedling development is affected by seed vigor as NaCl concentration increases.

Regarding the collar diameter (Figure 3B), higher values were observed at potential -0.2 MPa, which did not differ from potentials 0 MPa and -0.4 MPa. This result demonstrates that, under moderate stress (-0.2 MPa), *P. pendula* may have favored the collar diameter development as an attempt to acclimatize to the salinity environment, to the detriment of the increase in seedling length. However, it is observed that this behavior is not verified at the most negative potentials (-0.4 and -0.6 MPa), probably due to the reduced ability to tolerate the stress intensity. The seedling dry mass values (Figure 3B) decreased as osmotic potentials became more negative.

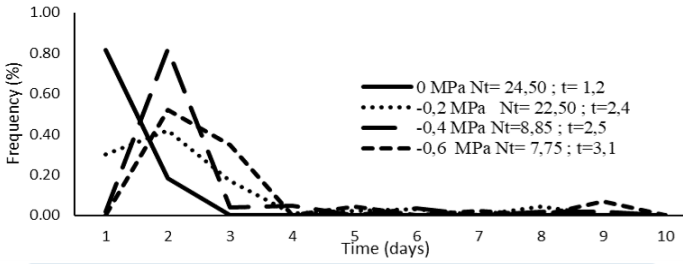

Figure 2: Relative frequencies of Parkia pendulous germination of plants under saline stress with NaCl (t = Average germination time; Nt = total number of seeds germinated in the period).

The biplot graphic representation and the linear correlation express the correlation of TSP, PA, TSS and Proline variables with the principal components (Figure 5).

Thus, the linear correlation coefficients between each variable allow explaining the discriminatory power of each variable within a principal component. Therefore, based on the linear correlations between biochemical variables and exposure time (days) under salt stress, it was observed that TSP presented

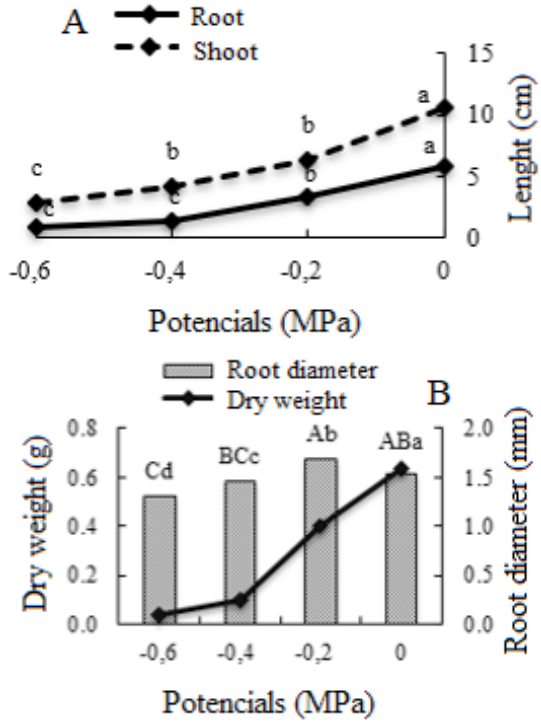

Figure 3: Root length and Shoot lenght (cm) **(A)** and Root diameter (mm) and Dry weight of seedlings **(B)** of *Parkia pendula*, subjected to saline stress with NaCl. The same capital letters for the slash and lowercase letters for the line do not differ from each other (P < 0,05), by Tukey test.

The PCA results, correlation coefficients and eigenvalues, indicate that 35.87% of the total variability can be explained by the first principal component (PC1); the second main component (PC2) can explain 29.77% of the variability, totaling 65.64% of the variability contained in the original **values (Table 1 and Figure 4)**.

Table 1: Eigenvalues, amount of explained variance, correlation coefficients and eigenvectors between the biochemical variables and times (days) in saline stress and the three first principal components.

Components	**PC1**		**PC2**	
Eigenvalue	1.44		1.19	
Explained variance (%)	35.87		29.77	
Cumulative variance (%)	35.87		65.64	
Correlation (eignvector)				
TSP	**0.81**	(45.86)	0.26	(5.55)
PA	0.30	(6.10)	**-0.70**	(-40.54)
TSS	**-0.83**	(-47.83)	-0.05	(-0.20)
Proline	-0.05	(-0.21)	**0.80**	(53.70)
Interpretation	The TPS content in contrast with TSS content		The PA in contrast with Proline content.	

significant positive correlation ($p < 0.05$), while in contrast, TSS presented significant negative correlation ($p < 0.05$) in PC1 (Figure 5). On the other hand, Proline presented significant positive correlation coefficient ($p < 0.05$), in contrast to PA, which presented significant negative correlation ($p < 0.05$) in PC2.

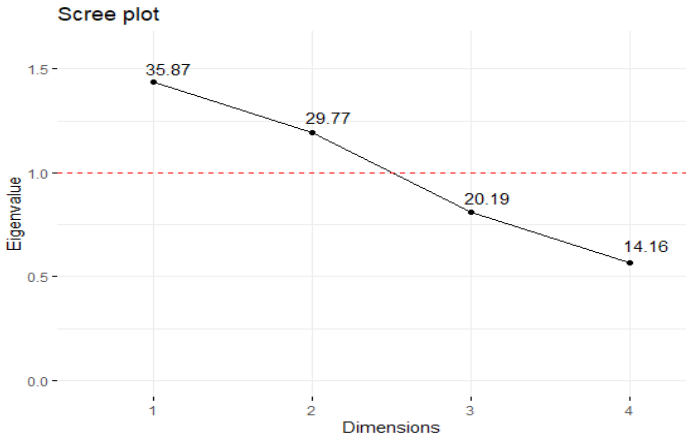

Figure 4: Screeplot of the dimensions used in the principal components analysis (PCA), indicating the percentage of explained variances used for interpreting the results to biochemical variables.

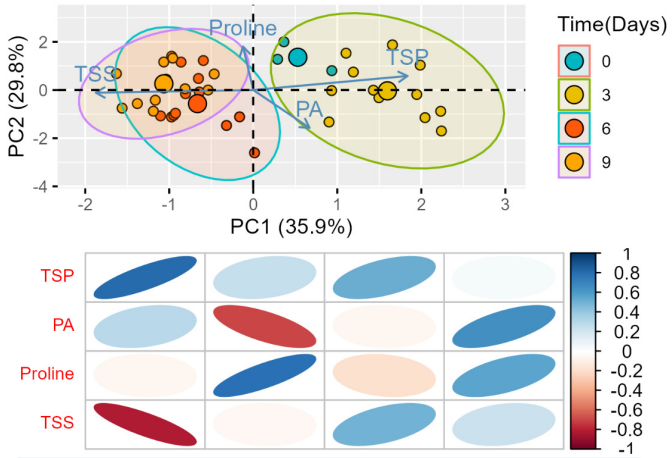

Figure 4: Biplot graphic of the first and second principal components from the PCA with all observations in the time (days) and correlation matrix between the biochemical variables and time (days) with the principal components. TSP= Total Soluble Protein (mg g^{-1} fresh weight); PA= Peroxidase Activity (H_2O_2 consumed minutes mg fresh weight); Proline (µg g^{-1} dry weight); TSS= Total Soluble Sugar (mg glucose g^{-1} dry weight).

Discussion

The reduction in the germination rate of *P. pendula* in the study (Figure 1A) was also observed in *Enterolobium schomburgkii* seeds (Benth.) at NaCl potentials from -0.2 MPa [19], while in *Apuleia leiocarpa* seeds, reduction in normal seedlings due to water and salt stress was observed [20]. These results are justified by the NaCl accumulation as a function of imbibition, causing damage to the embryo, with the intensification of ions in the protoplasm, promoting physiological changes and seed death [21]. These differences can be explained by the fact that the first changes in biochemical processes usually occur before the occurrence of declines in germination capacity.

Similarly, the lower GSI results observed in the present study **(Figure 1B)** are consistent with what has been described for other forest species such as *Gliricidia sepium* [22], *Apuleia leiocarpa* [20] and *Enterolobium contortisiliquum* [8]. The reduced GSI values are a reflection of the excess of Na+ and Cl- ions in the medium, which causes changes in metabolism, reduced water absorption and toxicity [23].

The highest MGT values provided at the most negative potentials -0.4 and -0.6 MPa **(Figure 1B)** are directly related to lower water availability, as a consequence of salinity, which increases the completion time of the seed germination process, being corroborated by [24], who also observed the highest MGT values in *Jatropha curcas* L. seeds with increased NaCl concentration. Therefore, there is reduction in seed germination due to the toxic effect of ions, so there is difficulty in absorbing important nutrients caused by the high salt concentration, promoting changes and impairing metabolic actions, consequently increasing germination time and reducing root elongation [25].

The reduction in seedling length as a result of salt stress, as observed in **Figure 3A**, was also observed in *Caesalpinea ferrea* [26], *Delonix regia* [27], *Erythrina velutina* Willd. [28] and *Albizia lebbeck* (L.) Benth. This is justified by the high salinity that causes reduction in the water potential in the growth medium, resulting in reduction in cell turgor [29]. Therefore, reduced cell turgidity tends to interrupt cell division, at the same time reducing cell growth elongation, slowing down or delaying plant development [28,29]. [30] reported that the transfer of dry mass from seed reserve tissues to the embryonic axis is considered a way of analyzing seed vigor through the seedling dry weight. This reduction in dry mass as a result of salt stress was also evidenced in *Vigna unguiculata* (L.) Walp [31] and *Erythrina velutina* Willd. [28]. This reduction in dry mass accumulation is a consequence of lower seedling growth and seed vigor and is related to the difficulty of *P. pendula* in osmotically adjusting to salinity with NaCl.

Regarding PCA, it was inferred that PC1 presented the highest correlation coefficients for biochemical variables in order of importance, first the TSS contents (-0.83) and then the TSP contents (0.81) (Table 1 and Figure 5). The results indicate that the TSP levels grouped 0 days and 3 days, justifying that with longer exposure times under salt stress, the action of proteolytic enzymes occurs, degrading amino acids and causing reduction in protein [32], and its content in seeds is related to vigor and genetic factors, being influenced by the characteristics of the environment during its formation [33].

In this context, it should be highlighted that initially in the germination process, TSS consumption starts quickly at the least negative potentials (0 and -0.2 MPa), being reduced at the most negative potentials, with soluble sugar reserves being used as source of energy for germination and as substrate for the formation of cell structures. This behavior corroborates [33], whose greater capacity to mobilize sugar and other reserves in seeds during germination results in seedlings with better initial performance, explaining the results.

The results also suggest that with longer exposure to stress (6 and 9 days), TSS consumption tends to decrease - probably due to the delay in the germination process as a result of the lower volume of solution absorbed by the seed tissues and the toxic effect promoted by salts, and that the lowest TSS levels recorded under higher stress intensity are due to osmoregulation. Therefore, evidence indicates that the TSS biochemical variable tends to be an excellent indicator in *Parkia pendula* seeds and seedlings under salt stress.

Similarly, the result of the importance of PC2 variables indicated that the highest correlation coefficients were Proline (0.80), followed by PA (-0.70) **(Table 1 and Figure 5)**. The positive correlation value is consistent, as Proline acts as an osmotic regulatory substance and tends to be stored preferentially in plant vacuoles [34] and under stress, they are transported to the cytoplasm, which reduces the osmotic potential, contributing to keep the cell protoplasm swollen [35]. Therefore, the

increase in Proline content under salt stress conditions was also observed in *Hymenaea courbaril* L. seeds [36]. The negative correlation value of the enzyme activity at 6 and 9 days may be related to the toxic effect of the accumulation of Na+ and Cl- ions caused by salt stress. Therefore, the oscillation in the activity of the peroxidase enzyme may be related to the change in the developmental phase of *P. pendula* at this saline potential (>0.2 MPa), since the plant material analyzed in this period corresponded to formed seedlings, with increased peroxidase activity in environment with high salt concentration, indicating protection against the stress factor [37].

Conclusion

The study with *Parkia pendula* seeds and seedlings revealed that salt stress with NaCl negatively affected the germination process, reducing the percentage values and GSI, increasing MGT, mainly at potential -0.6 MPa, validating the first hypothesis that *P. pendula* has no tolerance to salt stress.

The increase in salt stress was determinant in the reduction of the growth variables root and shoot length, collar diameter and seedling dry mass.

The collection intervals and different osmotic potentials changed the TSS, TSP, proline and PA contents compared to control in *P. pendula* seeds and seedlings, corroborating the second hypothesis that increasing the exposure time of seeds to salt stress changed the biochemical variables.

The results indicate that sugar and soluble protein reserves are source of energy to promote germination and substrate for the formation of cell structures, and the increase in Proline levels and peroxidase activity indicates osmoprotective action in adaptation to abiotic stress. Therefore, the study indicated that TSS at longer collection intervals and at more negative osmotic potentials is a good indicator of salt stress in *P. pendula* seeds and seedlings.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contribution statement

Luciano de Souza Maria - Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – original draft. Juliana Pereira Santos - Review & editing, Validation, Software, Methodology, Data curation. Elisabeth Emilia Ribeiro Teixeira - Conceptualization, Formal analysis, Investigation, Resources, Supervision, Validation, Writing – review & editing. Lúcia Filgueiras Braga - Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

References

1. Oliveira LC, Hopkins M. Parkia in Flora and Funga do Brasil. In: Botanical Garden of Rio de Janeiro, 2022.
2. Marangon C. External morphology of seeds and seedlings of Parkia pendula Benth. ex Walp. 2018; 2-10.
3. Pinedo GJV, Ferraz and IDK. HYDROCONDITIONING OF Parkia pendula [BENTH ex WALP]: SEEDS WITH PHYSICAL DORMENCE OF AMAZONIAN TREE. R Tree. 2008; 32: 39-49.
4. Tamires OB, Silva KRS, Teixeira DTF. Nitrogen Metabolism and Antioxidant System in Young Plants of Parkia pendula Submitted to Drought. Journal of Agricultural Science. 2018; 11: 459.
5. Pamplona ALQ, Costa EN, Carvalho JSB. Physiological performance of seeds and seedlings of three forest species subjected to KCl salinity. Research, Society and Development. 2021; 10: e33210413904.
6. Soliman MH, Alayafi AAM, El Kelish AA, Abu-Elsaoud AM. Acetylsalicylic acid enhance tolerance of Phaseolus vulgaris L. to chilling stress, improving photosynthesis, antioxidants and expression of cold stress responsive genes. Botanical Studies. 2018; 59: 6.
7. Elkelish AA, Soliman MH, Alhaithloul HA, El-Esawi MA. Selenium protects wheat seedlings against salt stress-mediated oxidative damage by up-regulating antioxidants and osmolytes metabolism. Plant Physiology and Biochemistry. 2019; 137: 144-153.
8. Dutra TR, Massad MD, Moreira PR, Ribeiro ESM. Effect of salinity on germination and initial growth of seedlings of three forest tree species. Brazilian Forest Research. 2017; 37: 323.
9. Santos RP, Gonçalves JCG, Borges EEL. Morphoanatomy, physiology and biochemistry of Parkias seeds., Novas Ediç. 2017.
10. Betoni R, Scalon S de PQ, Mussury RM. Salinity and temperature on seed germination and vigor of mutambo (Guazuma Ulmifolia Lam.) (Sterculiaceae) seeds. Tree Magazine. 2011; 35: 605-616.
11. Labouriau LG, Viladares MEB. On the germination of seeds of [the fiber plant] Calotropis procera (Ait.) Ait f Anais. 1976.
12. Maguire JD. Speed of Germination—Aid In Selection And Evaluation for Seedling Emergence And Vigor1. Crop Science. 1962.
13. Nakagawa J. Seed vigor tests. In: In: VIEIRA, R.D.; CARVALHO NM (ed) FUNEP. Jaboticabal. 1994; 164.
14. Lima GPP, Brazil OG, Oliveira AM de. POLYAMINE AND PEROXIDASE ACTIVITY IN BEAN (Phaseolus vulgaris L.) GROWN UNDER SALINE STRESS. Scientia Agricola. 1999; 56: 21-26.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976; 72: 248-254.
16. DuBois M, Gilles KA, Hamilton JK. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry. 1956; 28: 350-356.
17. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973; 39: 205-207.
18. Johnson RA, Wichern DW. Applied multivariate statistical analysis, 6a Ed. Upper Saddle River, NJ 07458, 2007.
19. Braga LF, Sousa MP, Almeida TA. Enterolobium schomburgkii (Benth.) Benth. seed germination under saline stress and polyamine application. Brazilian Journal of Medicinal Plants. 2009; 11: 63-70.
20. Spadeto CP, Lope JC, Mengard LHG, Matheus MT, Bernardes P. SALINE AND WATER STRESS ON GERMINATION OF SEEDS OF GARAPA (Apuleia leiocarpa (VOGEL.) J. F. Macbr.) Cristiani. Biosphere Encyclopedia. 2012; 8: 539-551.

21. Freitas AR, Lopes JC, Matheus MT. Overcoming dormancy in jatobá seeds. Brazilian Forest Research. 2013; 33:85-89.

22. Farias SGG, Freire ALO, Santos DR, Bakke IA, Silva RB. EFFECTS OF WATER AND SALINE STRESS ON GLIRICIDIA SEED GERMINATION [Gliricidia sepium (JACQ .) STEUD .] 1. Revista Caatinga. 2009; 22: 152-157.

23. Guedes RS, Alves EU, Viana JS. Germination and vigor of Apeiba tibourbou seeds subjected to water stress and different temperatures. Forest Science. 2013; 23:45-53.

24. Andréo-Souza Y, Pereira AL, da Silva FFS, Ribeiro RC, Evangelista MRV et al. Effect of salinity on physic nut (Jatropha Curcas L.) seed germination and seedling initial growth. Brazilian Seed Magazine. 2010; 32: 83-92.

25. Cavallaro V, Barbera AC, Maucieri C. Evaluation of variability to drought and saline stress through the germination of different ecotypes of carob (Ceratonia siliqua L.) using a hydrotime model. Ecological Engineering. 2016; 95: 557-566.

26. de Freitas RMO, Nogueira NW, de Oliveira FN. Effect of irrigation with saline water on emergence and initial growth of jucá seedlings. Caatinga Magazine. 2010; 23:54-58.

27. Nogueira NW, Suerda J, Lima S De. Effect of salinity on emergence and initial growth of flamboyant seedlings. Brazilian Seed Magazine. 2012; 34: 466-472.

28. Silva ECA da, Costa JR da S, Costa PCF da. Influence Of Salinity On Emergence And Early Growth Of Mulungu. Agricultural Science Magazine 2019; 17:63.

29. Naseer MN, Rahman FU, Hussain Z. Effect of Salinity Stress on Germination, Seedling Growth, Mineral Uptake and Chlorophyll Contents of Three Cucurbitaceae Species. Brazilian Archives of Biology and Technology. 2022; 65: 1-10.

30. Guedes RS, Alves EU, Gonçalves EP. Seedling length test in the evaluation of the physiological quality of Erythrina velutina Willd seeds Seedling length test in the evaluation of the physiological quality of Erythrina velutina Willd seeds. 2009; 793-802.

31. Deuner C, Maia MS, Deuner S, Almeida AS, Menechello GE. Viability and antioxidant activity of seeds of cowpea genotypes submitted to saline stress. Brazilian Seed Magazine. 2011; 33: 711-720.

32. Cardoso KPS, Palheta JG, de Sousa J do CM. Physiological and biochemical metabolism in Jatoba plants (Hymenaea courbaril L.) affected by water stress and flooding. Australian Journal of Crop Science. 2017; 11: 844-852.

33. Henning FA, Mertz LM, Jacob EA. Chemical composition and reserve mobilization in soybean seeds with high and low vigor. Bragantia. 2010; 69: 727-734.

34. Yang X, Lu M, Wang Y. Response Mechanism of Plants to Drought Stress. Horticulturae. 2021; 7.

35. Ribeiro RC, Matias JR, Pelacani CR, Dantas BF. Activity of antioxidant enzymes and proline accumulation in Erythrina velutina Willd. seeds subjected to abiotic stresses during germination. Journal of Seed Science. 2014; 36: 231-239.

36. Nascimento HHC do, Santos CA dos, Freire CS. Osmotic Adjustment In Jatobá Seedlings Submitted To Salinity In Hydroponic Medium. Tree Magazine. 2015; 39: 641-653.

37. Harter LSH, Harter FS, Deuner C. Salinity and physiological performance of mogango seeds and seedlings. Brazilian horticulture. 2014; 32:80-85.