UFSM

Ciência Florestal

ISSN 1980-5098
Open access

Ci. Fl., Santa Maria, v. 32, n. 4, p. 2199-2218, Oct./Dec. 2022 • https://doi.org/10.5902/1980509867600
Submitted: 8th/09/2021 • Approved: 30th/08/2022 • Published: 23rd/11/2022

Artigos

Effect of *seed priming* with NaCl on the induction of salinity tolerance in *Myracrodruon urundeuva* Allemão *in vitro*

Efeito do *seed priming* com NaCl na indução de tolerância à salinidade em *Myracrodruon urundeuva* Allemão *in vitro*

Lindomar Maria de Souza[I], **Emanuella Maria da Conceição**[II], **Marta Ribeiro Barbosa**[I], **Luiz Palhares Neto**[III], **Ana Maria Maciel dos Santos**[I], **Robson Antonio de Souza**[I], **Laureen Michelle Houllou**[I]

[I]Centro de Tecnologias Estratégicas do Nordeste, Recife, PE, Brazil
[II]Escola de Referência em Ensino Médio Silva Jardim, Recife, PE, Brazil
[III]Universidade Estadual do Sudoeste da Bahia, Jequié, BA, Brazil

ABSTRACT

Soil salinization is an environmental factor that frequently occurs in semi-arid regions around the world and *seed priming* technique is one of the alternatives to obtain the greatest establishment of seedlings in the field, through the induction of tolerance to environmental stresses. This research aimed to evaluate the effect of *seed priming* with NaCl on the induction of salinity tolerance in *Myracrodruon urundeuva* Allemão *in vitro*. The research was carried out at the Center for Strategic Technologies of the Northeast, in Recife / PE-Brazil *M. urundeuva* seeds were disinfected and submitted to two treatments: water (control) or NaCl (2mM), and inoculated in WPM medium at three saline concentrations (0.0; 25.0 and 50.0 mM NaCl) for 45 days.Plant height, number of leaves, fresh biomass (total, shoot, and root), shoot/root ratio, sodium and potassium and Na/K ratio, antioxidant enzyme activity, and peroxide content were evaluated malondialdehyde hydrogen. The data were subjected to variance analysis and the results were compared using the Student-Newman-Keuls test at 5% probability, using the R software. *Seed priming* with NaCl promoted a beneficial effect on the height of seedlings exposed to salinity. On the other hand, height was inversely proportional to saline concentrations, regardless of *seed priming*. The shoot/root ratio was also lower in seedlings cultivated under 25 and 50 mM NaCl. Despite being favorable for most growth variables, *seed priming* with NaCl induced an increase in lipid peroxidation in seedlings that were not exposed to salinity, which is linked to a decrease in the activity of antioxidant enzymes. The antagonistic response to NaCl stimulation between growth parameters and plant defense observed in the present research raises the need for further complementary studies that make it possible to delineate the metabolic alterations of *M. urundeuva* against a chemical stimulus to induce tolerance to a given stress.

Keywords: Caatinga; Tree; Soil salinization; Antioxidant enzymes

RESUMO

A salinização do solo é um fator ambiental que ocorre frequentemente em regiões semi-áridas ao redor do mundo e a técnica de *seed priming* é uma das alternativas para se obter o maior estabelecimento de mudas no campo, através da indução de tolerância aos estresses ambientais. Este trabalho objetivou avaliar o efeito do *seed priming* com NaCl na indução da tolerância à salinidade em *Myracrodruon urundeuva* Allemão *in vitro*. A pesquisa foi realizada no Centro de Tecnologias Estratégicas do Nordeste, em Recife / PE-Brasil. Sementes de *M. urundeuva* foram desinfestadas e submetidas a dois tratamentos: água (controle) ou NaCl (2mM), e inoculadas em meio WPM em três concentrações salinas (0.0; 25.0 and 50.0 mM NaCl) por 45 dias. Foi avaliada a altura, número de folhas, biomassa fresca (total, parte aérea e raiz), razão parte aérea / raiz, sódio, potássio, razão Na / K, atividade de enzimas antioxidantes, teores de peróxido de hidrogênio e malondialdeído. Os dados foram submetidos à análise de variância e os resultados comparados pelo teste Student-Newman-Keuls a 5% de probabilidade, utilizando o software R. O *priming* com NaCl promoveu efeito benéfico na altura de mudas expostas à salinidade. Todavia, a altura foi inversamente proporcional às concentrações salinas, independentemente do *priming*. A razão parte aérea / raiz foi menor nas mudas cultivadas sob NaCl 25 e 50 mM. Apesar de ser favorável para a maioria das variáveis de crescimento, o *seed priming* com NaCl induziu aumento na peroxidação lipídica em mudas não expostas à salinidade, o que está relacionado à diminuição da atividade de enzimas antioxidantes. A resposta antagônica à estimulação do NaCl entre parâmetros de crescimento e defesa das plantas na presente pesquisa levanta a necessidade de novos estudos complementares que possibilitem delinear as alterações metabólicas de *M. urundeuva* frente a um estímulo químico para induzir tolerância a um determinado estresse.

Palavras-chave: Caatinga; Árvore; Salinização do solo; Enzimas antioxidantes

1 INTRODUCTION

The Caatinga ecogeographic domain is exclusive to the Brazilian territory, predominantly in the northeast region of the country, and represents about 13% of the national territory, occupying an area of 912,000 km2 rich in biodiversity (BARROS *et al.,* 2021). The main characteristic of Caatinga environment is the poorly developed soils, with an intense dry period, with almost no rain for 8 to 10 months throughout the year (AQUINO *et al.,* 2021). Due to anthropogenic exploration as well as the edaphoclimatic characteristics of the semi-arid region, the Caatinga is in a condition of vulnerability, highly threatened in the scenario of rising global temperatures, putting this region on alert (NOBRE, 2011) due to the risks of desertification and salinization of soils (OLIVEIRA *et al.,* 2013; NOBRE, 2011).

The process of soil salinization is a frequent environmental factor in semi-arid regions around the world, caused by low rainfall and high evaporation rate, being aggravated by the inadequate use of the soil in production processes. Even native species, which are more adapted to the environment, may have the germination process and/or seedling survival impeded in more extreme conditions of soil salinity (RIBEIRO *et al.,* 2017). This fact can make programs unfeasible to recover degraded areas with saline soils, especially when natural regeneration techniques are used through the dispersion of propagules in the environment or even the direct seeding of forest species (GONÇALVES *et al.,* 2020).

Among the alternatives to obtain the greater establishment of seedlings in the field, the *seed priming* technique can provide tolerance to various environmental stresses through the induction of cellular memory, which accompanies specific epigenetic changes as well as plant physiology (REHMAN *et al.,* 2014). On occasion, *seed priming* establishes a long-term somatic memory accompanied by specific changes in the epigenome that comprise differences in chromatin states, transcriptional responsiveness, as well as the physiology of the whole plant. Thus, this technique can promote increases in the activities of several enzymes, as well as physiological adjustments that allow plants to survive a given stress condition (ACHARYA *et al.,* 2020). Allied to this, there is an easier water absorption by the seed, which activates the pre-germinative metabolism from DNA repair processes and modulations in the redox system, promoting the induction of tolerance to abiotic stresses during germination (NÓBREGA *et al.,* 2021).

One of the most used chemical agents in inducing tolerance to abiotic stresses is sodium chloride (NaCl), which can induce tolerance and increase the percentage of seed germination under salinity conditions when used in *seed priming* (STASSINOS *et al.,* 2021).

In the Caatinga restoration of native forests, the proper choice of species is essential to obtain good results. Popularly known as aroeira, *Myracrodruon urundeuva* Allemão is among the options of native trees from the Caatinga to be used in reforestation processes (DRUMOND *et al.,* 2016). Seedlings are obtained from seed germination. However, areas with saline soils can limit the establishment and maintenance of seedlings in the field.

M. urundeuva is a native tree of Brazil with distribution in the North, Northeast, Midwest, Southeast, and South of the country and occurs in Caatinga vegetation, Cerrado, and Atlantic Forest. It is widely used in the reforestation of native forests. However, its wood is economically exploited due to its physical-mechanical qualities and can be used for the production of stakes, beams, slats, rafters, floor battens, firewood, and charcoal. In addition to its use in the wood industry, this species has wide application in folk medicine in the treatment of inflammation, stomach ulcers, colds, rheumatism, among other diseases (PAREYN *et al.,* 2018), as well as in the commercial production of herbal products, is widely used by local populations in an exploratory and non-renewable manner. However, these activities, mostly predatory, are resulting in rapid loss of species, elimination of ecological processes and formation of extensive desertification nuclei in various locations region (SILVA *et al.,* 2012). *M. urundeuva* has multiple uses, being considered a reference tree species in studies with plants under environmental stress (CAPO *et al*., 2022).

Considering the problem of soil salinization in the semiarid region, as well as species conservation and restoration of degraded areas, this research aimed to evaluate the effect of *seed priming* with NaCl in inducing in vitro salinity tolerance in *M. urundeuva* Allemão. Initially, the following questions were asked: 1) Does *seed priming* have a beneficial action on the growth of *M. urundeuva* under salinity conditions? 2) *Seed priming* with NaCl is effective in inducing salt stress tolerance and activating the enzymatic antioxidant defense system in *M. urundeuva* seedlings?

2 MATERIAL AND METHODS

The study was carried out at the Laboratory of Research Applied to the Biofactory of the Northeast Strategic Technologies Center (CETENE), located in Recife-PE-Brazil. Diaspores of *Myracrodruon urundeuva* were collected at the José Nilson de Melo Experimental Station belonging to the IPA (Agronomic Institute of Pernambuco), in the municipality of Caruaru (PE), under geographic coordinates 8 ° 14 'and 35 ° 55' in December 2015.

Diaspores were used as explants, which were disinfected in alcohol (70%) for one minute and then in sodium hypochlorite (2.5%) for 15 minutes. Then, the explants were washed three times in sterile distilled water.

2.1 *Priming* application

After rinsing, the seeds were soaked in the following solutions: sterile distilled water or 2 mM NaCl solution, where they remained for 1 h under gentle agitation.

Then, the seeds were inoculated in test tubes containing 10 ml of Woody Plant Medium (WPM) (LLOYD; MCCOWN, 1981), plus 0.1 g. inositol L^{-1}, 30 g. L^{-1} sucrose and 5.5 g. L^{-1} of agar. The saline concentrations tested were: 0.0; 25.0 and 50.0 mM NaCl. After inoculation, the test tubes containing the diaspores were kept in a growth room at 25 ± 2°C in the dark for 45 days. After emergence, the seedlings were transferred to shelves with white LED lamps, with a light intensity of 42 μmol m2 s^{-1}. The seedlings were kept under these conditions for 45 days.

2.2 Development and growth parameters

2.2.1 Biochemical Analysis

The seedling height was considered from the largest root to the shoot, fresh biomass (total, shoot and root) was obtained using a precision scale and the number of leaves/plant was obtained by counting (transformed data √x+1).

Enzymatic activities of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.1), and catalase (CAT, EC 1.11.1.6) were analyzed at 45 days. For this, 0.2 g of *M. urundeuva* leaves were macerated in liquid nitrogen, and then 2 mL of extraction buffer (pH 7.0) containing potassium phosphate, 1,4-dithiothreitol, and ethylene diaminetetraacetic acid were added. Subsequently, the macerate was centrifuged at 10,000 g 4 °C for 20 minutes and the supernatant was used for the analysis of enzymatic activities.

The enzymatic activity of SOD was performed according to Giannopolitis and Reis (1977). A mixture of the reagents (1765 µl 85 mM sodium phosphate buffer, 780 µl 50 mM methionine, 225 µl 1 mM nitroblue tetrazolium, 30 µl 10 mM EDTA, and 150 µl 0.1 mM riboflavin was added to 50 µL of sample extract in test tubes. The tubes were exposed to lighting with fluorescent lamps (30 watts) for 5 minutes. Afterward, the tubes were kept in the dark until the moment of reading, which was performed in a spectrophotometer at 560 nm, and the results were expressed in U.SOD g^{-1} fresh mass (FM). APX activity was performed as described by Nakano and Asada (1981). An aliquot (75 µL) of the extract received 1335 µL of buffer (0.05 M potassium phosphate, 0.001 M EDTA, pH 6.0), 75 µL (0.01 M) ascorbate and 15 µL of hydrogen peroxide (0 .1M). Readings were performed in a spectrophotometer at 290 nm. The activities were calculated from the molar extinction coefficient for ascorbate (2.8 $mM^{-1}cm^{-1}$) and expressed in µmol AsA g^{-1} of fresh biomass per minute.

The CAT activity followed the methodology proposed by Berrs and Sizer (1952). To the sample extracts (50 µL) were added 1390 µL of monobasic potassium phosphate buffer (50 mM - pH 7.0) and 60 µL of hydrogen peroxide (500 mM). Readings were performed in a spectrophotometer at 240 nm. The enzymatic activity was expressed in nmol H_2O_2 g^{-1} of fresh mass per minute and for the calculation of the activities, 36 mM^{-1} cm^{-1} was considered as molar extinction coefficient.

Measurements of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents were performed with the same extract, which was prepared with 0.2 g of macerated leaves and homogenized in 2.0 ml of trichloroacetic acid (1%) and subsequently centrifuged at 2000 x*g* for 10 minutes at 4°C. The MDA analysis was performed according to Heath and Packer (1968). Approximately 250 µL of the extract was added to cryogenic tubes containing 1 mL of thiobarbituric acid (0.5%) and trichloroacetic acid (20%) solution. The tubes were kept in a dry bath for 30 minutes at 95°C and then cooled in an ice bath and centrifuged again for 10 minutes at 2000 *g*. Readings were performed at 535 and 600 nm and results were expressed in nmol. g^{-1} of fresh biomass. The H_2O_2 content was determined according to the method by Loreto and Velikova (2001). An aliquot of the extract (75 µL) was added to 1.75 mL of potassium phosphate buffer (10 mM, pH 7.0) and 1.5 mL of potassium iodide (1M). The H_2O_2 content was measured at 390 nm in a spectrophotometer and expressed in µmol. g^{-1} of fresh biomass.

2.2.2 Na and K content

The seedlings of *Myracrodruon urundeuva* were dried in forced aeration ovens at 60 ºC until constant weight. Then, the material was crushed and submitted to digestion in sulfuric acid and hydrogen peroxide in a digester block at 350ºC, according to the methodology proposed by Thomas *et al.* (1967). For the quantification of sodium and potassium contents, flame spectroscopy was used (EMBRAPA, 2009), and the results were expressed in mg. g. fresh mass$^{-1.}$

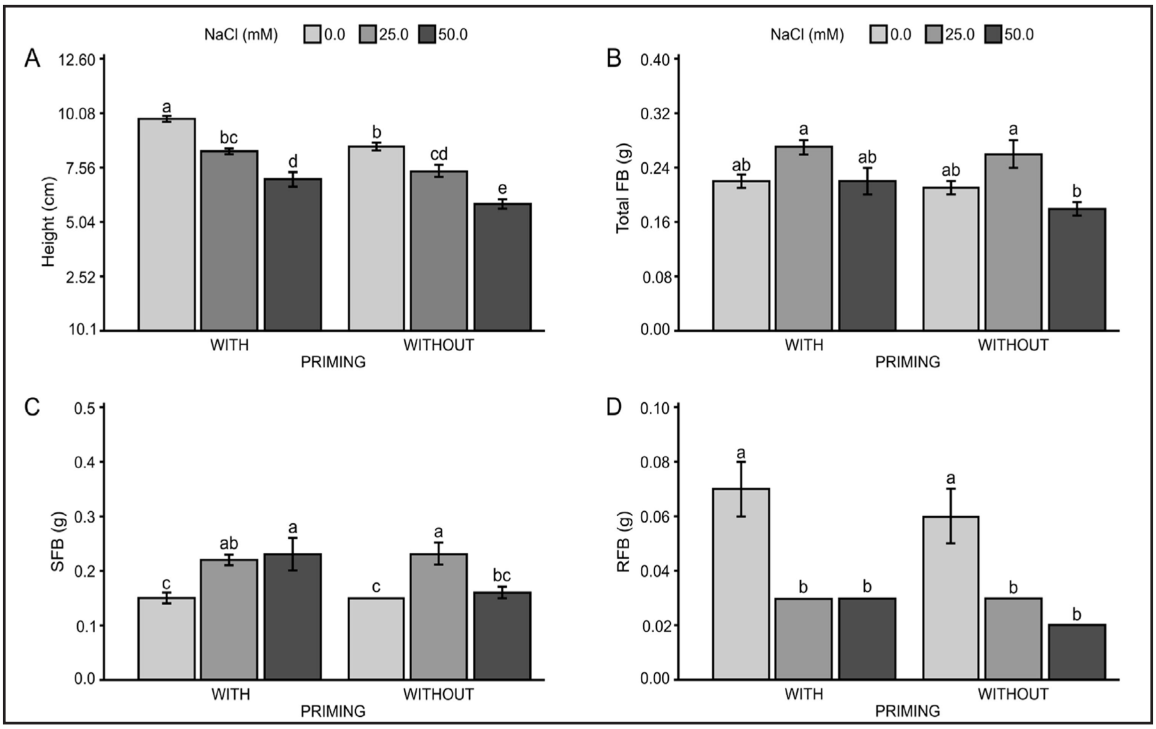
2.2.3 Statistical analysis

The experimental design was completely randomized, consisting of a 2x3 factorial scheme, 2 *seed priming* concentrations (control and 2M NaCl), and 3 saline levels (0.0; 25.0 and 50.0 mM NaCl), totaling 6 treatments with 24 reps. The data were subjected to analysis of variance (ANOVA), respecting the prerequisites (normality and homogeneity), and the means were compared using the Student-Newman-Keuls (SNK) test at a 5% probability level. Person correlation, principal component analysis (PCA), and cluster analysis were performed, all performed with the aid of the R software.

3 RESULTS AND DISCUSSION

Among the chemical agents widely used in *seed priming*, NaCl is the most common, being able to induce tolerance under salinity conditions (STASSINOS *et al.,* 2021). Seedling height was inversely proportional to saline concentration in both priming groups. The seedlings that received the conditioning with 0mM NaCl showed greater height than the untreated seedlings. (Figure 1A).

Figure 1 – Effect of *seed priming* on height (A), total fresh biomass (Total FB) (B), shoot fresh biomass (SFB) (C) and root fresh biomass (RFB) (D) of *Myracrodruon urundeuva* Allemão seedlings submitted to three levels of salinity (0.0; 25.0 and 50.0 mM NaCl) *in vitro* for 45 days



Source: Authors (2021)

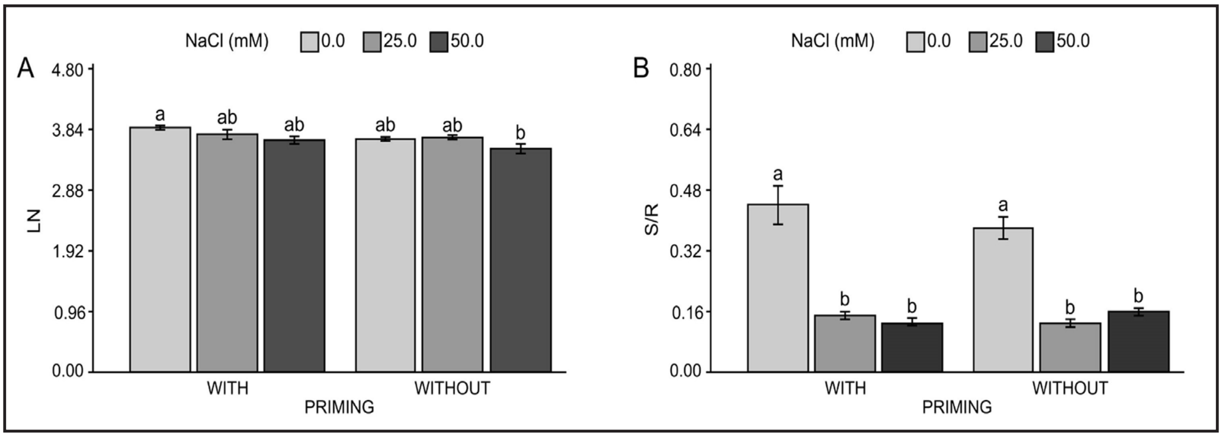
Studying the initial growth of *Cereus jamacaru* seedlings, Nobrega *et al*. (2021) also observed that pre-treatment of seeds with eliciting substances can increase seedling growth.The total fresh biomass (TFB) of the seedlings decreased in the saline treatment with 50.0 mM NaCl in the group that did not receive the *seed priming*, while the seedlings from the pre-treated seeds maintained the total fresh biomass even in the highest salt concentration (Figure 1B). *Seed priming* also favored the increase in shoot fresh biomass (SFB), while seedlings from treatment without *priming* showed a decrease in the mean of this variable when subjected to a saline concentration of 50.0 mM NaCl (Figure 1C). Root fresh biomass (RFB) was reduced in saline concentrations of 25.0 and 50.0 mM of NaCl, regardless of *priming* application (Figure 1D). Assessing the physiological behavior of *Myracrodruon urundeuva* under salinity conditions, Oliveira *et al*. (2019) suggest that the use of this species in saline soils is limited. The authors tested different osmotic potentials with polyethylene glycol (PEG) and NaCl. The authors found that the osmotic threshold for germination of *M. urundeuva* seeds in NaCl was -0.43 MPa. Therefore, more studies are needed.

The *seed priming* technique is a viable and low-cost technology that makes it possible to reduce the adverse effects of salinity on the physiological and biochemical aspects of plants (KHAN *et al.,* 2021). The beneficial effects of *seed conditioning* with NaCl were demonstrated by Abol-Hasani *et al*. (2020), by decreasing the harmful effects of salinity in *Dracocephalum moldavica* plants. The authors emphasized the increase in biomass production, decrease in membrane damage, electrolyte leakage, and increase in leaf area. Preconditioning with NaCl favored the maintenance of biomass production in sugarcane plants and induction of tolerance when they were exposed to salinity (MELO *et al*., 2014).

In the case of *M. urundeuva* in the present research, the excess of salts was not favorable to the accumulation of fresh root biomass, which is probably linked to the osmotic effect of the saline media solutions, caused by the decrease in the free energy of water, directly influencing the cellular water status (AMANIFAR; TOGHRANEGAR, 2020). Despite the eliciting effect of the H_2O_2 in attenuating the harmful effects of salinity, a decrease in seedling growth of *M. urundeuva* under salinity conditions was observed (RODRIGUES *et al*., 2020) The seedlings from the *seed priming* treatment kept the mean leaves number (LN) similar regardless of the saline concentration (Figure

2A). On the other hand, in seeds that did not receive *priming*, the seedlings presented a reduction in the number of leaves when submitted to 50.0 mM of NaCl. The shoot/root ratio (SRR) was reduced in seedlings cultivated with 25.0 and 50.0 mM of NaCl, regardless of *seed priming* application (Figure 2B).

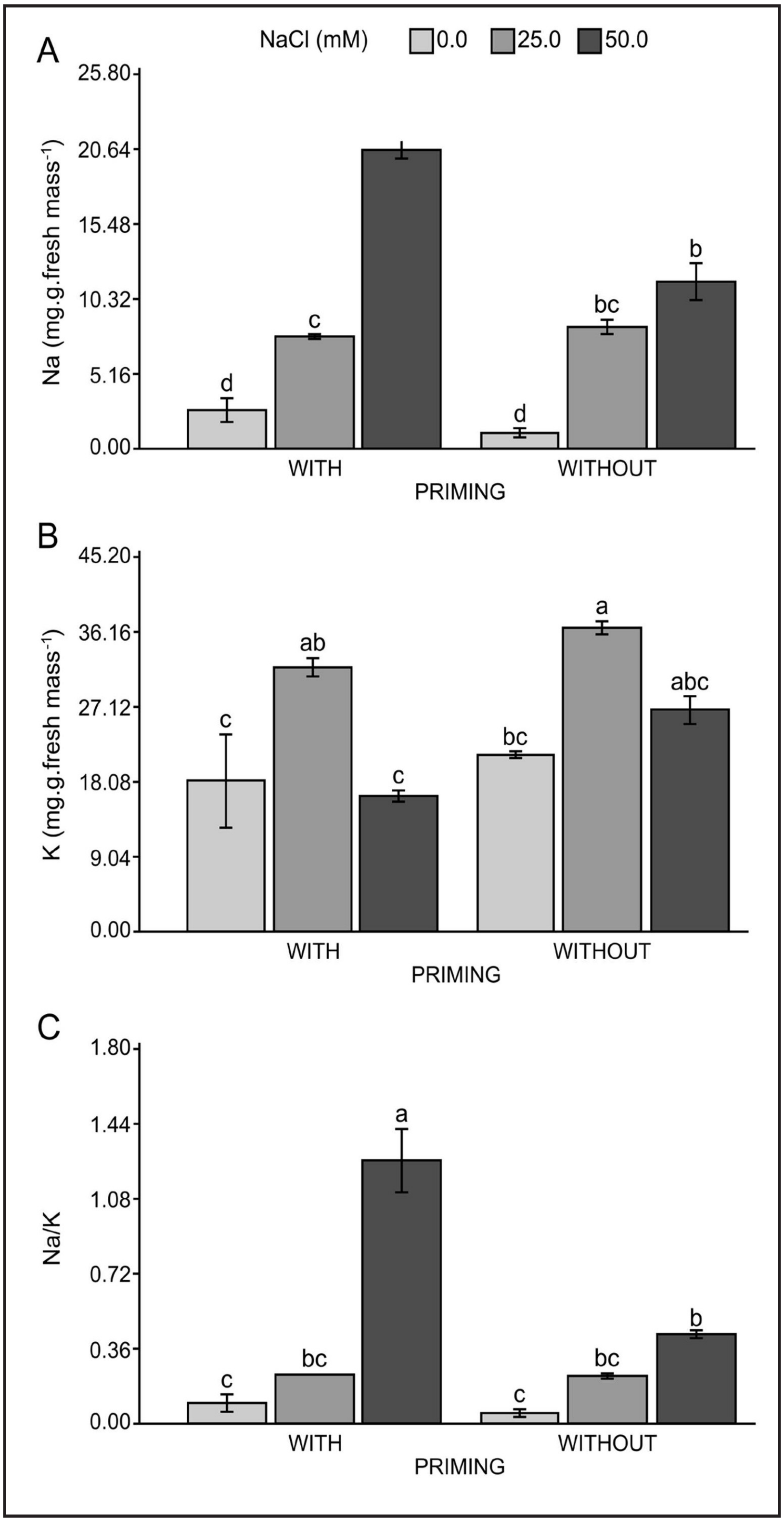
Figure 2 – Effect of *seed priming* on the number of leaves (LN) (A) and shoot/root ratio (S/R) (B) in *Myracrodruon urundeuva* Allemão seedlings submitted to three levels of salinity (0.0; 25.0 and 50.0 mM NaCl) *in vitro* for 45 days



Source: Authors (2021)

The effect of NaCl on growth is one of the most studied topics in plant physiology. The growth inhibition and the plant production reduction could be a result of solution changes in osmotic potential, caused by excess salts and/or their toxic effect (ISAYENKOV; MAATHUIS, 2019). Thus, the decrease in plant growth, in addition to being linked to the cytotoxic effect of sodium, is closely linked to drastic changes in the water status of the plant, which can be considered the primary cause of productivity losses, directly affecting the basic processes of cell division and expansion (HERNÁNDEZ, 2019). Sodium levels increased at the expense of saline concentrations in the culture medium, where the highest average was found in plants subjected to 50.0 mM NaCl and receiving *seed priming* (Figure 3A). Regarding potassium contents, the highest averages were verified in plants submitted to 25.0 mM of NaCl regardless of priming treatment (Figure 3B). The Na / K ratio was higher in pretreated plants that were subjected to 50.0 mM NaCl (Figure 3C).

Figure 3 – Effect of *seed priming* on sodium (Na) content [mg. g. fresh mass^{-1}] (A), potassium (K) [mg. g. fresh mass^{-1}] (B) and Na / K ratio (C) in *Myracrodruon urundeuva* Allemão seedlings submitted to three levels of salinity (0.0; 25.0 and 50.0 mM NaCl) *in vitro* for 45 days

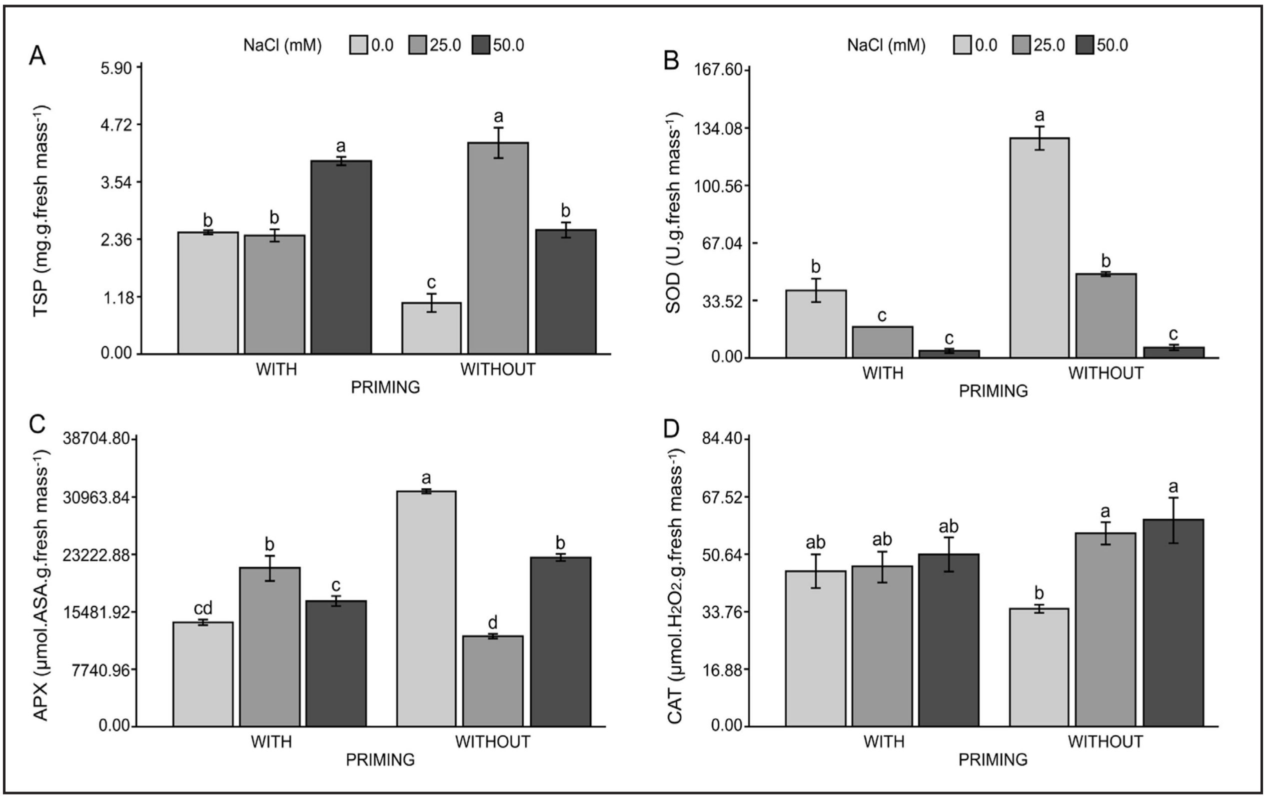


Source: Authors (2021)

Under salinity conditions, sodium can reduce the absorption of nutrients such as potassium (OLIVEIRA *et al.,* 2018). In the present research, it was found that *M. urundeuva* seedlings were not able to promote an efficient ionic adjustment, which allowed greater absorption of sodium at the expense of potassium.

The total soluble protein (TSP) content was altered as a function of priming treatments and saline concentrations in the culture medium (Figure 4A). The application of *seed priming* with NaCl favored the increase in the content of total soluble proteins in seedlings subjected to 50.0 mM of NaCl. However, seedlings from seeds that did not receive priming and that were subjected to the same saline concentration showed a decrease in the mean values for this variable (Figure 4A). Under conditions of salt stress, several nitrogenous compounds can be accumulated among the main ones are proteins, a multigenic trait of tolerance to salt stress (HERNÁNDEZ, 2019).

Figure 4 – Effect of *seed priming* on total soluble protein content (TSP) (A), on the activity of superoxide dismutase enzymes (SOD) (B), ascorbate peroxidase (APX) (C) and catalase (CAT) in *Myracrodruon urundeuva* Allemão seedlings submitted to three levels of salinity (0.0; 25.0 and 50.0 mM NaCl) *in vitro* for 45 days



Source: Authors (2021)

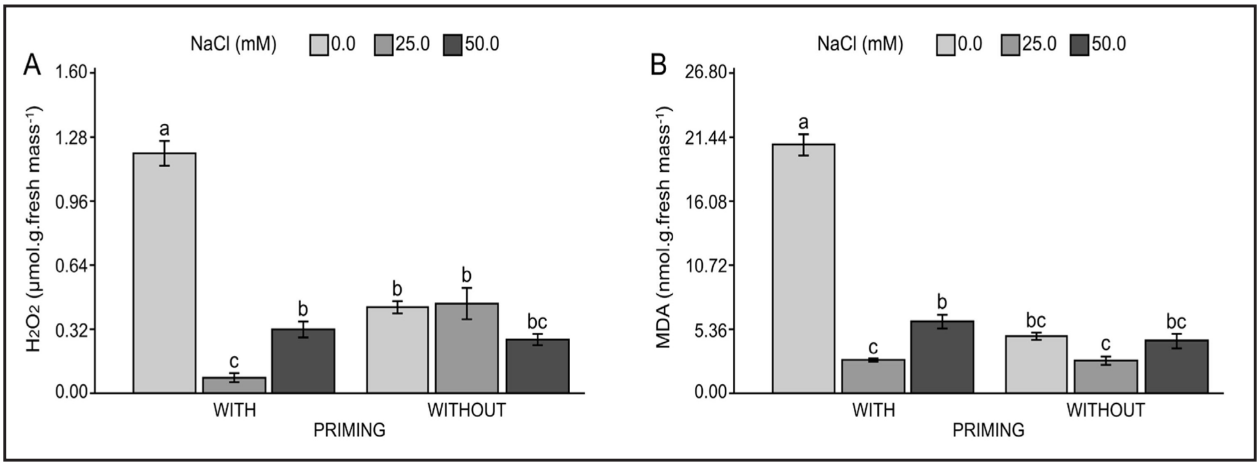
Proteins play several roles in plant survival, including the defense function, and these represent the main changes in the total soluble protein content in plants (KOSOVÁ *et al.,* 2018). The response in the protein content of plants under salinity conditions can vary greatly according to the degree of tolerance that each species presents (gene expression), even suggesting the participation of some polypeptides in the cellular osmotic adjustment (HERNÁNDEZ, 2019).

In adverse environmental conditions, plants use antioxidant defense systems (enzymatic and non-enzymatic) to prevent severe damage caused by oxidants in their cell structures. Among the main enzymes that make up the enzymatic antioxidant defense system are superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) which act on the front line of plant defense, mitigating the adverse effects of stress as well as preventing oxidative stress (SOUZA *et al.,* 2020).

The SOD activity in seedlings showed an inversely proportional relationship with salinity, regardless of priming treatment, that is, the higher the salinity, the lower the activity of this enzyme. However, seedlings from seeds that were priming with NaCl showed a marked decrease in the activity of this enzyme (Figure 4B). APX activity was more expressive in the group that did not receive *seed priming* with NaCl, especially in the saline control. On the other hand, the CAT activity in this same treatment was lower, indicating a more effective and joint action of SOD and APX (Figure 4B, C and D). The CAT activity did not show differences between the seedlings of saline treatments that received priming with NaCl (Figure 4D).

Tolerance to environmental stresses has been associated with efficient antioxidant defense (SOUZA *et al.,* 2020), through the upregulation of the activity of antioxidant enzymes (SOD, CAT, POD, GR, etc.), where each enzyme performs a specific function in the detoxification of reactive oxygen species (ROS) (ABOGADALLAH, 2010). Although SOD catalyzes the conversion of the superoxide anion ($O_2^{.-}$) to H_2O_2, in the present study this enzyme did not present a strong or significant correlation with the H_2O_2 contents (r= 0.19). On the other hand, H_2O_2 showed a strong and positive correlation (0.78, 0.77, 0.67 and 0.66) with the growth parameters. This might suggest that this molecule had most of its origin in the apoplast region through NADPH-dependent oxidases and some cell wall peroxidases. This region is where the loosening of the wall for cell expansion occurs (SILVEIRA *et al.,* 2017). Salinity caused by excess NaCl commonly causes physiological stress, contributing to an imbalance in the redox system and leading to an increase in ROS content, increasing damage to biological membranes (MORAIS *et al.,* 2019). However, in the present research, it was observed that the contents of H_2O_2 and MDA had the highest means in the saline control in the group of seeds that were primed with NaCl (Figures 5A, 5B).

Figure 5 – Effect of *seed priming* on hydrogen peroxide content (H_2O_2) (A) and malondialdehyde (MDA) (B) in *Myracrodruon urundeuva* Allemão seedlings submitted to three levels of salinity (0.0; 25.0 and 50.0 mM NaCl) *in vitro* for 45 days

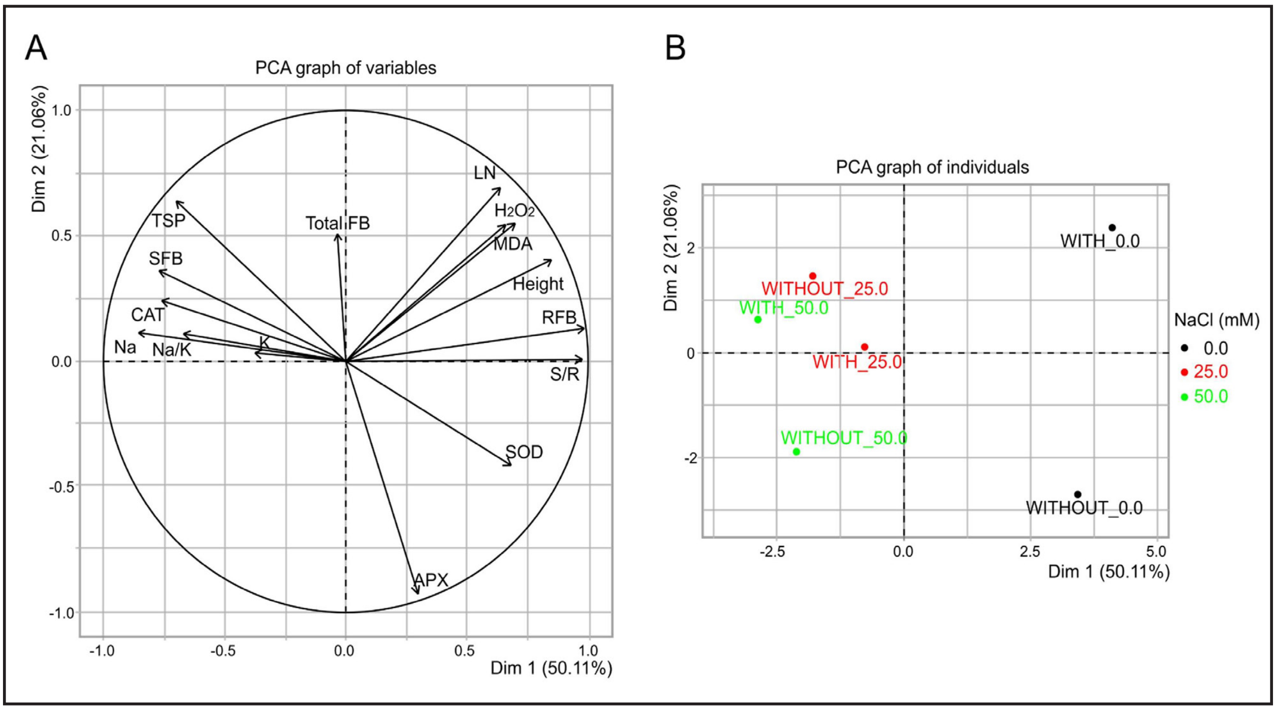


Source: Authors (2021)

The increase in the amount of salts level in the substrate solution can trigger an imbalance in the plant's redox system, causing stress, which in turn can damage plant growth and development (MORAIS *et al.,* 2019). However, the answers obtained in the present research show us that *priming* with NaCl was not effective in activating the enzymatic antioxidant defense system in seedlings that were not subjected to subsequent stress, not showing the desired signaling effect. On the contrary, it favored stress on seedlings that were not cultivated in a saline medium.

The principal components analysis (PCA) considering the two main axes, explain 71% of the variations presented in this study. Within the analyzed parameters, it is observed that dimension 1 explains 50.11% of the results (Figure 6A).

Figure 6 – Principal Component Analysis (PCA) in Myracrodruon urundeuva Allemão seedlings submitted to three levels of salinity in vitro for 45 days



Source: Authors (2021)

In where: Principal Component Analysis (PCA): Effect of *seed priming* on height, total fresh biomass (TFB), fresh shoot biomass (FSB), fresh root biomass (FRB), number of leaves (NL), sodium (Na) content, potassium (K) content, total soluble protein content (TSP), on the activity of superoxide dismutase enzymes (SOD), ascorbate peroxidase (APX) and catalase (CAT), on hydrogen peroxide content (H_2O_2) and malondialdehyde (MDA) in *Myracrodruon urundeuva* Allemão seedlings submitted to three levels of salinity (0.0; 25.0 and 50.0 mM NaCl) *in vitro* for 45 days.

The groupings observed in the PCA were important for a better understanding of the seedling responses to the imposed factors. The variables were grouped according to the correlation with the evaluated treatments and, thus, it is observed that salinity was the main factor with the distribution of variables within the quadrants (Figure 6B). In general, salinity negatively affected growth parameters and some biochemical variables, such as enzymatic activity of SOD and APX, as observed in the upper quadrants. The analysis shows the inversely proportional relationship between the soluble protein contents and the activity of SOD and APX enzymes, confirming that the total protein contents may vary as a function of the enzymatic activity. It also shows us the positive correlation between the H_2O_2 contents with most of the growth parameters in the upper right quadrant, as well as the positive correlation between the H_2O_2 and MDA contents, confirming that the increase in lipid peroxidation in this treatment was due to the increased concentrations of this ROS. The vectors position indicates that salinity had a detrimental effect on the seedling root system. The results clearly show that *seed priming* with NaCl caused a decrease in the activity of SOD and APX enzymes. Seedlings grown under salinity, regardless of priming, showed a positive correlation with Na and K contents, as well as with protein contents and CAT activity (Figures 6A, 6B).

4 CONCLUSION

Despite *Myracrodruon urundeuva* not tolerating environments with high salinity, this study proved some tolerance plasticity within the saline concentrations tested could be obtained. *Seed priming* with NaCl, despite being favorable when considering most growth and development variables, induced an increase in lipid peroxidation in seedlings that were not exposed to salinity, which is linked to a decrease in the activity of antioxidant enzymes. This antagonistic response to NaCl stimulation between growth parameters and plant defense raises the need for further studies to complement this one, with a broader framework of analyzes in order to delineate *M. urundeuva* strategies in a more integral way when a chemical stimulus is directed to the embryo.

REFERENCES

ABOL-HASAN, F. S.; ROSHANDEL, P. Induced changes by NaCl-*Seed priming* in *Dracocephalum moldavica* plants upon salinity. **Journal of Horticulture and Postharvest Research**, Birjand, v. 3 (Special issue: Abiotic and biotic stresses in horticultural crops), p. 29-42, 2020.

ABOGADALLAH, G. M. Insights into the significance of antioxidative defense under salt stress. **Plant Signaling & Behavior**, Texas, v. 4, n. 5, p. 369-374, 2020.

AQUINO, D. N.; ANDRADE, E. M.; SOUZA FILHO, E. T.; CAMPOS, D. A. Impacto de Secas e Antropização na Dinâmica da Cobertura Florestal em Fragmento do Domínio Fitogeográfico da Caatinga. **Revista Brasileira de Geografia Física**, Pernambuco, v. 3, n.14, p.1675-1689, 2021.

ACHARYA, P.; JAYAPRAKASHA, G. K.; CROSBY, K. M.; JIFON, J. L.; PATIL, B. S. Nanoparticle-Mediated *Seed Priming* Improves Germination, Growth, Yield, and Quality of Watermelons (*Citrullus lanatus*) at multi-locations in Texas. **Scientific Reports**, Londres, n. 10, p. 1-16, doi:10.1038/s41598-020-61696-7, 2020.

AMANIFAR, S.; TOGHRANEGAR, Z. The efficiency of arbuscular mycorrhiza for improving tolerance of *Valeriana officinalis* L. and enhancing valerenic acid accumulation under salinity stress. **Industrial Crops and Products**, v.147, p. 1-13, doi:10.1016/j.indcrop.2020.112234, 2020.

BARROS, A. V.; MELO, B. K. C.; COSTA, T. N. B. G.; CAMPOS, D. M. O.; OLIVEIRA, C. B. S.; OLIVEIRA, J. I. N. As riquezas da Caatinga e seu potencial farmacológico: uma revisão sistemática. **SAJEBTT**, Rio Branco, v.8, n. 1, p. 771-791, 2021.

BERRS, L. S. J. R.; SIZER, I. W. A espectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. B. C. v. 195, n. 1, p. 133-140, 1952.

CAPO, L. F. M.; MORAES, M. L. T.; ZULIAN, D. F.; WREGE, M. S.; PORTELA, R. M.; CAMBUIM, J.; SILVA, A. M.; SOARES, M. T. A. S.; SOUSA, V. A.; AGUIAR, A. V. Natural distribution of *Myracrodruon urundeuva* Fr. All. in Brazil at current and future climate scenarios due to global climate change. **Revista Árvore**, v. 46, p. 1-11, 2022.

DRUMOND, M. A.; KILL, L. H. P.; RIBASKI, J.; AIDAR, S. T. Caracterização e usos das espécies da Caatinga subsídio para programas de restauração florestal nas unidades de conservação da Caatinga (UCCAs). **Embrapa Semiárido**, Pernambuco, p. 37, 2016.

Embrapa – Empresa Brasileira de Pesquisa Agropecuária. **Manual de análises químicas de solos, plantas e fertilizantes** / editor técnico, Fábio Cesar da Silva. 2. ed. rev. ampl. - Brasília, DF: Embrapa Informação Tecnológica, p. 627, 2009.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases I. Occurrence in higher plants. **Plant Physiol**. v. 59, n. 2, p. 309-314, 1977.

GONÇALVES, M. P. M.; FELICIANO, A. L. P.; SILVA, A. P.; SILVA, L. B.; SILVA, K. M.; SILVA JÚNIOR, F. S.; GRUGIKI, M. A.; SILVA, M. I. O. Influência de diferentes tipos de solos da Caatinga na germinação de espécies nativas. **Brazilian Journal of Development**, Curitiba, v. 6, n. 1, p.1216-1226, 2020.

KOSOVÁ, K.; VÍTÁMVÁS, P.; URBAN, M. O.; PRÁŠIL, I. T.; RENAUT, J. Plant Abiotic Stress Proteomics: The Major Factors Determining Alterations in Cellular Proteome. **Frontiers in Plant Science**, v. 9, n. 122, p. 1-22. doi:10.3389/fpls.2018.00122, 2018.

KHAN, A.; SHAFI, M.; BAKHT, J.; ANWAR, S.; KHAN, M. O. Effect of salinity (NaCl) and *seed priming* ($CaCl_2$) on biochemical parameters and biological yield of Wheat. **Pakistan Journal of Botany**, v. 53, n. 3, p. 779-789, doi:10.30848/pjb2021-3, 2021.

HEATH, R. L.; PACKER, L. Photoperoxidation in isolated chloroplast. Kinetics and stoichiometry of fatty acid peroxidation. **Arch. Biochem. Biophys.** v. 125, p. 189-198, 1968.

HERNÁNDEZ, J. A. Salinity Tolerance in Plants: Trends and Perspectives. **International Journal of Molecular Sciences,** v. 20, n. 2408, p. 1-8. doi:10.3390/ijms20102408, 2019.

ISAYENKOV, S. V.; MAATHUIS, F. J. M. Plant Salinity Stress: Many Unanswered Questions Remain. **Frontiers in Plant Science,** v.10, n. 80, p. 1-21. doi:10.3389/fpls.2019.00080, 2019.

LORETO, F.; VELIKOVA, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quences ozone products, and reduces lipid peroxidation of cellular membranes. **Plant Physiol.** v. 127, p. 1781 - 1787, 2001.

LLOYD, G.; MCCOWN, B. H. Commercially-feasible micropropagation of Mountain Laurel, Kalmia latifolia, by shoot tip culture. **Proc. Int. Plant Prop. Soc.** v. 30, p. 421-427, 1981.

MELO, G. M.; BARBOSA, M. R.; DIAS, A. L. F.; WILLADINO, L.; CAMARA, T. R. Pré-condicionamento *in vitro* de plantas de cana-de-açúcar (*Saccharum* spp.) para tolerância ao estresse salino. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v.18, p. S27–S33, 2014.

MORAIS, M. B.; BARBOSA-NETO, A. G.; WILLADINO, L.; ULISSES, C.; CALSA JUNIOR, T. Salt Stress Induces Increase in Starch Accumulation in Duckweed (*Lemna aequinoctialis*, Lemnaceae): Biochemical and Physiological Aspects. **Journal of Plant Growth Regulation**, n. 38, p. 683-700, 2019.

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. **Plant Cell Physiol.** v. 22, p. 867-880, 1981.

NOBRE, P. Mudanças climáticas e desertificação: os desafios para o Estado Brasileiro. In: LIMA, R. C. C.; CAVALCANTE, A. M. B.; MARIN, A. M. P. Desertificação e mudanças climáticas no semiárido brasileiro. **Instituto Nacional do Semiárido (INSA)**, Campina Grande, p. 25 – 35, 2011.

NÓBREGA, J. S.; NASCIMENTO, R. G. S.; SILVA, R. T.; FIGUEIREDO, F. R. A.; BEZERRA, A. C.; LOPES, M. F. Q.; ALVES, E. U.; BRUNO, R. L. A. Ácido salicílico atenua o efeito do estresse hídrico na germinação e crescimento inicial de plântulas de *Cereus jamacaru* DC. **Scientia Plena**, Sergipe, v.17, n. 4, p.1-8, 2021.

OLIVEIRA, G. M.; SILVA, F. F. S. D.; ARAUJO, M. D. N.; COSTA, D. C. C. D.; GOMES, S. E. V.; MATIAS, J. R. DANTAS, B. F. Environmental stress, future climate, and germination of *Myracrodruon urundeuva* seeds. **Journal of Seed Science**,v. 41, n. 1, p. 32 - 43. http://dx.doi.org/10.1590/2317-1545v41n1191945, 2019.

OLIVEIRA, C. S.; GOMES, I. S.; PACHECO, J. S.; RIBEIRO, D.; MATOS, F. S. Disponibilidade de cálcio e crescimento de mudas de eucalipto sob estresse salino. **Revista Agrarian**, Mato Grosso do Sul, v. 11, n. 42, p. 299-306, 2018.

OLIVEIRA, L. S.; DIAS, P. C.; BRONDANI, G. E. Micropropagação de espécies florestais brasileiras. **Pesquisa Florestal Brasileira,** Colombo, v. 33, n. 76, p. 439 – 453, 2013.

PETROV, V. D.; BREUSEGEM, F. V. Hydrogen peroxide - a central hub for information flow in plant cells. **AoB Plants, pls**, n.14, p.1-13, 2012.

REHMAN, H. U.; NAWAZ, Q.; BASRA, S.M.A.; AFZAL, I.; YASMEEN, A.; HASSAN, F. U. Seed Priming

Influence on Early Crop Growth, Phenological Development and Yield Performance of Linola (*Linum usitatissimum L.*). **Journal of Integrative Agriculture**, v.13, n. 5, p.990–996, 2014.

RIBEIRO, R. C.; DANTAS, B. F.; MATIAS, J. R.; PELACANI, C. R. Efeito do estresse salino na germinação e crescimento inicial de plântulas de *Erythrina velutina* Willd. (Fabaceae). **Gaia Scientia**, v. 11, n. 4, p. 65-78, 2017.

RODRIGUES, M. H. B. S.; SILVA, J. N.; ALVES, E. U.; ALCANTARA, B. R. L. Hydrogen peroxide as a mitigation of salt stress on the germination of *Myracroduon urundeuva* (Allemão) Engl. Seeds. **Scientia Forestalis**, v. 49, n.130, p.1-11, 2020.

SILVA, M. I. G.; MELO, C. T. V.; VASCONCELOS, L. F.; CARVALHO, A. M. R.; SOUSA, F. C. F. Bioactivity and potential therapeutic benefits of some medicinal plants from the Caatinga (semi-arid) vegetation of Northeast Brazil: a review of the literature. **Revista Brasileira de Farmacognosia**, v. 22, n. 1, p. 193 – 207, 2012.

SOUZA, L. M.; BARBOSA, M. R.; MORAIS, M. B.; PALHARES NETO, L.; ULISSES, C.; CAMARA, T. R. Biochemical and morphophysiological strategies of *Myracrodruon urundeuva* plants under water deficit. **Biologia Plantarum**, v. 64, p. 20-31, 2020.

SILVEIRA, N. M.; MARCOS, F. C. C.; FRUNGILLO, L.; MOURA, B. B.; SEABRA, A. B.; SALGADO, I.; MACHADO, E. C.; HANCOCK, J. T.; RIBEIRO, R. V. S-nitrosoglutathione spraying improves stomatal conductance, rubisco activity, and antioxidant defense in both leaves and roots of sugarcane plants under water déficit. **Physiol Plant**, v.160, n.4, p.383-395, 2017.

STASSINOS, P. M.; ROSSI, M.; BORROMEO, I.; CAPO, C.; BENINATI, S.; FORNI, C. Enhancement of *Brassica napus* Tolerance to High Saline Conditions by *Seed Priming*. **Plants**, v.10, n.403, p. 01-16, 2021.

PAREYN, F. G. C.; ARAÚJO, E. L.; DRUMMOND, M. A.; MIRANDA, M. J. A. C.; SOUZA, C. A.; SILVA, A. P. S.; BRAZOLIN, S.; MARQUES, K. K. M. Plantas para o futuro - Região Nordeste: *Myracrodruon urundeuva* aroeira. In: **Espécies Nativas da Flora Brasileira de Valor Econômico Atual ou Potencial Plantas para o Futuro:** Região Nordeste, p.766 - 772. 2018.

THOMAS, R.L.; SHEARRD, R.W.; MOYER, J.R. Comparison of conventional and automated procedures for N, P and K analysis of plant material using a single digestion. **Agronomy Journal**, Madison, v.59, p.240-243, 1967.

Authorship Contribution

1 Lindomar Maria de Souza

Biologist, Dr., Researcher

https://orcid.org/0000-0003-2916-4271 • lindomarsouza.ufrpe@gmail.com

Contribution: Conceptualization, Investigation, Methodology, Supervision

2 Emanuella Maria da Conceição

Biologist, Dr., Professor, Researcher
https://orcid.org/0000-0001-9491-4177 • profmanubios@gmail.com
Contribution: Data curation, Investigation, Methodology

3 Marta Ribeiro Barbosa

Forestry Engineer, Dr., Researcher
https://orcid.org/0000-0003-0469-8859 • martaribeirobarbosa@gmail.com
Contribution: Formal Analysis, Writing – original draft, Writing – review & editing

4 Luiz Palhares Neto

Biologist, Dr., Researcher
https://orcid.org/0000-0002-5801-8313 • netopalhares1@gmail.com
Contribution: Formal Analysis, Writing – original draft

5 Ana Maria Maciel dos Santos

Agronomist, Dr., Researcher
https://orcid.org/0000-0001-6756-2743 • agrom1960@yahoo.com.br
Contribution: Writing – original draft

6 Robson Antonio de Souza

Biologist, Researcher
https://orcid.org/0000-0001-6886-468X • robson.cetene@gmail.com
Contribution: Methodology, Formal Analysis

7 Laureen Michelle Houllou

Biologist, Dr., Researcher
https://orcid.org/0000-0001-5290-5969 • laureen.houllou@cetene.gov.br
Contribution: Project administration, Writing – original draft, Writing – review & editing

How to quote this article

Souza, L. M.; Conceição, E. M.; Barbosa, M. R.; Palhares Neto, L.; Santos, A. M. M.; Souza, R. A.; Houllou, L. M. Effect of seed priming with NaCl on the induction of salinity tolerance in Myracrodruon urundeuva Allemão in vitro. Ciência Florestal, Santa Maria, v. 32, n. 4, p. 2199-2218, 2022. DOI 10.5902/1980509867600. Available from: https://doi.org/10.5902/1980509867600.