

## Physiological performance of *Tabebuia aurea* seeds subjected to abiotic stresses

### Desempenho fisiológico de sementes de *Tabebuia aurea* submetidas a estresses abióticos

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**ABSTRACT** - Seeds subjected to abiotic stresses may have their physiological performance compromised, impairing the multiplication of species. Thus, the objective was to evaluate the germination and initial development of *Tabebuia aurea* seedlings subjected to water and salt stresses. Water stress was simulated with two osmotic agents (PEG 6000 and mannitol) at five osmotic potentials (0.0, -0.2, -0.4, -0.6 and -0.8 MPa). For salt stress, three osmotic agents (NaCl, KCl and CaCl<sub>2</sub>) were used at five concentrations (0, 6, 12, 18 and 24 dS m<sup>-1</sup>). The experiments were conducted under a completely randomized experimental design, with four replicates of 25 seeds, in a factorial scheme (osmotic agents × potentials), and the data were subjected to analysis of variance and regression. The variables analyzed were germination, first germination count, germination speed index, root and shoot length, and seedling root and shoot dry mass. The results showed that water and salt stresses reduced the germination and vigor of *T. aurea* seeds, with water stress being more harmful to the species, especially when induced by PEG 6000. Of the salts used, the one that most harmed the species was CaCl<sub>2</sub>, followed by NaCl and KCl.

**RESUMO** - Sementes submetidas a estresses abióticos podem ter seu desempenho fisiológico comprometido, prejudicando a multiplicação das espécies. Dessa forma, objetivou-se avaliar a germinação e o desenvolvimento inicial de plântulas de *Tabebuia aurea* submetidas aos estresses hídrico e salino. Para tanto, foi simulado o estresse hídrico com dois agentes osmóticos (PEG 6000 e manitol) em cinco potenciais osmóticos (0,0; -0,2; -0,4; -0,6 e -0,8 MPa). No estresse salino, foram utilizados três agentes osmóticos (NaCl, KCl e CaCl<sub>2</sub>) em cinco concentrações (0; 6; 12; 18 e 24 dSm<sup>-1</sup>). Os testes foram executados sob delineamento experimental inteiramente casualizado, com quatro repetições de 25 sementes, em esquema fatorial (agentes osmóticos × potenciais), sendo os dados submetidos a análise de variância e regressão. As variáveis analisadas foram germinação, primeira contagem de germinação, índice de velocidade de germinação, comprimento radicular e da parte aérea, massa seca de raiz e da parte aérea das plântulas. Os resultados demonstraram que os estresses hídrico e salino reduziram a germinação e vigor de sementes de *T. aurea*, sendo o hídrico mais danoso à espécie, principalmente quando induzido por PEG 6000. Dos sais empregados, o que mais prejudicou a espécie foi o CaCl<sub>2</sub>, seguido pelo NaCl e KCl.

**Keywords:** Bignoniaceae. Salinity. Water stress. Semi-arid region.

**Palavras-chave:** Bignoniaceae. Salinidade. Estresse hídrico. Semiárido.

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## INTRODUCTION

The Caatinga ecosystem is characterized by a semi-arid, hot climate with low rainfall (AZERÊDO; PAULA; VALERI, 2016). Its vegetation is composed of multi-purpose species with easy adaptation to different conditions of soil and natural environments of the semi-arid region.

Among the important species for the Caatinga ecosystem, *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S.Moore, popularly known in Portuguese as craibeira and belonging to the Bignoniaceae family, stands out. It has timber, landscape and medicinal potential and is indicated for reforestation (LORENZI, 2014). As its main means of reproduction is by seeds, the germination and initial development of the seedling can be decisive for its multiplication (BRITO et al., 2020).

Under natural conditions, unfavorable abiotic factors, such as fluctuations in the presence or amount of light, water, oxygen, carbon dioxide, salts and temperature, commonly occur, which can generate stress for the seeds, impairing their physiological performance during germination (TAIZ et al., 2017). Thus, in arid and semi-arid regions, such as in the Brazilian Northeast, the occurrence of water and salt stresses is quite common, since in this area there is low rainfall and high evapotranspiration, favoring these stresses.

For this reason, plant production becomes dependent on adequate management, with irrigation and salinity correction practices to minimize the



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occurrence of water and salt stresses (SOUSA et al., 2018; LEAL et al., 2019). Tests with simulation of water and salt stress in the laboratory have been used to identify species that are capable of developing in stressful environments.

The initial stage of plant development is one of the most affected by water and salt stresses, because the germination process begins with the absorption of water by the seeds, allowing the rehydration of tissues and intensification of respiratory processes and other metabolic pathways, which result in the development of the embryonic axis (BEWLEY et al., 2013). However, at highly negative osmotic potentials, the absorption of water by the seeds is impaired, thus hindering the occurrence of germination (VALDOVINOS et al., 2021).

The absorption of ions by cells reduces their osmotic potential, leading to water restriction. In addition, the presence of salts inside cells can reduce seed vigor and cause cytotoxicity (LEAL et al., 2019). Thus, the energy expenditure is directed to performing osmotic adjustment, as a survival strategy, and thus plant development is reduced (LIANG et al., 2018; FERREIRA et al., 2021). Considering that water and salt stresses are limiting factors to the germination and development of plant species, it is essential to know how these factors limit the tolerance and adaptive capacity of species under these conditions.

In this context, studies on the physiological performance of *T. aurea* seeds subjected to abiotic stresses are fundamental, considering that under these conditions they can hinder or compromise the reproduction of the species. Therefore, the objective was to evaluate the germination and initial development of *T. aurea* seedlings subjected to water and salt stresses.

## MATERIAL AND METHODS

*T. aurea* seeds were obtained from ripe fruits harvested from 20 trees existing at the central campus of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil (5°12'11" S latitude; 37°19'34" W longitude and approximate altitude of 19 m), between November and December 2020. According to Köppen's classification, the local climate is BSw<sup>h</sup>, dry and very hot, with two climatic seasons: a dry one, which usually comprises the period from June to January, and a rainy one, between February and May (CARMO FILHO; ESPÍNOLA SOBRINHO; MAIA NETO, 1991).

After harvest, the fruits were subjected to natural drying in the shade for six days at room temperature (27.5 °C; 68.9% RH). Then, the seeds were extracted and cleaned manually and left for three days in the same environment. After drying, the moisture content was determined (DIAS et al., 2010), and the seeds were placed in plastic bottles and stored in a controlled environment (17 °C ± 2 °C; 55% RH) until the experiment was carried out.

Two experiments were carried out in a completely randomized design, with four replicates of 25 seeds per treatment: the first with water stress in a 2 × 5 factorial

scheme (polyethylene glycol – PEG 6000 and mannitol × 0.0 – distilled water; –0.2; –0.4; –0.6 and –0.8 MPa); and the second with salt stress in a 3 × 5 factorial scheme (sodium chloride - NaCl, potassium chloride - KCl and calcium chloride - CaCl<sub>2</sub> × 0.0 - distilled water; 6; 12; 18 and 24 dS m<sup>-1</sup>).

Mannitol solutions were prepared according to Van't Hoff's formula:  $\psi_s = -RTC$  (MPa), where:  $\psi_s$ : osmotic potential (atm); R: universal constant of ideal gases (0.082 atm. L mol<sup>-1</sup> °K<sup>-1</sup>); T: temperature (°C) and C: concentration (mol L<sup>-1</sup>) (SALYSBURY; ROSS, 1992). PEG 6000 solutions were obtained according to the recommendation of Villela, Doni Filho and Siqueira (1991). Van't Hoff's formula was also used in the saline solutions, which were adjusted with the aid of a conductivity meter (dS m<sup>-1</sup>).

The seeds were subjected to asepsis before setting up the germination test. For this, a solution was prepared with 100 mL of distilled water and five drops of neutral detergent, where the seeds were immersed for 10 minutes. They were then washed three times in running water and rinsed twice in distilled water for complete removal of the solution (BRASIL, 2013).

The germination test was conducted in paper towel roll substrate and moistened with the solution of each treatment, in an amount equivalent to 2.5 times the weight of the dry paper. The rolls were placed in germination chambers at 25 °C (BRASIL, 2013) with photoperiod of 12 h, and kept for 14 days. Evaluations consisted of the following analyses:

Germination - evaluations carried out at 10 and 14 days after sowing, but with adaptations regarding the test period. The final evaluation was advanced from 21 (BRASIL, 2013) to 14 days due to the development of the seedlings in paper roll.

Germination speed index - obtained by daily counts of the number of germinated seeds from the 1st to the 14th day after sowing, according to the methodology recommended by Maguire (1962).

Shoot length and root length - at the end of the germination test, the hypocotyl (measured from the collar to the apex of the seedling) and the primary root (measured from the collar to the tip of the main root) of normal seedlings were measured with a ruler graduated in millimeters, with results expressed in cm.seedling<sup>-1</sup>.

Shoot and root dry mass - at the end of the germination test, normal seedlings were cut at the collar to separate roots and shoots. These parts were placed in Kraft-type paper bags and dried in a forced air circulation oven at 65 °C until reaching constant mass. Then, they were weighed on an analytical scale (0.001 g), and the results were expressed as g.seedling<sup>-1</sup>.

The results of the experiments were analyzed separately, and the data were subjected to analysis of variance through the F test, at 5% probability level. In case of significance, they were subjected to regression analysis (quantitative variables) and Tukey test at 5% probability level (qualitative variables). Statistical analyses were performed in Sisvar software (FERREIRA, 2019).

## RESULTS AND DISCUSSION

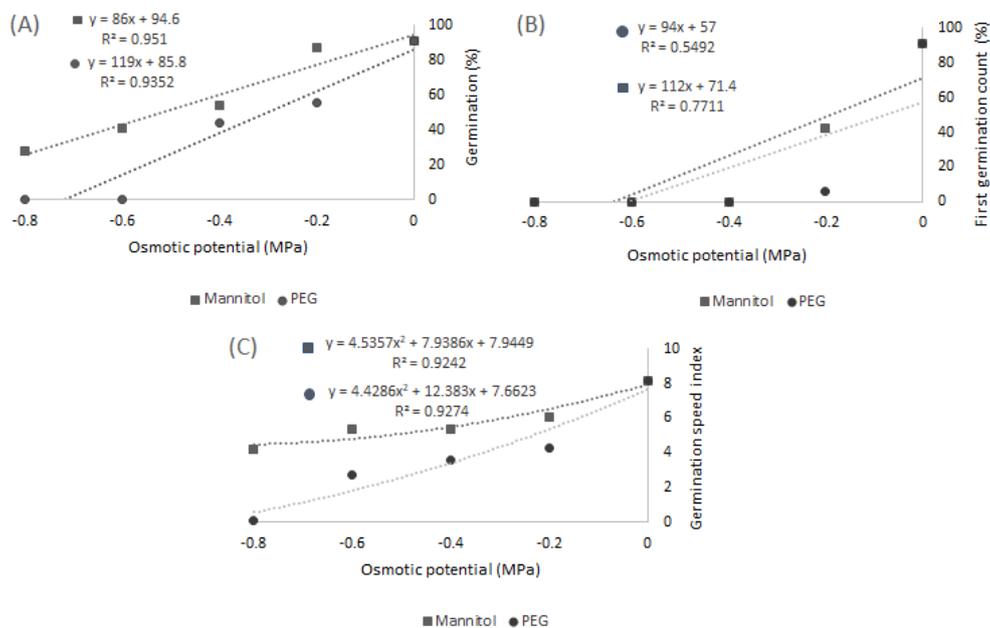
### Water stress

From the potential of  $-0.2$  MPa, there were significant reductions in germination, first count and germination speed index of *T. aurea* seeds (Figure 1). Germination decreased linearly according to the decrease of the osmotic potential of the solutions. This reduction was more considerable when PEG 6000 was used from the level of  $-0.6$  MPa, with no germination occurring at the most negative potential ( $-0.8$  MPa). Thus, the maximum limit of tolerance to water stress simulated with PEG 6000 was observed at the level of  $-0.72$  MPa. However, for mannitol it was not possible to find the maximum limit of tolerance, since the lowest potential tested ( $-0.8$  MPa) was not sufficient to stop seed

germination, which was equal to 28% (Figure 1A).

The first germination count of *T. aurea* seeds showed a marked reduction from the level of  $-0.2$  MPa for both osmotic agents. For this variable, there was no germination from the levels of  $-0.4$  MPa (PEG 6000) and  $-0.6$  MPa (mannitol) (Figure 1B). This was probably due to the physiological drought caused by the reduction in the osmotic potential. This causes the seeds to remain longer in phase II of the imbibition process, leading to delay and reduction in germination (BEWLEY et al., 2013).

The results obtained for the germination speed index (GSI) were similar to those of first germination count, with decreases from  $-0.2$  MPa, and the reduction at this potential was 48% with PEG 6000 and 25% with mannitol, compared to the control (0 MPa) (Figure 1C).



**Figure 1.** Germination (A), first germination count (B) and germination speed index (C) of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore seeds subjected to water stress.

The reduction in germination speed may be related to the delay in phase III, whose event requires greater availability of water for the primary root to protrude (TAIZ et al., 2017). Thus, as there was restriction of water absorption, caused by the reduction in the water potential of the solution, physiological processes were delayed or prevented, thus reducing the germination speed of the seeds, as also verified by Moura et al. (2011) in seeds of *Mimosa caesalpinifolia*. These results point to the harmful effects caused by the low availability of water to the seeds during germination. However, it is possible to observe a higher tolerance of *T. aurea* to water stress simulated with mannitol, for which the results of germination, first germination count and GSI were better than those obtained with PEG 6000.

The higher sensitivity of seeds to PEG 6000, when

compared to mannitol, can be attributed to the high viscosity and high molecular weight of PEG solutions. Thus, water absorption by the seeds becomes difficult and  $O_2$  absorption is compromised, leading to greater water restriction (ANTUNES et al., 2011). As a consequence, it can reduce germination and initial development of seedlings (VALDOVINOS et al., 2021). On the other hand, mannitol solutions have low viscosity and, therefore, are more absorbed by the seeds, thus reducing the effect of water restriction, consequently resulting in less drastic effects during the germination process (FERREIRA et al., 2017).

The high sensitivity of seeds caused by the use of PEG 6000, when compared to mannitol, has also been verified in other forest species. The germination and GSI of *Samanea tubulosa* seeds were extremely affected by PEG 6000 from

−0.3 MPa, while in mannitol, greater effects on germination were observed from −0.9 MPa (SANTOS JUNIOR; SILVA, 2020). Similarly, *Handroanthus impetiginosus* under PEG 6000 and mannitol at temperatures of 25 and 30 °C showed greater sensitivity to the former from the level of −0.6 MPa, for both temperatures (SANTOS et al., 2018).

Due to the delay in seedling development caused by water stress, a large number of seedlings with development of only the primary root was observed in the first count; for this reason, they were not considered normal seedlings. At the end of germination, some of these seedlings had the other essential structures developed, hence being considered normal seedlings according to the criteria established in the Rules for Seed Analysis (BRASIL, 2009). This explains the difference found between germination and first germination count, pointing out that the conditions of water stress hindered the germination process and delayed the development of seedlings. Under more negative potentials, germination was impeded because they compromised the sequence of metabolic events, for exceeding the minimum water potential required for this process (BEWLEY et al., 2013; COLMAN et al., 2014).

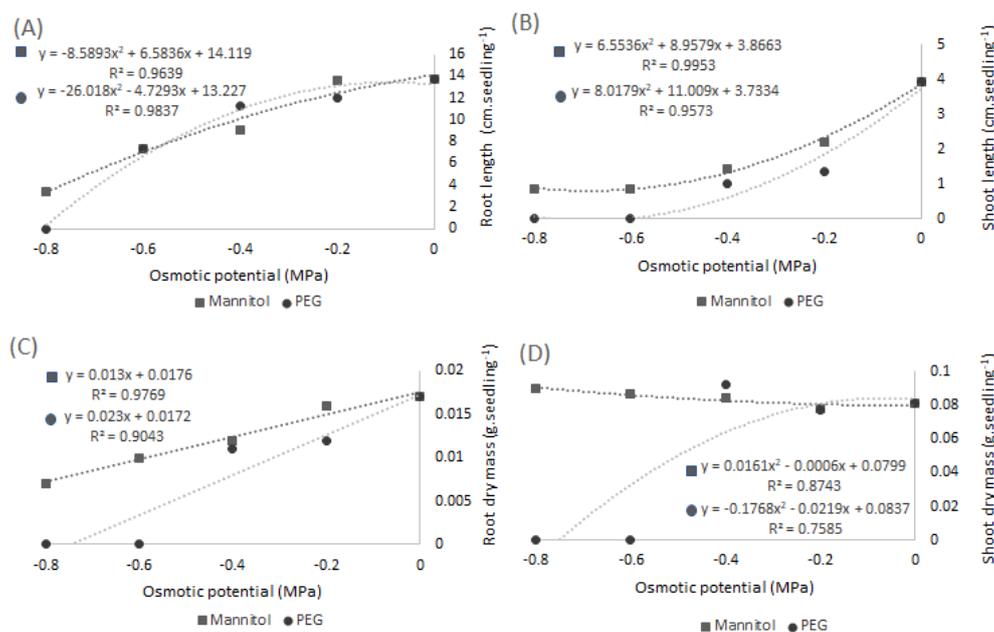
Studies involving abiotic stresses in forest seeds have been reported, showing that the tolerance limit varies between species. Some are more sensitive, such as *Pityrocarpa moniliformis*, whose maximum limit of seed germination in PEG 6000 solutions occurred at −0.1 MPa (MATOS et al., 2021). In *Combretum leprosum*, a decrease in germination from −0.2 MPa was observed in PEG 6000 solutions, under different temperatures (LEAL et al., 2020). In *H. impetiginosus*, the range of tolerance in PEG 6000 solutions was also between −0.6 and −0.8 MPa, while in *T. rosealba* and *H. chrysotrichus*, it ranged from −0.8 to −1.0 MPa (VALDOVINOS et al., 2021).

Root length and shoot length were reduced with the decrease in the osmotic potential of the substrate (Figure 2). The reduction was gradual for seedling root length with the use of mannitol as the osmotic potentials decreased, while with PEG 6000, the reduction was more pronounced from −0.4 MPa. As there was no germination in PEG 6000 at −0.8 MPa, there was also no root and shoot development in this treatment.

The reductions were more pronounced in shoot length than in root length, from −0.2 MPa in both osmotic agents. This is due to the restriction in water absorption necessary for seedling development. Thus, as a way to ensure survival, plants invest more in root growth, in order to improve water absorption efficiency (SILVA et al., 2019). This mechanism works as an adaptation of the species to water deficit and other conditions of abiotic stresses.

Water stress simulated with PEG 6000 resulted in shorter shoot length when compared to that simulated with mannitol, with no development of seedling shoots from −0.6 MPa (Figure 2B). This result highlights the effects caused by water stress, due to the reduction in cell expansion and inadequate turgor (TAIZ et al., 2017). Similar findings were observed by Matos et al. (2021) in *P. moniliformis* seedlings subjected to water stress with PEG 6000, which caused a decrease in root length and shoot length of seedlings as the osmotic potentials became more negative, from −0.2 MPa.

In *H. impetiginosus* seedlings, Santos et al. (2018) also found reduction in root length and shoot length as the water potential of PEG 6000 and mannitol solutions reduced from −0.2 MPa, at temperatures of 25 and 30 °C. In *C. leprosum*, Leal et al. (2020) found reductions in seedling root length and shoot length as the osmotic potential with PEG 6000 decreased.



**Figure 2.** Root length (A), shoot length (B), root dry mass (C) and shoot dry mass (D) of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore seedlings subjected to water stress.

Root dry mass decreased with the reduction of the water potential used. This variable showed linear behavior for both osmotic agents. As observed for the other variables, PEG 6000 caused a greater decrease in root dry mass than mannitol (Figure 2C).

Shoot dry mass of the seedlings showed a peculiar behavior (Figure 2D). While the use of PEG 6000 caused a marked reduction due to the increase in water restriction from  $-0.2$  MPa, with mannitol this variable remained constant (Figure 2D). The decrease in shoot dry mass with the use of PEG 6000, as a function of the reduction in the water potential of the solution, may be associated with the reduction in the speed of physiological and biochemical processes. In addition, under this condition of water deficit, hydrolysis and mobilization of reserves in the seeds during the germination process are usually difficult (SOUSA et al., 2018; LEAL et al., 2020). Similarly, the water stress simulated by PEG 6000 caused a reduction in the dry mass of roots and shoots of *P. moniliformis* seedlings (MATOS et al., 2021). In *C. leprosum* seedlings, the water potential of  $-0.2$  MPa with PEG 6000 caused a reduction in shoot dry mass, at temperatures of 25, 30, 35 and 20-30 °C, and an increase in root dry mass, but more negative potentials caused its reduction (LEAL et al., 2020).

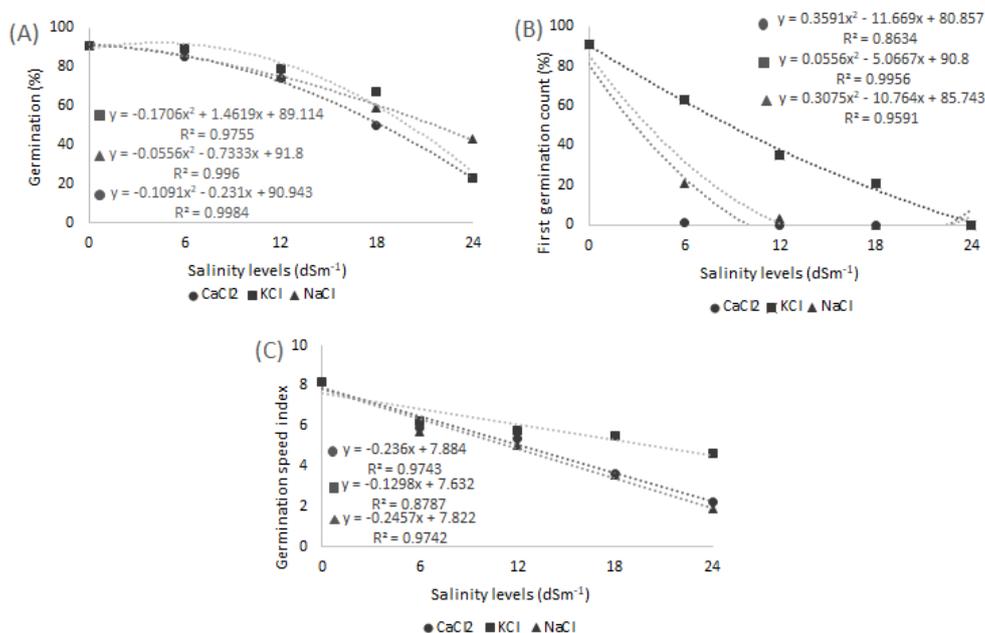
In general, the seeds showed better physiological performance when germinated in a substrate moistened with water, without water stress. This can be explained by the restriction in water absorption caused by the reduction of

water potential, which directly influences the physiological processes that promote germination (BEWLEY et al., 2013). In addition, different germination responses of *T. aurea* seeds were obtained with water stress induced by mannitol and PEG 6000, which can be attributed to the permeability of the seed coat against low-molecular-weight solutes. Thus, mannitol may have been absorbed by the seeds, thus reducing the effect of water restriction by increasing the water potential of the solutions (FERREIRA et al., 2017). Consequently, *T. aurea* seeds showed higher tolerance to water stress with mannitol than with PEG 6000.

### Salt stress

The germination of *T. aurea* seeds gradually decreased with the increase in salinity with the saline agents NaCl, KCl and CaCl<sub>2</sub>. However, germination above 50% was observed at the level of 18 dS m<sup>-1</sup> for the three agents, indicating low sensitivity of the species to salinity (Figure 3A).

Germination in saline medium (KCl) resulted in a quadratic curve, with a slight increase at the level of 6 dS m<sup>-1</sup> and a subsequent decrease, reaching a 75% reduction at 24 dS m<sup>-1</sup>, when compared to the control. Under CaCl<sub>2</sub> and NaCl salts, the reduction occurred gradually. However, NaCl affected germination less at levels of higher concentration, with a 53% reduction in germination at 24 dS m<sup>-1</sup>, versus 75% when using CaCl<sub>2</sub>, compared to the control.



**Figure 3.** Germination (A), first germination count (B) and germination speed index (C) of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore seeds subjected to salt stress.

Most forest species have their development affected by salinity, especially in the germination, emergence and initial growth stages, whose steps are usually the most affected by the presence of saline agents, as observed here in *T. aurea* seedlings. The increase in the salt concentration of the solutions caused a reduction in the water absorption capacity of the seeds, due to the decrease in their water potential. Thus, there were reductions in germination capacity and germination speed, although they showed high vigor. In addition, under high concentrations of salts, there may also have been a toxic effect or the rupture of seed coat layers, causing damage to the embryo (CRUZ et al., 2020).

Studies on the effects of different salts on seed germination have been conducted to evaluate the tolerance of species under these conditions. For *Ochroma pyramidale*, it was found that the maximum limit of tolerance to salt stress simulated with NaCl, CaCl<sub>2</sub> and KCl was -0.6 MPa, and its development was more affected by CaCl<sub>2</sub> (CRUZ et al., 2020). In *S. tubulosa* seeds subjected to salt stress simulated with NaCl and KCl, there was a decrease in germination from -0.6 MPa, and the effect was more drastic in substrate with NaCl, causing a 68% reduction at the potential of -1.2 MPa, while under KCl the reduction was 49% at the same level, compared to the control (SANTOS JUNIOR; SILVA, 2020). Low tolerance to salt stress simulated by NaCl and CaCl<sub>2</sub> in the germination stage was observed in *P. moniliformis*, whose reductions were 45% and 30% at concentrations of -0.1 MPa and -0.4 MPa, respectively, and this species showed moderate tolerance to KCl, with a reduction of 40% at -0.8 MPa (MATOS et al., 2021).

The values of first germination count of *T. aurea* seeds under salinity conditions decreased significantly from 6 dS m<sup>-1</sup>, indicating that the increase in the concentration of salts causes a delay in the germination of seeds of this species. The effects were more severe with CaCl<sub>2</sub> and NaCl, for which the germination values reached zero already at 12 dS m<sup>-1</sup>. On the other hand, the seeds were less sensitive to KCl when compared to the other osmotic agents. Although this agent also caused a gradual reduction in the results of first count, zero germination of the seeds occurred only at the level of 24 dS m<sup>-1</sup> (Figure 3B).

The differences observed between the percentages of germination and first counts of *T. aurea* seeds subjected to salt stress are due to the poor development of the seedlings in the first count, showing only the emergence of primary root, so they were not considered normal seedlings. At the end of the germination test, the formation of the hypocotyl-radicle axis was observed, indicating that the seedlings were able to have full development, despite the delay caused by salt stress.

The increase in the salt concentration of the solutions linearly reduced the germination speed index of *T. aurea* seeds, resulting in lower values for seeds subjected to CaCl<sub>2</sub> and NaCl salts (Figure 4C). When comparing the results obtained at the level of 24 dS m<sup>-1</sup> with those in the control, the reductions in GSI were 77% with NaCl, 73% with CaCl<sub>2</sub> and 43% with KCl. In a similar study with *O. pyramidale* seeds,

GSI was reduced by 80%, 68% and 36% under the water stress of -0.4 MPa with CaCl<sub>2</sub>, NaCl and KCl, respectively (CRUZ et al., 2020). This finding indicates the need to evaluate the effect that each salt can cause, since tolerance to the cationic nature of water can have different responses for distinct species (LEAL et al., 2019).

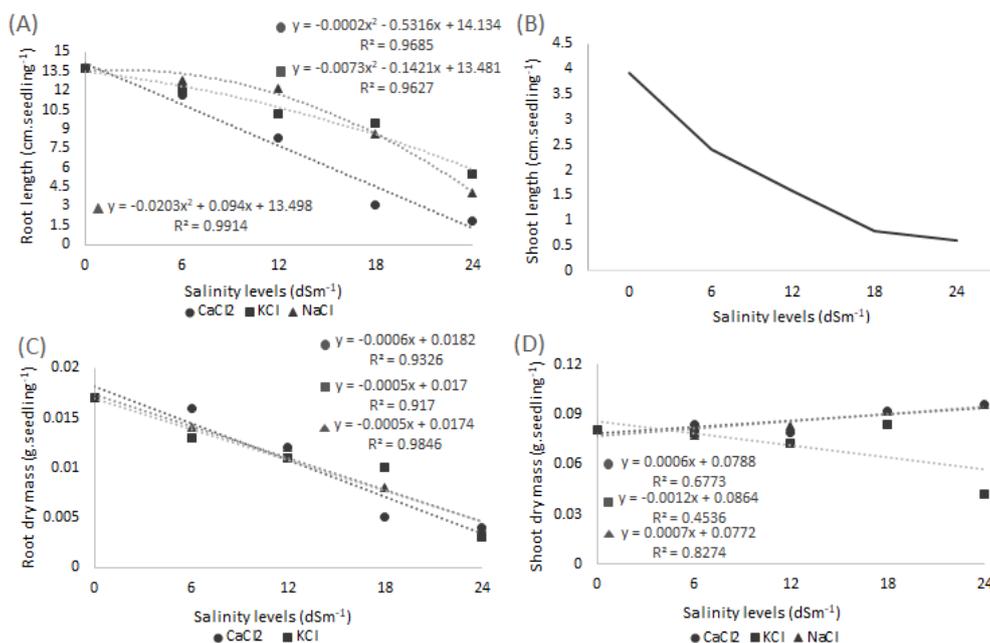
Excess of salts causes dehydration of cells, cytotoxicity, and reductions in metabolic activity and synthesis of tissues in the seeds, due to the decrease in the water potential of the water and consequent difficulty for seedling roots to absorb water (TAIZ et al., 2017). This results in a reduction in germination speed or even in the inability to germinate. Thus, even if germination occurs, it can harm its establishment, causing the seeds that produced the root to not be able to develop, thus inducing an increase in the formation of abnormal seedlings and dead seeds (BEWLEY et al., 2013; NOGUEIRA et al., 2020).

The three saline agents (NaCl, CaCl<sub>2</sub> and KCl) reduced the length of the roots of *T. aurea* seedlings as salinity increased. The most severe reduction was observed with CaCl<sub>2</sub>, which resulted in root length of 1.88 cm.seedling<sup>-1</sup> at 24 dS m<sup>-1</sup>, which represents a reduction of 86% when compared to the control. For KCl and NaCl, the reductions from 0 to 24 dS m<sup>-1</sup> were 60 and 70%, respectively (Figure 4A).

The reduction of root system works as a mechanism of tolerance to salinity, as it limits the entry of water and, consequently, the entry of salts, thus avoiding the occurrence of toxicity caused by excess of specific ions (OLIVEIRA et al., 2016; SÁ et al., 2016). In a similar study, Leal et al. (2019) observed reductions of 3% in the root length of *C. leprosum* seedlings under water stress with CaCl<sub>2</sub> at 6.5 dS m<sup>-1</sup>, while in KCl and NaCl there was no root formation at the levels of 6.5 dS m<sup>-1</sup> and 5.5 dS m<sup>-1</sup>, respectively. For *S. tubulosa* seeds, Santos Junior and Silva (2020) found that there was a reduction in the root length of seedlings under water stress from -0.6 MPa in NaCl and KCl.

The shoot length of *T. aurea* seedlings did not differ statistically between the osmotic agents KCl and NaCl, being higher than the values found with CaCl<sub>2</sub> (Table 1).

Considering the variations between the levels tested, there was a reduction in the length of shoots of *T. aurea* seedlings, as the salt concentration of the solutions increased, with a reduction of 38% at the level of 6 dS m<sup>-1</sup>, compared to the control (Figure 4B). This reduction in shoot length may be due to the decrease in cell expansion caused by physiological drought, in addition to the toxic effect of the presence of ions in the protoplasm (TAIZ et al., 2017). Similarly, Nogueira et al. (2020) observed a reduction of approximately 50% in the shoot length of *Piptadenia stipulacea* seedlings irrigated with saline water at 6.5 dS m<sup>-1</sup> NaCl, compared to those obtained at 0.5 dS m<sup>-1</sup>. Leal et al. (2019) also found reduction in the shoot length of *C. leprosum* seedlings with the increase in the concentration of saline solutions, a reduction of 70% at 6.5 dS m<sup>-1</sup> with CaCl<sub>2</sub>, compared to the control treatment (0 dS m<sup>-1</sup>).



**Figure 4.** Root length (A), shoot length (B), root dry mass (C) and shoot dry mass (D) of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore seedlings subjected to salt stress.

**Table 1.** Individual effect of osmotic agents on shoot length (SL) of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore seedlings subjected to salt stress.

Saline agent	SL (cm. seedling <sup>-1</sup> )
KCl	2.035 a
NaCl	1.885 a
CaCl <sub>2</sub>	1.690 b

Means followed by the same letter, in the column, do not differ from each other by Tukey test at 5% probability level.

As the salinity levels increased, there was a linear reduction in the root dry mass of *T. aurea* seedlings, with similar responses for the three agents tested (Figure 4C). Although there was a reduction in the shoot dry mass of *T. aurea* seedlings subjected to salt stress with KCl, the opposite effect occurred with CaCl<sub>2</sub> and NaCl, under which the dry mass was directly proportional to the increase in salinity levels (Figure 4D). These results differ from those found by Nogueira et al. (2020), who observed a progressive reduction in the root and shoot dry mass of *P. stipulacea* seedlings, as salinity (NaCl) levels increased. Likewise, Cruz et al. (2020) observed a decrease in the dry mass of *O. pyramidale* seedlings subjected to salinity with NaCl, CaCl<sub>2</sub> and KCl, from the potential of -0.1 MPa, and the most pronounced reduction was found with the use of CaCl<sub>2</sub>, which led to reduction of 71% at -0.4 MPa compared to the control. Leal et al. (2019) also found reduction in the root dry mass accumulation of *C. leprosum* subjected to salt stress with NaCl, KCl and CaCl<sub>2</sub>; the effects occurred gradually and were more severe under KCl and NaCl, with null values at 5.5 dS m<sup>-1</sup>. In the same study, exponential effect was caused

by the three agents on shoot dry mass, with reductions of 46%, 99.9% and 100% under CaCl<sub>2</sub>, KCl and NaCl at 6.5 dS m<sup>-1</sup>, respectively.

The damage caused by the presence of salts to *T. aurea* seeds and seedlings can be attributed to the destabilization of the osmotic potential and membrane balance of the plants caused by the concentration of ions in the protoplasm, inducing their metabolism to repair cellular structures, consequently reducing their development. This works as a mechanism of adaptation to stress, ensuring conditions for the maintenance of vital activities, even if in a limited way (FERREIRA et al., 2017; LIMA; PAREYN; DRUMMOND, 2018).

The salt that most impaired the germination and initial development of *T. aurea* was CaCl<sub>2</sub>, followed by NaCl and KCl. This can be attributed to disturbances in the protoplasm caused by excess Cl<sup>-</sup> and Na<sup>+</sup> ions, which affect enzyme activity and energy production, thus limiting nitrogen assimilation (CRUZ et al., 2020).

The negative effects caused by the CaCl<sub>2</sub> solutions

may also be associated with the fact that  $\text{Ca}^{2+}$  is a secondary messenger of signal translation, and fluctuations in the concentration of cytosolic  $\text{Ca}^{2+}$  can alter the activity of several antioxidant enzymes and some plant hormones. The effects caused by NaCl solutions may be associated with the presence of sodium ions inside the cells, which can destabilize the osmotic potential and the balance of plant membranes, consequently reducing plant development (CRUZ et al., 2020; FERREIRA et al., 2017).

Although the increase in salt concentration caused negative effects on the germination and vigor of *T. aurea* seeds, even using high levels ( $24 \text{ dS m}^{-1}$ ), it was not possible to find the maximum tolerance limit of the species using the saline agents  $\text{CaCl}_2$ , NaCl and KCl. This indicates that the species has tolerance to salt stress, hence conferring adaptive character, demonstrating greater capacity of seedlings for survival and establishment in a saline environment. Therefore, these stressors are not considered limiting factors to the propagation of the species.

## CONCLUSIONS

Water and salt stresses reduce the germination and vigor of *T. aurea* seeds, and water stress is the most harmful to the species. *T. aurea* seeds are more sensitive to PEG 6000-induced than to mannitol-induced water stress, and their maximum tolerance limit in PEG 6000 is  $-0.72 \text{ MPa}$ . Of the salts used, the one that most harms the species is  $\text{CaCl}_2$ , from the level of  $6 \text{ dS m}^{-1}$ , followed by NaCl and KCl.

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