GERMINATION AND INITIAL GROWTH OF Sesbania PUNICEA (Cav.)Benth.: INFLUENCE OF SALINITY, FLOODING AND LIGHT¹

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ABSTRACT – Sesbania punicea (Cav.) Benth. has been recommended for the recovery of degraded areas. This study aimed at verifying the effect of salinity in the germination of *S. punicea* seeds in different hydric and luminosity conditions and assessing the influence of salinity and flooding on the growth and concentration of photosynthetic pigments. After the morphometric measurements of the fruits and seeds, we performed germination tests in different salt concentrations (0,5, 10 and 15gL⁻¹NaCl), keeping the seeds in a humid substrate (normoxy) or submerged (flooding), in the presence or absence of luminosity. There was a decrease in the percentage of germination and germination speed index (GSI) of seeds that were kept submerged in the dark. The average germination time (AGT) decreased up to the concentration of 10gL⁻¹NaCl when the seeds were submitted to submersion in the presence of light. The growth and the production of leaves of seedlings exposed to flooding decreased as the salt concentration increased. The concentrations of photosynthetic pigments of seedlings of *S. punicea* may be limited by the presence of salts concentrations in the soil.

Keywords: Seeds; Seedlings; Degraded areas.

GERMINAÇÃO E CRESCIMENTO INICIAL DE Sesbania punicea Benth: INFLUÊNCIA DA SALINIDADE, ALAGAMENTO E LUZ.

RESUMO – **Sesbania punicea** tem sido indicada para a recuperação de áreas degradadas. Este estudo teve como objetivo verificar o efeito da salinidade na germinação de sementes de **S. punicea** em diferentes condições hídricas e de luminosidade, bem como avaliar a influência da salinidade e alagamento no crescimento e concentração de pigmentos fotossintéticos. Após a realização das medidas morfométricas dos frutos e sementes, realizamos testes de germinação em diferentes concentrações de sal (0, 5, 10 e 15gL⁻¹NaCl), mantendo as sementes em um substrato úmido (normoxia) ou submersas (alagamento), na presença ou ausência de luminosidade. Houve uma diminuição na porcentagem de germinação e índice de velocidade de germinação (IVG) de sementes que foram mantidas submersas no escuro. O tempo médio de germinação (TMG) diminuiu até a concentração de 10gL⁻¹NaCl quando as sementes foram submetidas à submersão na presença de luz. O crescimento e a produção de folhas de plântulas expostas ao alagamento diminuíram à medida que a concentração de sal aumentou. As concentrações de pigmentos fotossintéticos de ocupação de novas áreas degradadas por **S. punicea** pode ser limitada pela concentração de sais no solo.

Palavras-Chave: Áreas degradadas; Plântulas; Sementes.



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1. INTRODUCTION

The germination process is a critical stage for plant establishment, where reactivation of metabolic activity with increased vacuolization and reserve mobilization of endosperm cell are the first signs that a seed has transitioned to germination (Penfield, 2017). The sequence of physiological and biochemical events during the germination of seeds is influenced by several abiotic factors, such as light, temperature and hydric saturation of the soils which can restrain or inhibit germination (Oliveira et al., 2015; Oliveira and Gualtieri, 2016).

Hydric saturation of the soil restrains the amount of oxygen and, within short time, may generate a hypoxic environment or even an anoxic one. Metabolic changes in the germinative process can occur as a function of oxygen depletion in the medium. Conditions of anoxia may accelerate the rate of anaerobic fermentation and result in the depletion of reserve carbohydrates, and consequently interfere with the germination of the seeds (Ferreira et al., 2009). Salinity is another important factor that interferes in the germination of seeds, growth and reproduction of the plants (Parida and Das, 2005). In saline soils, the germination and survival of the seedlings are conditioned by the capacity to accumulate salts in the cell juice, as a mechanism to compensate for the low osmotic potential and ensure water absorption (Willadino and Camara, 2010).

Optimal conditions for the development of species, viability and seed germination rate can be used to predict the impact that some species may cause in the ecosystems. Therefore, succession, natural regeneration and rehabilitation studies of degraded areas have germination and the establishment of seedlings as their guiding principles (Gomes and Fernandes, 2002).

Sesbania punicea (Cav.) Benth. (Fabaceae), a species originally from South America, is distributed throughout the phytogeographic domains of Atlantic Forest, Pampa and Pantanal (Bergmann, 2014; Queiroz, 2018). It is considered an invasive exotic species in some places of North America, Europe, South Africa and Australia, to where it was taken as an ornamental plant and then quickly spread (Woodward and Quinn, 2011; WIDEpac, 2012). The species has been cultivated as an ornamental plant, showing excessive success in its naturalization, with propagation often facilitated by natural perturbations such as fires, storms, change in the use of land and excessive building in formerly forested

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land, where the plant settles and spreads in clearings (Ulibarri et al., 2002). As it tolerates poor soil very well and can easily adapt to any climate, this species can infest humid or flooded areas, including irrigated rice cultures (Kissmann and Groth, 1992).

Because of their high survival rates in perturbed environments, some species of the genus *Sesbania* have been recommended for recovering degraded areas in Brazil (Chaves et al., 2003). For instance, *S. virgata* has been indicated for recovering riparian forests (Silva et al., 2011). However, the use of species of this genus in environmental recovery projects should be analyzed cautiously because of the easy spread and infesting characteristics for the species (WIDEpac, 2012).

Sesbania punicea is a shrub or small tree and can grow up to five meters high. It has paripinnate leaves, each with 7-16(18) pairs of oppositely arranged leaflets, sometimes with thorny stipules and small stipules, and slender stems. Blooming occurs in spring and summer with big, showy, red-orange flowers, producing fruit in autumn. The fruit is a straight, pendulous, tetragonal and dehiscent legume, which produces 3-9(10) seeds (Ulibarri et al., 2002; Bergmann, 2014). This is a fast-growing species that produces a large amount of seeds that can spread to long distances through water currents (Hunter and Platenkamp, 2003; Woodward and Quinn, 2011). The species reproduces only through seeds, whose viability, in a seed bank, is no longer than 2-3 years (Hoffmann and Moran, 1998).

Although the occurrence of S. punicea is facilitated by environmental perturbations, making it an infesting species, studies that approach the requirements for the germination of its seeds and its adaptability to stressful environmental conditions is necessary for understanding the development and establishment of this species. Such information helps environmental managers make decisions about the necessity of intervention for species control or the use of the species in vegetal restoration projects. Two limiting factors for the establishments of many species in the coastal region of southern Brazil can be cited: the variations in hydrological conditions and the presence of salinity in the soil. Therefore, this study aims to investigate the effect of salinity during the germination of Sesbania punicea seeds in different hydric and luminosity conditions and assess the influence of salinity and flooding on the growth.



2. MATERIALS AND METHODS

2.1. Place and collection of seeds

We obtained seeds of *S. punicea* from fruits of 20 different matrices present in the Environmental Protection Area (APA) of Verde Lagoon, located in the city of Rio Grande, RS, Brazil (32°06' and 32°09'S to 52°10' and 52°11'W). Samples of the plants were collected, mounted herbarium specimens and deposited in the Herbarium HURG of the University Federal of Rio Grande–FURG, as voucher specimens under voucher number HURG 2662.

Fruits in similar ripeness status were collected in April 2012 and taken to the Laboratory of Vegetal Physiology of the Federal University of Rio Grande -FURG, where we carried out the experiments. After collecting the fruits, we mixed them and manually opened them for the seeds. The seeds that were dark-colored stained or had holes were discarded, and those that were intact and light-colored were packed in kraft paper envelopes and stored in a dry and airy place until the beginning of the experiments, a week later. In order to assess the reproductive investment of the species, an aliquot of 200 fruits was taken from the total number of fruits collected. We performed morphometry measurements of fruits and seeds, taking into consideration: length and width of the fruit (cm), number of seeds per fruit, size (cm) and mass (g) of the seeds. We weighed the seeds using an analytical balance accurate to 0.1mg, and we took the measurements with calipers.

2.2. Germination Tests

Taking into account that the seeds of the genus *Sesbania* show different levels of tegument dormancy (Ferreira et al., 2005), the seeds were scarified with number-eight wood sandpaper, according to the recommendation of Fowler and Bianchetti (2000). Germination tests were performed on eight samples of 25 seeds, for each of the four treatments: normoxy/light and normoxy/dark; flooded/light and flooded/dark. The conditions of each treatment were: (a) sowing the seeds in "gerbox" type boxes, on a sheet of blotting paper moistened with 25 mL of saline solution with concentrations of 0,5,10 and 15gL⁻¹NaCl, and transferred to a germination chamber (SL-224 Solab) at 25°C, with a photoperiod of 12h light/12h dark (NR-L, normoxy/light treatment) or 24h dark (NR-D, normoxy/dark

treatment); (b) sowing the seeds in Falcon pipes (50 mL) filled with 25 mL of saline solution with concentrations of 0, 5, 10 and 15gL⁻¹NaCl, and transferred to a germination chamber (SL-224 Solab) at 25°C, with a photoperiod of 12h light/12h dark (FL-L, flooded/light treatment) or 24h dark (FL-D, flooded/dark treatment).

We observed germination daily for 30 days, and the seeds that showed a minimal root protrusion of 2mm were considered germinated (Rehman, 1996). The treatments whose germination occurred in the dark were counted with safety green light (Amaral-Baroli and Takaki, 2001). In order to check the possibility of dormancy induced by low osmotic potential, the seeds that did not germinate after 30 days of incubation in salinities higher than zero were submitted to the procedure of salt stress relief (Khan and Gul, 2006). For this, the seeds were washed with distilled water and placed in distilled water for germination, under the same conditions described above, for 15 more days.

Germination was analyzed by the following parameters: percentage of germination (G); germination speed index (GSI), given by the equation $GSI = \Sigma(G1/T1 + ... + Gi/Ti)$, where G1 to Gi represents the number of seeds germinated each day and T1 to Ti is the time in days (Oliveira et al., 2009); average time of germination (AGT) according to the equation $ATG = \Sigma(n x d)/N$, where *n* is the number of seeds germinated within a time interval, *d* is the period of incubation, in days, at the moment of germination, and *N* is the total number of germinated seeds in the treatment (Brenchley and Probert, 1998).

2.3. Growth responses of the seedlings

In order to analyze the initial growth of the seedlings of *S. punicea* in different hydric and saline concentrations, after scarification the seeds were placed in a clear polystyrene vase. This vase contained 0.5Kg of soil collected in the Environmental Protection Area (APA) of Verde Lagoon, previously dried at 200°C for two hours (Lima Junior, 2010). After sowing the seeds, we used the following treatments: normoxy/light treatment with daily irrigation with 25mL of salt solution with 0, 5, 10 and 15 gL⁻¹NaCl, and flooded/light treatment, keeping 1cm layer of water over the substratum, under the same salt concentrations (0, 5, 10 and 15gL⁻¹NaCl). The seedlings were kept under these conditions for 60 days, and after that, we analyzed the total number of leaves and the height of the plants.

2.4. Extraction and quantification of photosynthetic pigments

At the end of the growth experiment, seedlings with 60 days of growth were used for the extraction and quantification of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids). For this, only seedlings originating from the normoxy / light treatment, submitted to different concentrations of NaCl, were used. Due to low production of leaves by plants growing in flooding conditions, we have not performed the extraction and quantification of photosynthetic pigments for this treatment.

After gauging the fresh mass, we macerated the samples with acetone 80%. The macerate obtained was filtered and the volume of acetone was filled up to 100mL. After that, a Biospetro SP-22 spectrophotometer was used at wavelengths of 663nm, 645nm and 470nm. The concentrations of the pigments were determined according to per the formulae of Arnon (1945) for chlorophylls and Lichtenthaler (1987) for carotenoids.

2.5. Data analysis

This study used a completely randomized experimental design. After verifying the normalization of the data, the germination percentages were transformed by using the expression arcsen v %, and the growth values were transformed in v x for their normalization. However, the data presented here are not transformed. For the analysis of the germination and growth data, a regression analysis was applied for to verify the differences between the averages in the treatments. When there was significance at 5%, the germination data were adjusted by orthogonal polynomials. The data of concentration of pigments were submitted to variance analysis by F test and the means were compared by Tukey's test, with an error probability of 5%. The quantitative characteristics were submitted to descriptive analysis for obtaining their respective means.

3. RESULTS

We collected 1500 fruits of *S. punicea* from 20 matrices present in the study area, totaling approximately 7600 seeds, whose weight was 0.532Kg. Fifty-five percent of the seeds collected were intact and 45% were predated, with holes in the endocarp caused by driller insects. Table 1 shows the descriptive analysis of the morphometric measurements of the fruits and seeds.

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Table 1 – Morph metric measurements of fruits and seeds
of Sesbania punicea collected in the Environmental
Protection Area (APA) of Verde Lagoon ($N = 200$).
Tabela 1 – Medidas morfométricas de frutos e sementes de
Sesbania punicea coletados na Área de Proteção
Ambiental (APA) da Lagoa Verde ($N = 200$).

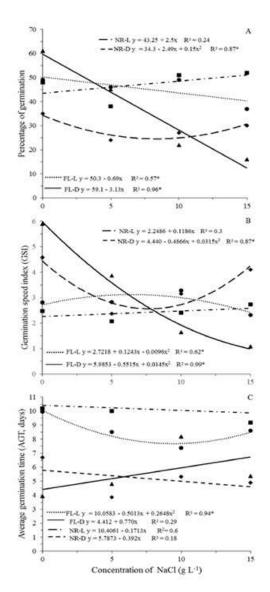
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Characteristic	Mean
(A) Fruits	
Length (cm)	8.09 ± 1.19
Width (cm)	1.41 ± 0.15
Average number of seeds	6.67 ± 1.53
(B) Seeds	
Size (cm)	0.6 ± 0.01
Individual fresh mass (g)	$0.07{\pm}0.01$
Average mass of 100 seeds (g)	7.11±0.01

Figure 1 shows the germinative behavior of the seeds of *S. punicea* in relation to the salt concentration. The germination of seeds that had been kept submerged (flooded) and in the dark adjusted to the linear model and showed a tendency to decrease as the salt concentration increased. There was a 73% decrease in the germination of seeds kept under these conditions for the concentration of 15 gL⁻¹NaCl when compared to the control (0gL⁻¹ NaCl).

Seeds that had been kept under normoxy conditions in the dark showed quadratic adjustment for the variable germination, with a high coefficient of determination ($R^2 = 0.87$). The point of minimal response for seeds under these conditions was 24%. On the other hand, seeds kept submerged and in the presence of light, like those kept submerged in the dark, showed a decrease in germination up to the highest salt concentration. There was a decrease of approximately 25% in the germination of seeds under the concentration of 15gL⁻¹NaCl, in comparison with those under concentration of 0gL⁻¹NaCl (Figure 1A). There was no significant difference (P>0.05) in the average germination time (AGT) of seeds and in the germination speed index (GSI) under the influence of normoxy/light treatments (Figures 1A and 1B).

For other treatments, GSI data adjusted to the quadratic model (Figure 1B). There was a tendency for decreasing germination speed index in seeds kept submerged in the dark up to the concentration of 15gL⁻¹NaCl. Seeds kept submerged with light showed their point of maximum response in concentration of 6.5gL⁻¹NaCl with a subsequent tendency to decrease up to the highest salt concentration. However, seeds kept in normoxy in the dark showed a tendency for decreasing germination speed index up to the point of minimum





- Figure 1– Germination (A), germination speed index (B) and average germination time (C) of *Sesbania punicea* submitted to different hydric and luminosity conditions in different NaCl concentrations. FL-L, flooded treatment in light; FL-D, flooded treatment in the dark; NR-L, normoxy treatment in light; NR-D, normoxy treatment in the dark (*Significant at 5%).
- Figura 1 Germinação (A), índice de velocidade de germinação (B) e tempo médio de germinação (C) de Sesbania punicea submetidos a diferentes condições hídricas e de luminosidade em diferentes concentrações de NaCl. FL-L, tratamento inundado na luz; FL-D, tratamento inundado no escuro; NR-L, tratamento normoxia em luz; NR-D, tratamento normoxia no escuro (* Significativo em 5%).

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response (2.6gL⁻¹NaCl), with a subsequent tendency to increase up to the highest salt concentration (15gL⁻¹NaCl).

Seeds under light submersion conditions presented significant differences ($R^2 = 0.94$), in the average germination time (AGT) with increasing NaCl concentration (Figure 1C). The seeds under these conditions showed a decrease in the average germination time up to the concentration of $10gL^{-1}$ NaCl with a subsequent increase up to the highest concentration. There was no significant difference (P>0.05) in the average germination time of seeds under the influence of normoxy/dark and flooded/dark treatments. There were no answers in germination in seeds submitted to salt stress relief and germinated for 15 days using distilled water in conditions of flooded or normoxy dark/light treatments.

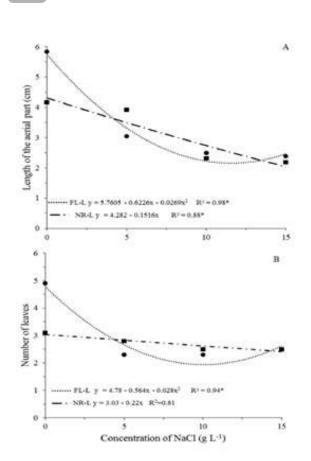
The length of the aerial part of seedlings under normoxy conditions decreased linearly up to concentration 15gL⁻¹NaCl, with a 48% decrease between the highest and lowest concentrations (0gL⁻¹NaCl). Seedlings under the influence of flooding showed quadratic adjustment for the height of aerial part, with a decrease in the height of the aerial part up to the point of minimal response of 11.6 gL⁻¹NaCl (Figure 2A). The number of leaves of plants under the influence of flooding adjusted to the quadratic model with high coefficient of determination (R² = 0.94), with the point of minimal response in the concentration of 10.1 gL⁻¹NaCl. There was no significant difference (P>0.05) in the number of leaves of plants under the influence of the normoxy treatment (Figure 2B).

The concentrations of photosynthetic pigments in seedlings of *S. punicea* that had been kept under normoxy conditions with light are shown in Table 2. There was no significant difference (P>0.05) between the values of chlorophylls and carotenoids in the different salt concentrations tested.

4. DISCUSSION

The fruits of Sesbania punicea, collected in the APA (Environmental Protection Area) of Verde Lagoon, showed seeds with little variation in average size $(0.6 \pm 0.01 \text{ cm})$ and weight $(0.07 \pm 0.01 \text{ g})$, with a high predation rate (45%). The average fresh mass of 100 seeds was very similar to the values found by Poletto et al. (2007) for the species Sesbania virgata. According to Hoffmann and Moran (1998), S. punicea presents

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- Figure 2 Length of the aerial part (A) and number of leaves (B) of plants of *Sesbania punicea* submitted to different hydric conditions and NaCl concentrations. FL, flooded treatment; NR-L, normoxy treatment (*Significant at 5%).
- Figura 2 Comprimento da parte aérea (A) e número de folhas (B) de plantas de Sesbania punicea submetidas a diferentes condições hídricas e concentrações de NaCl. FL, tratamento inundado; NR-L, tratamento normoxia (* Significativo em 5%).

a high fruit production as a survival strategy, since the seeds undergo strong predation pressure, besides having a short life in the seed bank.

The excess of water during germination may impose anoxic conditions to the seeds. Seeds of many terrestrial plants that display high germination rates in soil do not germinate in water, because they can quickly lose viability under such conditions (Parolin, 2001). According to Okamoto and Joly (2000), for the species *Inga sessilis*, anoxia can be lethal to the seeds. Although flood conditions have influenced the resumption of embryo growth, there was a relative increase in the percentage of germination of seeds stored in

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these conditions, when compared to those germinated in normoxy. Seeds of *Tabebuia cassinoides*, typical species of environments with flooding, did not germinate under conditions of total submersion and anoxic, but under normoxic conditions or partial submersion the seeds reached 100% of germination (Kolb and Joly, 2010). Seedlings of *Tabebuia aurea*, another species characteristic of flooded environments, survive in conditions of stress by flooding and anoxic for more than 48 hours, reducing its metabolic activity (Oliveira and Gualtieri, 2016).

An important restriction resulting from excess water is an inadequate supply of oxygen to submerged plant tissues. Anaerobic conditions result in a rapid change in the expression of genes, which play a vital role in the metabolism of cells partially deficient in oxygen (Jackson and Colmer, 2005). The root system of the *T. cassinoides* plants presented high amounts of ethanol under conditions of soil water saturation, corroborated by an increase in ADH activity (Kolb and Joly, 2009). Seeds of *T. cassinoides* submerged and in anoxia showed activation of glycolytic pathway after a day of soaking and ethanol was the main product formed, along with an expressive production of lactate and malate (Kolb and Joly, 2010).

The saline stress affects the germination of seeds because of the osmotic effect, ion toxicity, or both. The plant can have various mechanisms for dealing with damage caused by salt, such as modifications in development, activity of antioxidant enzymes and hormonal production (Zhang et al., 2010). The effect of NaCl on germination of seeds of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* was due to both osmotic stress and ion toxicity (Song et al., 2005).

The presence of dissolved osmolytes in the water causes a decrease in their hydric potential, being first perceived by the root system of the plants, inducing osmotic stress and reducing water availability (Acosta-Motos et al., 2017), which can also affect seed germination due to the low availability of water free. The decrease in the number of germinated seeds with the increase of salt concentration as well as the absence of germination in the salt stress relief tests may represent an indication of the damage caused by salt to the embryo of *S. punicea*. Some studies have shown how germination can be inhibited under high concentration of salt and



 Table 2 – Chlorophyll a, b and total chlorophyll, and carotenoid pigments, expressed in mg g⁻¹ fresh mass, in leaves of Sesbania punicea submitted to normoxy and different concentrations of NaCl.

 Tabela 2 – Clorofila a, b e clorofila total e pigmentos carotenóides, expressos em mg g-1 de massa fresca, em folhas de Sesbania punicea submetidas a normoxia e diferentes concentrações de NaCl.

NaCl (gL-1)	Chlorophylla	Chlorophyllb	Total chlorophyll	Carotenoids
0	$0.24^{\rm ns} \pm \ 0.0491$	$0.146^{\rm ns}\pm\ 0.0237$	$0.378^{\rm ns}\pm\ 0.0748$	$0.05^{\rm ns} \pm \ 0.0165$
5	0.221 ± 0.0321	0.125 ± 0.0232	0.346 ± 0.056	0.047 ± 0.004
10	0.188 ± 0.0639	0.116 ± 0.0622	0.315 ± 0.1204	0.046 ± 0.0165
15	0.141 ± 0.0434	$0.112\ \pm\ 0.0853$	0.296 ± 0.1281	0.029 ± 0.0135
CV	25.18	41.75	29.04	25.16

CV - Coefficient of variation. ^{ns} Not significant (p < 0.05).

that the seeds can germinate after saline stress conditions are removed (Khan et al., 2000, 2001; Song et al., 2005). However, this kind of response has been found in halophyte species.

Among the most common effects of soil salinity, growth inhibition by ions Na⁺ and Cl⁻ is considered the main cause of the decrease in the speed of physiological and biochemical processes and consequent reduction in plant growth (Flowers, 2005; Dhanapackiam and Muhammad Ilyas, 2010). The growth results observed in this study show the deleterious effect of salinity on the growth of seedlings of *S. punicea*. After 60 days of growth, there was an evident decrease in the size of seedlings and in the number of leaves produced as the concentration of saline solution increased.

Not all the species respond to salinity in the same manner. Tolerance varies in every culture and within the same species. The effect of salinity can be attributed to the water deficit caused by excess soluble salts in the root zone, causing a decrease in turgescence, reduction in cell expansion and consequent reduction in plant growth (Khalid and Silva, 2010). Osmotic balance is essential for plants growing in saline medium, it can affect almost all aspects of plant development including: germination, vegetative growth and reproductive development (Shrivastava and Kumar, 2015). We observed a similar result in our study. In spite of the variability of the samples, there was an evident decrease in growth as the salt concentration increased.

The photosynthetic pigments of plants of *S. punicea* kept in different salt concentrations and in the absence of flooding were not affected by the salt concentrations. Despite the fact that the values showed a tendency to decrease as the salt concentration increased, the high coefficient of variation made it impossible for

us to distinguish the means. Conversely, the concentrations of photosynthetic pigments of plants of *Bruguiera parviflora* cultivated in hydroponics (Parida et al., 2001), of the halophyte *Plantago coronopus* (Koyro, 2006), of *Glaucium flavum* (Cambrollé et al., 2011) and of the cowpea *Vignia sinensis* (Kandil et al, 2017) were affected by salt stress.

Sesbania punicea is a species whose distribution is facilitated by perturbations in the nature, such as land clearing (Ulibarri et al., 2002) and the presence of poor soils (Kissmann and Groth, 1992). The high tolerance to salinity during the germination of the seeds may represent another facilitator for the spread of the species in environments with stressful characteristics, such as saline soils. The germination responses of S. punicea observed in this study show a competitive advantage of the species for quickly occupying perturbed environments, even in saline soils. However, characterizing and using S. punicea as a species with a great potential for recovering degraded areas might not be effective due to salinity, as the growth of the seedlings was affected by the increase in the salt concentration. This fact might limit occupation and spread of the species in new colonized areas.

6. CONCLUSION

The responses of germination and the development of the seedlings of *S. punicea* were affected by hydric conditions. The presence of NaCl affected negatively the germinative process in normoxic conditions. Flooding decreases the percentage of germination as salt concentrations increase. There is a decrease in the growth of the seedlings and in the production of leaves as the salt concentration increases. Therefore, the colonization and occupation of new degraded areas by *S. punicea* may be limited by the presence of salt concentrations in the soil.

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