Production of *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson (Fabaceae) seedlings irrigated with saline water

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ABSTRACT: Saline irrigation water at high levels causes disturbance in the growth of more sensitive plants. The objective of this research was to evaluate the initial growth of *Pityrocarpa moniliformis* seedlings under different electrical conductivity in irrigation water through physiological and biochemical analyses. The experiment was conducted in a greenhouse with five water electrical conductivities (0.5, 2.0, 4.0, 6.0 and 8.0 dS m\(^{-1}\)) and four repetitions of 20 plants each, arranged in a randomized block design. Application of the treatments with irrigation water containing NaCl began at 30 days after sowing. To determine the behaviour of the species, the following variables were analysed: stem diameter, plant height, number of leaves, leaf area, Dickson quality index, shoot dry mass, root dry mass and total dry mass, as well as the biochemical variables, such as the concentrations of total soluble sugars, free proline and chlorophylls a and b. The increase in irrigation water salinity hampered the growth of *P. moniliformis* seedlings, with electrical conductivity of 0.5 dS m\(^{-1}\) being the limit for maximum production. The results also indicated that the deleterious effects of salt stress on *P. moniliformis* seedlings variables increase in concentrations of proline, total soluble sugars and betaine glycine.

Key words: dry forests, semi-arid region, salt stress, salinity, abiotic stress

HIGHLIGHTS:

Saline stress negatively affects the quality of *P. moniliformis* seedlings.
The phytomass of *P. moniliformis* seedlings is significantly reduced with the increase of salt stress in irrigation water.
The increase in salinity of irrigation water affects the levels of osmoregulators in *P. moniliformis* seedlings.

RESUMO: Água de irrigação salina a níveis elevados pode ocasionar transtorno no crescimento de plantas mais sensíveis. Objetivou-se avaliar o crescimento inicial de mudas de *Pityrocarpa moniliformis* irrigadas com água de diferentes conduittividades elétricas da água de irrigação, por meio de análises fisiológicas e bioquímicas. O experimento foi conduzido em casa de vegetação utilizando-se água de irrigação com cinco conduittividades elétricas (0,5; 2,0; 4,0; 6,0 e 8,0 dS m\(^{-1}\)), com quatro repetições de 20 plantas cada, dispostos em blocos ao acaso. Até 30 dias após a semeadura as mudas foram irrigadas duas vezes ao dia com água de abastecimento de baixa conduittividade elétrica (0,5 dS m\(^{-1}\)). Após esse período, quando as plantas estavam suficientemente desenvolvidas, iniciou-se a aplicação dos tratamentos com água de irrigação contendo NaCl. Aos 60 dias após a semeadura foram avaliadas as seguintes variáveis: diâmetro de colo, altura das plantas, número de folhas, área foliar, índice de qualidade de Dickson, massa seca da parte aérea, massa seca das raízes, massa seca total, bem como as avaliações bioquímicas de açúcares solúveis totais, teor de proline livre e clorofília “a” e “b”. O aumento da salinidade na água de irrigação prejudicou o crescimento das mudas de *P. moniliformis*, sendo a conduittividade elétrica de 0,5 dS m\(^{-1}\) o limite para máxima produção. Os resultados também indicaram que os efeitos deletérios do estresse salino nas mudas de *P. moniliformis* estimularam o aumento das concentrações de proline, açúcares solúveis totais e betaina glicina.

Palavras-chave: florestas secas, semi-árido, estresse salino, salinidade, estresse abiótico
**INTRODUCTION**

Drought and soil salinity are the main limiting factors in arid and semiarid regions for plant establishment (Sahito et al., 2013). NaCl-induced salt stress can pose a major threat to plant growth and development (Vecchio et al., 2018). It causes damage to the plant due to excess ions and water deficit (Taiz et al., 2017), which leads to reduction of plant growth and turgor pressure and causes changes in cell metabolism (Thalmann & Santella, 2017). Thus, plants remobilize their reserves to release energy, sugars and derived metabolites (Rodrigues et al., 2018).

Salt-resistant plants of the ‘Caatinga’ ecosystem can be used to reclaim degraded areas. In this context, the choice of species can determine the success of commercial, landscape and reforestation projects (Dantas et al., 2018). Recent studies carried out on forest species have highlighted promising results for their use in these environments (Leal et al., 2015; Leal et al., 2019; Lopes et al., 2019).

However, little is still known about ‘Caatinga’ cultivated plant species subjected to salt stress, in particular simulating field conditions. *Pityrocarpa moniliformis* is used as timber, medicine, forage, and in reclamation of degraded areas (Lorenzi, 2002). The species is very widespread in the ‘Caatinga’ of Northeastern Brazil, areas characterised by irregular rainfall, high irradiation, evapotranspiration and elevated concentration of salts in the soil (Guimarães et al., 2013). It is assumed that *P. moniliformis* has biological responses to different levels of soil salinity in the ‘Caatinga’ ecosystem (Pereira et al., 2016; Dantas et al., 2018).

The aim of this study was to evaluate the initial development and biochemical responses of *P. moniliformis* plants irrigated with water of different electrical conductivities through physiological and biochemical analyses.

**MATERIAL AND METHODS**

The study was conducted in a greenhouse at the Academic Unit Specialised in Agrarian Sciences (UAECIA) belonging to the Federal University of Rio Grande do Norte (UFRN), Macaíba, RN, Brazil (5° 53’ 9.77'' S, 35° 21’ 52.47'' W and 15 m altitude), from September to October 2018. The seeds were collected from native trees existing in the Rafael Fernandes experimental farm of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil (5° 11’ S, 37° 20’ W and 18 m altitude).

The experimental design was randomized blocks, formed by five treatments, with four repetitions of 20 plants each. The treatments consisted of five electrical conductivity of irrigation water: 0.5 (control), 2.0, 4.0, 6.0 and 8.0 dS m⁻¹ (Table 1). Prior to sowing, the seeds were scarified using a sandpaper (number 40) on the opposite part to the hilum. Sowing was performed in polyethylene plastic bags (1.2 L), using as substrate soil collected on the UAECIA campus, Macaíba, RN, Brazil, and the chemical analyses are presented in Table 1, which shows the initial analysis of the substrate (before the experiment) and the analyses at the end of the experiment, according to the treatment.

At 30 days after sowing (DAS), when the plants were sufficiently developed, irrigation was performed once a day using a watering can, with the same volume for all plots. Then the treatments were applied using saline water with NaCl at the defined electrical conductivity (EC), measured in a digital conductivity meter (MCA150 - TECNOPON).

At 60 DAS, the plants were analysed for the following variables: plant height (PH), measured with a ruler graduated in millimetres; number of leaves (NL); stem diameter (SD), measured with a digital calliper; leaf area (LA), measured by the disc method corrected according to Souza et al. (2012); Dickson quality index (DQI) (Dickson, 1960) and total dry mass (TDM), determined after the plant material was collected, separated into different parts (shoot and roots), placed in paper bags, dried in the oven at 65 °C until reaching constant weight and, subsequently, weighed on an analytical scale (0.0001 g), with the data expressed in g plant⁻¹.

In order to determine the biochemical variables, leaves were collected and the material was cut into smaller portions to collect the composite sample of each treatment and initially kept in a freezer. The absorbance of the chlorophyll solution was obtained by macerating 200 mg of plant material plus 4.5 mL of acetone PA and centrifugation (Arnon, 1949). Then, the supernatant was used for reading on a spectrometer at 663 nm for chlorophyll “a” and at 645 nm for chlorophyll “b”. Chlorophyll concentration was expressed in mg g⁻¹ of fresh matter (mg g⁻¹ FM).

For proline concentration determination, samples with 0.5 g of fresh material were manually homogenised, in porcelain mortar, with 10 mL of 3% sulphotosalicylic acid. Then, these samples were subjected to centrifugation at 5,000 rpm for 20 min, to compose the reaction, and placed in a test tube containing 2 mL of the supernatant, with the addition of 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. These solutions were maintained for 1 hour in boiling water bath (at

| ECw * (dS m⁻¹) | N (g kg⁻¹) | P (mg dm⁻³) | K⁺ (cmol dm⁻³) | Mg²⁺ (cmol dm⁻³) | Ca²⁺ (cmol dm⁻³) | Na⁺ (cmol dm⁻³) | pH (H₂O) | EC (dS m⁻¹) | ESP (%) |
|----------------|------------|-------------|---------------|----------------|----------------|----------------|----------|-------------|--------|
| Initial        | 0.42       | 0.7         | 0.11          | 0.4            | 1.1            | 0.02           | 7.1      | 0.06        | 2      |
| 0.5            | 0.14       | 4.3         | 0.12          | 0.4            | 1.0            | 0.18           | 6.9      | 0.11        | 9      |
| 2.0            | 0.7        | 2.0         | 0.10          | 0.3            | 0.7            | 0.83           | 7.1      | 0.22        | 43     |
| 4.0            | 0.7        | 0.5         | 0.07          | 0.3            | 0.6            | 1.33           | 6.8      | 0.40        | 58     |
| 6.0            | 0.7        | 0.6         | 0.07          | 0.4            | 0.5            | 1.55           | 6.7      | 0.48        | 54     |
| 8.0            | 0.7        | 3.4         | 0.07          | 0.1            | 0.4            | 1.99           | 6.7      | 0.51        | 73     |

ECw - Electrical conductivity of irrigation water
Soil analysis performed by the Soil Fertility and Plant Nutrition Laboratory DCAT/UFERSA/2019; pH - Hydrogen potential; EC - Electrical conductivity of the saturation extract of substrate; ESP - Exchangeable sodium percentage \([\text{Na}^+ / \text{Cation-Exchange Capacity}] \times 100\)
100 °C) and cooled by immersion in an ice bath, and colour intensity was measured at 520 nm. The absorbances obtained were compared with the standard proline curve, and the results were expressed in micrograms of proline per g of fresh material (Bates et al., 1973). The concentrations of total soluble sugars (TSS) were determined by the anthrone method (Yemm & Willis, 1954).

The results were subjected to analysis of variance and regression by F test at p ≤ 0.05, with the aid of the statistical program SISVAR (Ferreira, 2011). In case of significance, the model, linear or quadratic, was chosen, and the best fit was considered on the basis of biological expression.

**Results and Discussion**

The analysis of variance indicates that there were significant effects on plant height (PH), number of leaves (NL), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) (p ≤ 0.01) and on stem diameter (SD), leaf area (LA) and Dickson quality index (DQI) (p ≤ 0.05), indicating that salinity has a direct influence on the initial development of *P. moniliformis* seedlings (Table 2).

The results show reductions in the growth variables of *P. moniliformis* plants for all variables analysed, under salt stress (Figure 1).

Table 2. Summary of analysis of variance for plant height (PH), number of leaves (NL), stem diameter (SD), total dry mass (TDM) of *Pityrocarpa moniliformis* seedlings subjected to different electrical conductivity of irrigation water

| Sources of variation | DF | PH | NL | SD  | TDM | LA  | DQI |
|----------------------|----|----|----|-----|-----|-----|-----|
| Treatment            | 4  | 61.40** | 10.48** | 0.20* | 21.30** | 1.20* | 0.18* |
| Linear regression    | 1  | 207.8** | 36.86** | 0.25* | 77.44** | 4.82** | 0.61** |
| Quadratic regression | 2  | 14.51** | 3.17** | 0.48** | 4.12** | 0.00ns | 0.07ns |
| Blocks               | 3  | 2.72ns  | 0.22ns  | 0.02ns | 1.34*  | 0.03ns | 0.00ns |
| Residual             | 12 | 3.08  | 0.32  | 0.04  | 0.30  | 0.31  | 0.04  |
| CV (%)               | -  | 13.37 | 10.70 | 13.45 | 6.68  | 23.95 | 23.31 |

ns, *, ** - Not significant, significant at p ≤ 0.05 and p ≤ 0.01, respectively, by F test; DF - Degrees of freedom; CV - Coefficient of variation

Figure 1. Plant height (A), number of leaves (B), leaf area (C), stem diameter (D), total dry mass (E) and Dickson quality index (F) of *Pityrocarpa moniliformis* seedlings as function electrical conductivity of irrigation water (EC)
For plant height, there was significant reduction as a function of the increase in irrigation water salinity, with a 50% decrease at the highest salinity compared to the lowest salt concentration (0.5 dS m⁻¹) (Figure 1A).

The first regulatory strategy for tolerance to salt stress in order to reduce water losses by transpiration, due to increased salt concentration, is stomatal closure and reduction of water absorption by roots, which affects the development of plants throughout their cycle, as evidenced by Taiz et al. (2017). It is worth pointing out that the reduction of plant growth, as a consequence of decrease in turgor pressure and water absorption, is caused by changes in cellular metabolism, a strategy of plants for remobilising reserves to tolerate stress (Rodrigues et al., 2018). In this context, Brito et al. (2018) evaluated the height of *Schinopsis brasiliensis* Engl. plants and found greater growth (10.59 cm) when the substrate containing fertiliser salts was leached, compared to those grown in substrates without leaching (7.23 cm), demonstrating the effect of residues of irrigation water salts on the increase of substrate electrical conductivity.

The reduction in the number of leaves due to the increase in electrical conductivity of water negatively interfered with seedling quality (Figure 1B). The lowest value was obtained at salinity of 8.0 dS m⁻¹, with 3.1 leaves per plant, resulting in a reduction of more than 54% and, consequently, affecting the production of *P. moniliformis* seedlings. Based on soil analysis, it is possible to observe that the accumulation of salts in the substrate, and the excess of NaCl possibly reduced the contents of nutrients such as N, Mg, K and Ca (Table 1). A similar result was reported by Melloni et al. (2000), who observed leaf abscission as a strategy to reduce water loss, as evidenced by Taiz et al. (2017). These authors attribute this initial increment to a morphological response to stress, having this value as a tolerance limit.

A decreasing linear model was fitted to the values of leaf area (Figure 1C). Seedlings of *P. moniliformis* irrigated with water of electrical conductivity of 8.0 dS m⁻¹ had their leaf area reduced by 54% compared to those subjected to salinity of 0.5 dS m⁻¹. It was observed that there was no mitigation of stress, possibly because of the attempt to compensate for the effects of the salt, since leaf area was reduced as irrigation water salinity increased. This is the first line of defence of the plant against water depletion in the cell; leaf area is reduced and, consequently, the water supply tends to continue for some time (Taiz et al., 2017). Based on this fact, Brito et al. (2018) observed a 93.18% increase of leaf area in *Schinopsis brasiliensis* seedlings when they were produced in a leached substrate after fertiliser application, compared to the non-leached one for the highest dose. Leaf area is decisive for the success of seedlings in the field, as the increase in this variable associated with greater light interception is responsible for the increase in the production of plant biomass, as evidenced by Thalmann & Santelia (2017).

There was a reduction of 18.40% in stem diameter between plants under the highest (8.0 dS m⁻¹) and lowest (0.5 dS m⁻¹) salt concentrations was 10.88 and 5.46 g plant⁻¹, respectively, resulting in a reduction of 50.18% (Figure 1E). Similar results were obtained by Guimarães et al. (2013) in the initial growth of *Erythrina velutina* plants; these authors observed a linear reduction as salinity increased up to the level of 7.5 dS m⁻¹, and the stress was more pronounced from salinity of 2.5 dS m⁻¹.

In a study with *A. leprosum*, Leal et al. (2019) obtained null values for dry mass of seedlings irrigated with saline water of 6.5 dS m⁻¹. According to these authors, there was only radicle protrusion, hence insufficient to measure this variable. These results were also observed in *Erythrina velutina* seedlings by Lopes et al. (2019), who highlighted that reductions in shoot and total dry masses occurred due to the decrease in water absorption and increase in the toxicity of Na⁺ and Cl⁻ ions, which caused metabolic imbalances when accumulated in plant tissues.

The Dickson quality index (DQI) was linearly affected by the increase in salt concentration (R² = 0.80), and its highest value was found at EC of 0.5 dS m⁻¹ (Figure 1F). Despite the decreases in DQI in *P. moniliformis* seedlings from 0.5 dS m⁻¹, the values are still satisfactory to indicate quality seedlings. The lowest value obtained was 0.60 for seedlings irrigated with water of higher salt concentration (8.0 dS m⁻¹). This value is above the recommended minimum of 0.2 (Hunt, 2017).
The analysis of variance revealed significant effect (p ≤ 0.01) for proline (PRLN), total soluble sugars (TSS), glycine betaine (GB) and chlorophylls (CHL) “a” and “b” in *P. moniliformis* seedlings when exposed to different concentrations of NaCl (dS m⁻¹) (Table 3).

The levels of osmoregulators were affected by the increase in irrigation water salinity, as well as by the concentrations of chlorophyll “a” and “b” (Figure 2). Higher values of proline were observed with the increase of irrigation water salinity, indicating a positive quadratic model in response to the salinity levels. This demonstrates the greater amount of proline at the highest electrical conductivity of water irrigation, compared to that of the control (Figure 2A).

Possibly, at the electrical conductivity of 8.0 dS m⁻¹, the plant was under a high level of stress, which may have caused increased proline activity in the leaves and, consequently, lower response in the production of seedlings. This result is in agreement with Silveira et al. (2010), who stated that the negative effects of salt stress are actually a normal physiological response of plants to overcome an adverse condition.

Thus, much of the data suggests that proline concentrations increase after the occurrence of the effects caused by stress. This finding is in accordance with Ayala-Astorga & Alcaraz-Meléndez (2010), who found that the content of this amino acid

### Table 3. Summary of analysis of variance for proline (PRLN), total soluble sugars (TSS), glycine betaine (GB), chlorophyll a (CHL a) and chlorophyll b (CHL b) of *Pityrocarpa moniliformis* seedlings subjected to different electrical conductivity of irrigation water

| Source of variation | DF | PRLN | TSS | GB | CHL a | CHL b |
|---------------------|----|------|-----|----|-------|-------|
| Treatment           | 4  | 0.001** | 0.0031** | 0.0098** | 0.10** | 20.04** |
| Linear regression   | 1  | 0.006** | 0.012** | 0.038** | 0.38** | 14.60** |
| Quadratic regression| 1  | 0.0006** | 0.0003** | 0.0008** | 0.03** | 64.69** |
| Blocks              | 3  | 0.00**  | 0.003*  | 0.0009** | 0.001** | 0.86**  |
| Residual            |   | 0.00**  | 0.005   | 0.0015   | 0.002  | 3.37    |
| CV (%)              |   | 13.23  | 7.12   | 13.64    | 3.40   | 9.20    |

ns, *; ** - Not significant, significant at p ≤ 0.05 and p ≤ 0.01, respectively, by the F test; DF - Degrees of freedom; CV - Coefficient of variation

### Figure 2. Proline (A), total soluble sugars “TSS” (B), glycine betaine (C) and chlorophylls “a” and “b” (D) of *Pityrocarpa moniliformis* seedlings as function of electrical conductivity of irrigation water (EC)
increased significantly in *Paulownia tomentosa* Steud. plants exposed to increasing levels of sodium chloride.

The increase in electrical conductivity irrigation water led to a 95.83% increment in the concentrations of total soluble sugars, comparing the highest electrical conductivity to the control, hence evidencing that the increase in cell osmoprotectants is related to the effects of different stress-causing factors. According to Thalmann & Santelia (2017), sugars can derive from the assimilation of photosynthetic carbon or even accumulate due to the reduction of demand, as a consequence of growth limitation. The increase in sugar contents confirms the result found by Wang et al. (2013) in *Eutrema halophilum* (C.A.Mey.) Al-Shehbaz & Warwick plants. Likewise, Sahito et al. (2013) reported that there was an increase of sugar contents in *Acacia stenophylla* Benth. plants subjected to the extreme salinity of 16.67 dS m⁻¹.

Salt stress led to greater variation in glycine betaine activity at the four values (2.0; 4.0; 6.0; and 8.0 dS m⁻¹) electrical conductivities of irrigation water, equal to 8.5, 18.52, 23.33 and 34.28%, respectively, compared to that obtained at the values of 0.5 dS m⁻¹ (Figure 2C). It is suggested that the plant had a slightly positive biochemical reaction to the effects of salinity. Under this condition, there is disorder in the structural apparatus at the molecular and cellular levels, which may promote greater availability of osmotically active substances (Taiz et al., 2017). The effect of cell protection often results simply from a metabolic disorder caused by the deleterious effect of stress (Silveira et al., 2010). As reported by Akhtar et al. (2017), glycine betaine accumulation in *Typha domingensis* Pers. resulted from the salt stress. Brito et al. (2019) found that, after foliar application of glycine betaine in *Olea europaea* L. under rainfed conditions, its physiological activity increased in the short term.

Increase in irrigation water electrical conductivity caused reduction in chlorophyll “a” and initial increase in chlorophyll “b”, and linear and quadratic models were fitted to their data, respectively (Figure 2D). It is possible that excess of salts in irrigation water resulted in lower photosynthetic activity, so *P. moniliformis* plants were not able to respond to the increase in NaCl. These functional disorders and injuries are caused by the osmotic effect of the salt on chloroplast (Taiz et al., 2017). This fact is confirmed by Oliveira et al. (2018), who investigated the effects of salinity on photosystem II and chlorophyll concentrations in *Vigna unguiculata* (L.) Walp. subjected to the most severe treatment (12 dS m⁻¹), with a significant reduction in comparison to that of the control. Dias et al. (2019) found that the photosynthetic process in *Malpighia emarginata* D.C. was impaired by irrigation water with electrical conductivity of 3.8 dS m⁻¹.

Chlorophyll “b” concentrations were equal to 18.7 and 16.6 mg g⁻¹ in the control treatment and at the highest level of salinity, respectively (Figure 2D). These results are related to the fact that *P. moniliformis* seedlings had greater increments of growth at the lowest level of salinity (0.5 dS m⁻¹). It is also worth highlighting that there were higher values of chlorophyll “b” compared to chlorophyll “a”, which corroborates the data observed by Wang et al. (2013) in *Eutrema halophilum*. These authors found less chlorophyll “a” than chlorophyll “b” in the leaves due to the effect of salt stress, which also justifies better response of chlorophyll “b” to the stress, which was verified by the initial increment up to 4.0 dS m⁻¹. In this context, the concentrations of chloroplastic pigments, such as chlorophyll, can be used in important markers of plant acclimation that, in adverse situations, can result in the reduction of chlorophyll “a” and increase of chlorophyll “b” (Souza et al., 2011).

The results demonstrate the sensitivity of *P. moniliformis* to salt. This fact was confirmed by the decrease in physiological variables, growth and Dickson’s quality index. In addition, they were accompanied by an increase in proline, total soluble sugars and glycine betaine, showing the stress situation to which the species is submitted.

**Conclusions**

1. The increase in electrical conductivity in the irrigation water impairs the growth of *P. moniliformis* seedlings, with 0.5 dS m⁻¹ being the limit for maximum production.

2. The deleterious effects of salt stress on *P. moniliformis* seedlings stimulate the increase in the concentrations of proline, total soluble sugars and glycine betaine.

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