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Gas exchange of *Eucalyptus camaldulensis* Dehnh under saline water influence[1]

Gabriel Zapelini[2]; Marcio Carlos Navroski[3]; Mariane de Oliveira Pereira[4]; Tiago Krischnegg de Andrade[5]; Karollyne Renata Silva de Paula Baptista[6]; Ramon Silveira de Andrade[7]

Abstract: In view of the increasing expansion of the use of wood and non-timber products from the *Eucalyptus* genus, the identification and impact caused by the salinity of Brazilian soils in the development and growth of the species are of fundamental importance for a better utilization of salinized areas in the country. In order to contribute to the understanding of this problem, the present work evaluated the effect of different levels of salinity (brackish water) on gas exchange in *Eucalyptus camaldulensis* Dehnh plants. The levels analyzed were: 0, 50, 100, 200, 400 µM NaCl. A completely randomized design with 10 replicates (plants) was used, in which the gaseous exchanges were evaluated with the aid of a portable photosynthesis meter (IRGA), determined the values of photosynthesis (A), stomatal conductance (gs), rate transpiration (E), the relation between the intercellular and atmospheric concentration of CO² (Ci / Ca) and water use efficiency (WUE). Initially the seedlings did not undergo physiological changes, showing a certain tolerance of the species to high levels of salinity. Reduction in gas exchanges was only more noticeable after reapplication of the salt (after 7 days). Due to the results obtained, high tolerance of the species to salinity is observed.

Keywords: Salinity; Photosynthesis; Tolerance

Trocas gasosas de *Eucalyptus camaldulensis* Dehnh sob influência de água salobra

Resumo: Perante a crescente expansão do uso de produtos madeiráveis e não madeiráveis provindos do gênero *Eucalyptus*, a identificação e o impacto ocasionado pela salinidade dos solos brasileiros, no desenvolvimento e crescimento da espécie são de fundamental importância para o melhor aproveitamento das áreas salinizadas no país. Visando contribuir para o entendimento desta problemática, o presente trabalho avaliou o efeito de diferentes níveis de salinidade (água salobra) nas trocas gasosas em plantas de *Eucalyptus camaldulensis* Dehnh. Os níveis analisados foram: 0, 50, 100, 200, 400 µM de NaCl. Utilizou-se o delineamento inteiramente casualizado com 10 repetições (plantas), onde as trocas gasosas foram avaliadas com auxílio de medidor portátil de fotossíntese (IRGA), determinando-se os valores de fotossíntese (A), condutância estomática (gs), taxa transpiratória (E), relação entre a concentração intercelular e atmosférica de CO² (Ci/Ca) e eficiência no uso da água (WUE). Inicialmente as mudas não sofreram alterações fisiológicas, mostrando certa tolerância da espécie aos altos níveis de salinidade. Reduções nas trocas gasosas só foram mais perceptíveis após sete dias, com reaplicação do sal. Pelos resultados obtidos, percebe-se elevada tolerância da espécie à salinidade.

Palavras – chave: Salinidade; Fotossíntese; Tolerância

[1] Received on 08 August 2020 and accepted for **scientific article** publication on 21 September 2020.
[2] Forest Engineering student at Centro de Ciências Agroveterinárias - CAV-UDESC, Santa Catarina state, Brazil. E-mail: <zap1.gabriel@gmail.com>
[3] Forest Engineering, Dr. Professor at Centro de Ciências Agroveterinárias - CAV-UDESC, Santa Catarina state, Brazil. E-mail: <marcio.navroski@udesc.br>
[4] Forest Engineering, Dra. Centro de Ciências Agroveterinárias - CAV-UDESC, Santa Catarina state, Brazil. E-mail: <maripereira.florestal@gmail.com>
[5] Forest Engineering student at Centro de Ciências Agroveterinárias - CAV-UDESC, Santa Catarina state, Brazil. E-mail: <t.krischnegg@gmail.com>
[6] Forest Engineering, Master student at Centro de Ciências Agroveterinárias - CAV-UDESC, Santa Catarina state, Brazil. E-mail: <karollyne-silva@hotmail.com>
[7] Forest Engineering, Master student at Centro de Ciências Agroveterinárias - CAV-UDESC, Santa Catarina state, Brazil. E-mail: <ramon.andrade@edu.udesc.br>

Introduction

The growing expansion of the forestry industry in Brazil, promoted by the introduction of exotic species, has been the object of study and research, mainly due to the adaptation of these species to the climatic conditions in the country. The introduction of species in sites other than its place of origin is always a process that requires studies aimed at its survival, considering the productivity of the species in the region to be inserted.

In order to have a successful implantation of productive forest stands, a combination of several factors is necessary, such as genetic materials adapted to the location and edaphoclimatic conditions that favor the development of the species (BRUNINI and CARDOSO, 1998). However, with the expansion of forest plantations in less favorable areas, there is a need to know the behavior of different species. Among the exotic species introduced in Brazil, the genus *Eucalyptus* has been growing in prominence in reforestation programs, due to its rapid growth, easy handling, great rate of increment, biomass and mainly for its varied purposes in the industry.

Within the *Eucalyptus* genus, the *E. camaldulensis* species has been gaining space in the Brazilian market. The species is native to Australia, in the subtropical and temperate climate and occurs naturally at altitudes ranging from 30 m to 600 m above sea level. The annual rainfall index of the region of origin is between 250 mm to 600 mm with a dry period of 4 to 8 months, maximum temperature is 35°C and the minimum is 11°C. *Eucalyptus camaldulensis* is of great economic interest due to its ability to grow in poor soils, resistance to long periods of drought, tolerance to excessive rainfall, as well as hard and heavy wood (LIMA et al., 2010). Due to its physical-mechanical characteristics, *E. camaldulensis* wood has been used for civil construction, crossties, floors, framework, fences, turned wood, firewood, coal and in the manufacture of plywood (BOLAND et al., 2006).

The success of *E. camaldulensis* plantations as an exotic species is attributed to its superiority in wood production in relation to other species cultivated in adverse environmental conditions. This is because the species thrives in dry and sterile places, has tolerance to drought and high temperature, rapid growth and good regrowth capacity, tolerance to periodic flooding or waterlogging lasting 4 to 5 months, tolerance to soil salinity, some frost tolerance, in addition to the usefulness of its wood (ELDRIDGE et al., 1993).

Considering the adaptation of the species in forest areas in critical zones, it has been introduced in Brazil with high frequency, mainly in the Cerrado of Minas Gerais state and in São Paulo. Aiming to expand the region of the country in which the species is introduced, it is necessary study and researches the natural adversities that may or may not be tolerated by *E. camaldulensis*, and the physiological responses of the species. Scientific knowledge may subsidize some silvicultural treatments for the cultivation of the species.

One aspect that can influence directly in the development of the species is the salinity of the soil, usually caused by natural processes and, mainly, by the misuse of the land. Approximately 30 million hectares of soil on the planet are affected by salt, and 0.25 to 0.5 million hectares of productive area are lost each year, due to soil salinization (FAO, 2002).

The choice of tolerant species to critical environments, such as places with high levels of salinity, is of great importance for the success of the forestry activity. Thus, the present study aims to assess whether there is an effect of increased sodium chloride (NaCl) concentration on physiological parameters of *Eucalyptus camaldulensis* seedlings.

Material and methods

The experiment was carried out between October and December 2018, in a greenhouse in the forest nursery of the Center of

Agricultural Sciences - CAV (UDESC), located in the municipality of Lages, state of Santa Catarina, in the coordinates 27 ° 47'33 "S and 50 ° 18'4 "W. The site has an altitude of approximately 900 meters, whose climate, according to the Koppen classification, is temperate (humid mesothermal and mild summer) - Cfb, with average annual precipitation between 1300 and 1500 mm and average annual temperature of 15 ° C (ALVARES et al ., 2013). The region belongs to the hydrographic basin of the Uruguay River, a hydrographic sub-basin of the Pelotas River.

Seedlings of *Eucalyptus camaldulensis*, produced via cuttings, were put in 55 cm³ tubes filled with commercial substrate based on peat and pine bark. After 120 days, the seedlings were transferred to pots with five liters of capacity, filled with soil from the region. The pots remained in the shade house of the forest nursery for approximately five months. During the experiment, the plants were placed in a greenhouse, without any type of irrigation. The experimental design was completely randomized, with 10 repetitions (plants) per treatment, with the treatments being composed of different concentrations of sodium chloride - NaCl (0, 50, 100, 200 and 400 µM) diluted in distilled water.

The first application of the saline solution occurred on October 24, 2018, where 500 ml of the solution were applied per plant, in concentrations that varied according to the treatments. Subsequently, on October 30, a second application was performed, as the seedlings did not show any symptomatic variation initially. The only irrigation carried out during the experiment was with the saline solution, applied on the 7th day.

The gas exchange evaluations were performed with the aid of a portable photosynthesis meter, brand Liquor, model Li-6400xt, in five repetitions, where the following variables were measured, photosynthesis - A (µmol CO2 m^{-2} s^{-1}), stomatal conductance - gs (mol m^{-2} s^{-1}), transpiratory rate - E (mmol H2O m^{-2} s^{-1}), relation between intercellular and atmospheric concentration of CO^2 - Ci / Ca (µmol CO^2) and water use efficiency - WUE ([µmol m^{-2} s^{-1}] $^{-1}$). The photosynthetically active radiation used during the measurements was 900 µmol of photons m^{-2} s^{-1}. This value was determined by the luminous saturation curve measured for eucalyptus, between 800 and 1,000 µmol of photons m^{-2} s^{-1} (SILVA and KLAR, 2004). Photosynthetically active radiation (RFA) was provided by an artificial light source (LI-6400-40), with a blue light percentage of 10% of the total RFA.

The gas exchange evaluations were performed at 24 h (1 day) and 48 h (2 days), 7, 12 and 14 days after the first application of saline solution. For the evaluations, the same leaf from each plant was used throughout the experimental period. The readings were stable after 2-3 minutes. Water use efficiency (WUE) was calculated by dividing A by E.

The normality and homogeneity of the data variance were tested by the Kolmogorov-Smirnov and Bartlett tests, respectively. For the statistical analysis of the experiment, ANOVA analysis of variance (p <0.05) was used. When significant, the data were adjusted to the response surface graphs in the Statistica 6.0 software.

Results

The treatments differed for all physiological variables, with variations in adaptation and responses depending on the NaCl concentrations. When measuring the photosynthetic rate, a drop in photosynthesis is seen, mainly from the 7th day onwards when the second application of saline occurred (Figure 1). It is observed that, with the passage of days, under saline conditions, the photosynthetic rates were lower and lower, but even in high concentrations of NaCl, the plants have a capacity for tolerance to salinity.

The stomatal conductance (gs) of the plants was significantly reduced (Figure 2) by the salinity of the applied solution. There were linear decreases in gas due to the increase in the concentration of saline solution.

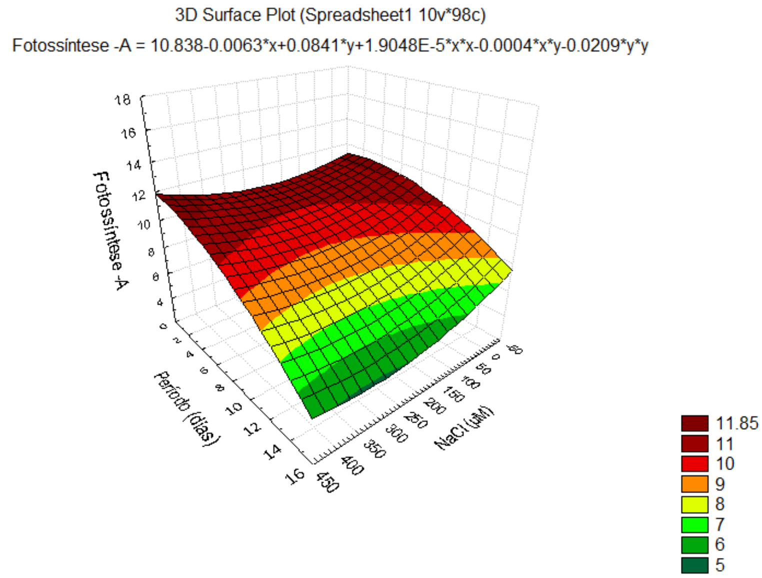

Figure 1 - Photosynthetic rate of *E. camaldulenis* seedlings as a function of application of different concentrations of saline solution (NaCl).
Figura 1 - Taxa fotossintética de mudas de *E. camaldulensis* em função da aplicação de diferentes concentrações de solução salina (NaCl).

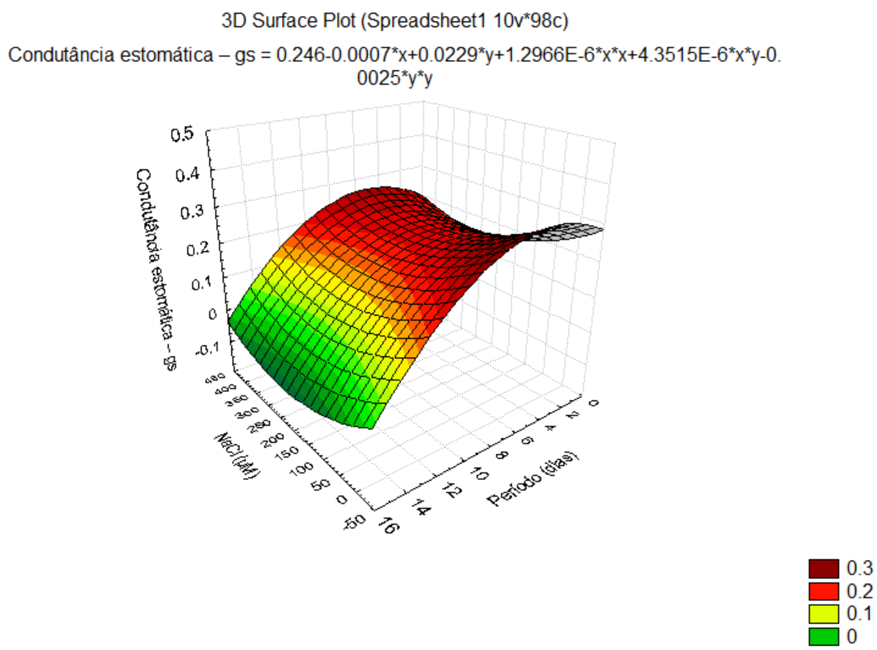

Figure 2 - Stomatic conductance (gs) of *E. camaldulensis* seedlings as a function of NaCl concentration variation.
Figura 2 - Condutância estomática (gs) de mudas de *E. camaldulensis* em função da variação da concentração de NaCl.

Transpiration (E) (Figure 3) had the same trend observed in stomatal conductance, with lower values after the first application, tending to decrease in the subsequent application.

Under saline conditions in the soil, the plants showed very efficient stomatal control since the lowest transpiratory rate was observed from the second application of the saline solution onwards.

During the evaluation period, there was a drop in the ci/ca ratio (Figure 4), due to stomatal closure, which in general ended up reducing the exchange of gases between the environment and the interior of the leaf.

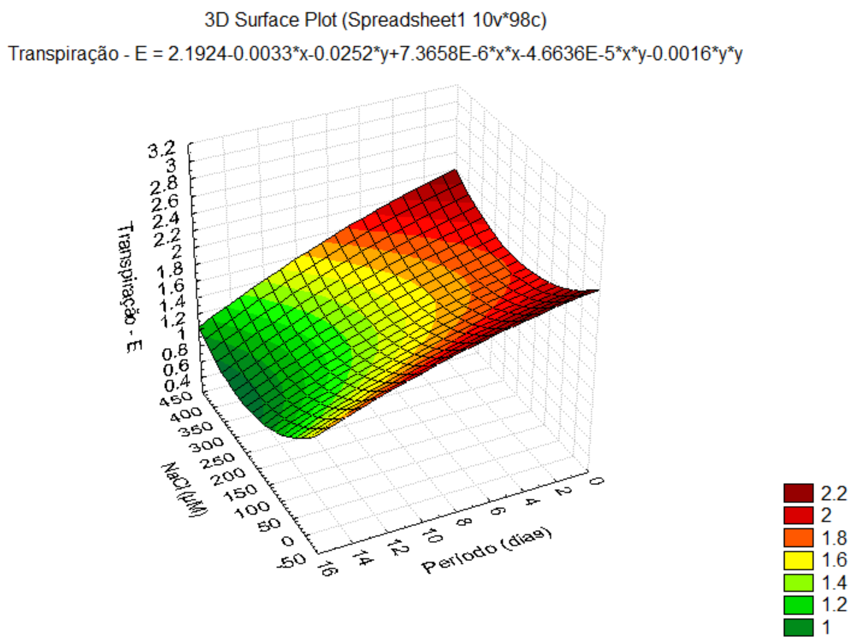

Figure 3 - Transpiration (E) of *E. camaldulensis* as a function of the different NaCl concentrations.
Figura 3 - Transpiração (E) de mudas de *E. camaldulensis* sob diferentes concentrações de NaCl.

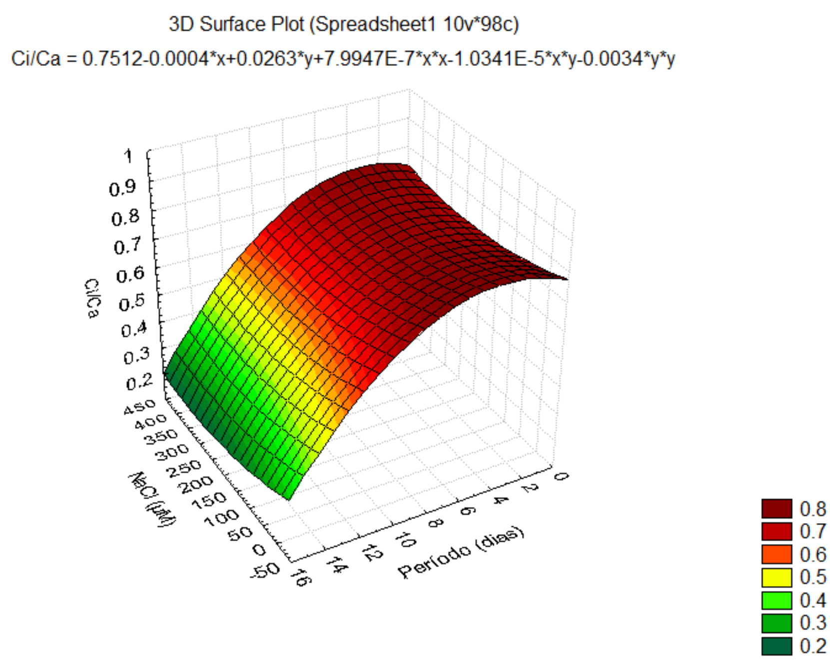

Figure 4 - Relationship between intercellular and atmospheric CO^2 (ci / ca) concentration in *Eucalyptus camaldulensis* as a function of days under salt stress and NaCl concentrations.
Figura 4 - Relação entre a concentração intercelular e atmosférica de CO^2 (ci/ca) em *Eucalyptus camaldulensis* em função dos dias sob estresse salino e concentrações de NaCl.

When analyzing the efficiency in the use of water (WUE) during the experimental period, it is noted that the plants subjected to the highest concentrations of salt (which reflects in water deficiency), had greater efficiency in the use of water (Figure 5). It was observed that the effects of different concentrations of saline solution on stomatal conductance were quite expressive, that is, when the seedlings received higher saline levels, there was better use of water.

In general, gas exchange was significantly affected after the second application of sodium chloride saline (NaCl). The water deficit caused by salinity was a limiting factor for stomatal opening, which contributed to the decrease in the assimilation of carbon by photosynthesis, because the higher the salinity, the lower the water availability, consequently, the values of A, gs and E will be smaller; water efficiency (WUE) increased when the saline concentrations were higher.

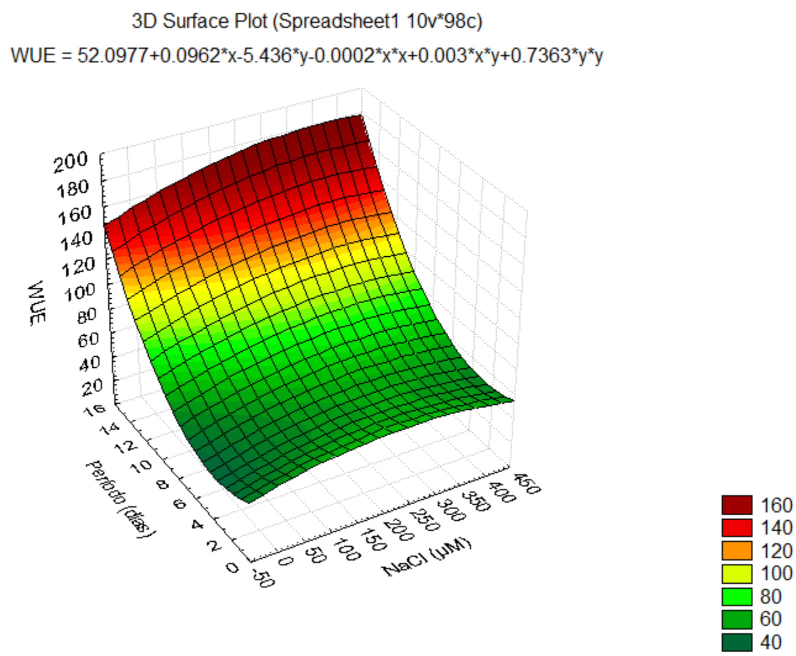

Figure 5 - Water use efficiency (WUE) in *Eucalyptus camaldulensis* under saline stress.
Figura 5- Eficiência no uso da água (WUE) em *Eucalyptus camaldulensis* sob estresse salino.

Discussion

The results of the present study demonstrate the potential for adaptation of the species *Eucalyptus camaldulensis* in places with greater salinity. The responses of plants to hydric stress, caused by salinity, included physiological changes such as stomatal closure, reduction in photosynthetic rates, changes in hormone levels and changes in the performance of genes. The photosynthetic rate (A) may or may not be related to stomata movement (SILVA and KLAR, 2004). The application of the saline solution shows that the increase in salinity levels reduced the stomatal conductance, which resulted in a decrease in the partial pressure of intercellular CO^2, negatively interfering in the assimilation of CO^2 by the photosynthetic apparatus (ANGELOCCI, 2002).

The plant response to water stress caused by salinity included physiological changes, such as stomate closure and reduction in photosynthetic rates. The decrease in stomatal conductance is directly related to the drop in the water potential of the leaves. Salinity reduces the photosynthetic rate per unit leaf area of most cultivated plants, due to the decrease in stomatal conductance (O'LARYARY, 1995). However, the reduction of stomatal opening can limit the rate of CO^2 diffusion into the leaf, with direct effects on photosynthesis and growth (EL - SHARKAWY, 2007).

The similarity between gs and E in the evaluations is expected, due to the closing of stomata, thus showing that with the passing of the day the perspiration ends up suffering a drop in its levels. This effect can cause osmotic stress, reducing the availability of water for individuals, consequently affecting gas exchange and plant growth.

The concentration of CO^2 is generally attributed to stomatal behavior, in which with the closure of stomata there is a greater resistance to the influx of CO^2 and, therefore, less concentration in the sub-stomatal chamber (PRAZERES et al., 2015). It is likely that efficiency in this case is linked to a lower rate of transpiration by plants.

The use of water comprises the processes of transpiration and evaporation of water in the soil, however, the WUE is sensitive to environmental conditions and changes (LACLAU et al., 2016). When evaluating *E. camaldulensis* plants under conditions of salt stress, it is important to differentiate the changes that occur during the individual's life and those that are the result of several cycles of natural or artificial selection of genes and, therefore, are part of the genetic structure of the species. In the first case, the changes occur as a result of temporary physiological changes, involving a series of metabolic processes in response to the presence of stress, giving rise to a process known as acclimatization. In the second, there are a series of definitive morphophysiological changes that lead to the evolution of the species, in a process called adaptation. Although the processes of acclimatization and adaptation involve changes in gene expression, only the adaptation mechanisms are transmitted from one generation to another (BOSCO, 2010).

Conclusions

Eucalyptus camaldulensis plants showed physiological resistance after application of sodium chloride saline (NaCl) to the substrate, especially in the first seven days, with low reduction of gas exchange.

Due to the physiological responses presented by the plants, such as the ability to tolerate salinity and the efficiency in the use of water, since with the higher the level of salinity, the better results were obtained, showing that there was better use of water, indicating satisfactory responses from *Eucalyptus camaldulensis* to high salinity conditions, indicating good adaptation of the species to soils with higher salinity.

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