

# Plant development, gas exchanges and pigments of *Mesosphaerum suaveolens* submitted to osmoconditioning and saline stress

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**Abstract** – Salinity is one of the main plant abiotic stresses affecting the establishment and development of crops. It is thus a matter of prime importance to search for technologies that minimize the damage caused by salinity. The aim of the present work was to evaluate the effect of salinity stress and osmotic conditioning of seeds on the biomass, gas exchanges and chlorophyll pigments in *Mesosphaerum suaveolens* (L.) Kuntze. The statistical design adopted was a randomized block design, combined according to the central composite design, referring to electrical conductivities of irrigation water and osmotic potentials, with minimum ( $-\alpha$ ) and maximum ( $\alpha$ ) values of 0.5 and 10.0 dS m<sup>-1</sup> and 0.0 and -1.0 MPa, respectively, totaling nine combinations. The characteristics of dry biomass, gas exchange and chlorophyll indices were evaluated at 45 days after the beginning of irrigation with saline water. The salinity of irrigation water severely affected the dry biomass and the gas exchanges of *M. suaveolens*. Irrigation water of electrical conductivity above 3.2 dS m<sup>-1</sup> caused reductions in chlorophyll *a*, *b* and total contents in *M. suaveolens* plants. Seed osmoconditioning did not attenuate the negative effects of saline stress on *M. suaveolens* plants.

**Keywords:** bamburral, chlorophyll, plant growth, photosynthesis, salinity

## Introduction

*Mesosphaerum suaveolens* (L.) Kuntze, also known as “bamburral” in the Brazilian Northeast, is a species belonging to the botanical family Lamiaceae, which presents subshrub growth habit and leaves with high aromatic potential (Sabóia et al. 2018). The species, besides being widely used by folk medicine in several regions of Brazil, is also rich in secondary compounds (alkaloids, flavonoids, tannins and others), which are used in the pharmaceutical and cosmetics industries (Alves et al. 2017, Bezerra et al. 2017).

The chemical quality of irrigation water is one of the factors that directly affect plants’ metabolic processes, especially in semi-arid regions, where water is scarce and, in many cases, saline (Guimarães et al. 2019). In the semi-arid region of Brazil, salinity is among the abiotic stresses that

most hinder the growth and production of crops (Sales et al. 2015).

Salinity can affect plants in two ways: by reducing the soil osmotic potential, thus reducing water and nutrient uptake, and by accumulating specific ions (Na<sup>+</sup> and Cl<sup>-</sup>), leading to toxicity and nutritional imbalance (Taiz et al. 2017). Osmotic and ionic effects on crops can cause changes in the plants’ physiological and biochemical functions (Ouhadach et al. 2018). Therefore, it is necessary to search for technologies that might attenuate these salinity stress negative effects on irrigated agricultural systems.

In this sense, seed osmoconditioning is a practice that has been adopted with the purpose of reducing the germination time, increasing the germinability, uniformity and

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vigor of the seedlings, which is advantageous in plants subjected to salt stress conditions (Cardoso et al. 2012). During physiological conditioning, seed hydration occurs slowly, which gives more time for the repair or reorganization of plasma membranes, allowing the formation of tissues in a more orderly manner, reducing the risk of damage to the embryonic axis (Windauer et al. 2007). Polyethylene glycol (PEG) has been widely used for the formulation of solutions with different osmotic potentials, simulating drought stress, which could induce secondary dormancy (Souza et al. 2011).

Thus, the aim of the present work was to evaluate the effect of salinity stress and osmotic conditioning of seeds on the biomass accumulation, gas exchanges and chlorophyll pigments in *M. suaveolens* plants.

## Materials and methods

The research was carried out in a greenhouse belonging to the Department of Plant Science and Environmental Sciences at the Federal University of Paraíba, located in the city of Areia, Paraíba, Brazil.

The statistical design adopted was a randomized block design, factorially combined according to the central composite matrix of Box, referring to electrical conductivities of irrigation water ( $EC_w$ ) and osmotic potentials, with minimum ( $-\alpha$ ) and maximum ( $\alpha$ ) values of 0.5 and 10.0  $dS\ m^{-1}$  and 0.0 and  $-1.0$  MPa, respectively, with four replicates consisting of two plants, totaling nine combinations (Mateus et al. 2001).

Saline water was prepared by adding sodium chloride (NaCl) to the water of the supply system ( $EC_w = 0.5\ dS\ m^{-1}$ ) in the proportions required. The salinity level of the waters was measured using the Instrutherm® (model CD – 860) micro-processed portable conductivity meter. Irrigation was performed daily, with the application of saline water 10 days after sowing. The irrigation volume applied was established through drainage lysimetry, from the difference between the amount applied and drained.

Osmoconditioning was performed by soaking seeds in PEG 6000 (Dinâmica®, Brazil) solutions. PEG 6000 was diluted in 200 mL of distilled water in the proportions required for each osmotic potential. The seeds were soaked for 8 hours at 25 °C, in containers covered with aluminum foil, being the amounts of PEG 6000 established according to Villela et al. (1991). Subsequently, the seeds were washed with distilled water.

The seeds used in the experiment were obtained from native plants found at the Novo Horizonte settlement, municipality of Várzea, Paraíba, Brazil. The seedlings were produced in 1.2 L capacity polyethylene bags, 10 seeds being sown per container. After seedling emergence, thinning was performed, keeping only the most vigorous plant in each container.

The polyethylene bags were filled with a substrate composed of soil (Latosol type), washed sand and tanned cattle manure in a 3:1:1 ratio. A substrate chemical analyse was

performed: pH = 7.8; organic matter ( $g\ kg^{-1}$ ) = 22.2; P ( $mg\ kg^{-3}$ ) = 85.3; K<sup>+</sup> ( $mg\ kg^{-3}$ ) = 693.6; Ca<sup>+2</sup> ( $cmol_c\ dm^{-3}$ ) = 2.9; Mg<sup>+2</sup> ( $cmol_c\ dm^{-3}$ ) = 1.59; Na<sup>+</sup> ( $cmol_c\ dm^{-3}$ ) = 0.23; H<sup>+</sup>+Al<sup>+3</sup> ( $cmol_c\ dm^{-3}$ ) = 0.0; Al<sup>+3</sup> ( $cmol_c\ dm^{-3}$ ) = 0.0; sum of bases ( $cmol_c\ dm^{-3}$ ) = 6.5; cation exchange capacity ( $cmol_c\ dm^{-3}$ ) = 6.5.

The evaluations were carried out at 45 days after the irrigation with saline water started. To determine root and shoot dry mass, these plant parts were packed in kraft paper bags and dried in a forced air circulation oven at 65 °C until reaching constant weight. Subsequently, the material was weighed, with results expressed in  $g\ plant^{-1}$ ; the total dry mass was obtained from the sum of the dry masses of the root and shoot, with the results in  $g\ plant^{-1}$ . Using the dry mass data, the shoot: root ratio was estimated (shoot dry mass/root dry mass).

The gas exchange evaluations were performed on the fourth leaf, from apex to base, between 9:00 and 10:00 a.m., using the portable infrared gas analyzer IRGA (model LI-6400XT, LI-COR®, Nebraska, USA) with 300  $mL\ min^{-1}$  air flow, 400  $\mu mol\ CO_2\ m^{-2}\ s^{-1}$  and a coupled light source of 1200  $\mu mol\ m^{-2}\ s^{-1}$ . The variables assessed were: net  $CO_2$  assimilation rate ( $A$ ) ( $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ ), stomatal conductance ( $g_s$ ) ( $mol\ H_2O\ m^{-2}\ s^{-1}$ ),  $CO_2$  concentration in intercellular spaces ( $C_i$ ) ( $\mu mol\ CO_2\ mol\ air^{-1}$ ), transpiration rate ( $E$ ) ( $mmol\ H_2O\ m^{-2}\ s^{-1}$ ) and foliar temperature (°C). From these variables, the water use efficiency ( $WUE: A/E$ ), intrinsic water use efficiency ( $iWUE: A/g_s$ ) and instantaneous carboxylation efficiency ( $iCE: A/C_i$ ) were calculated.

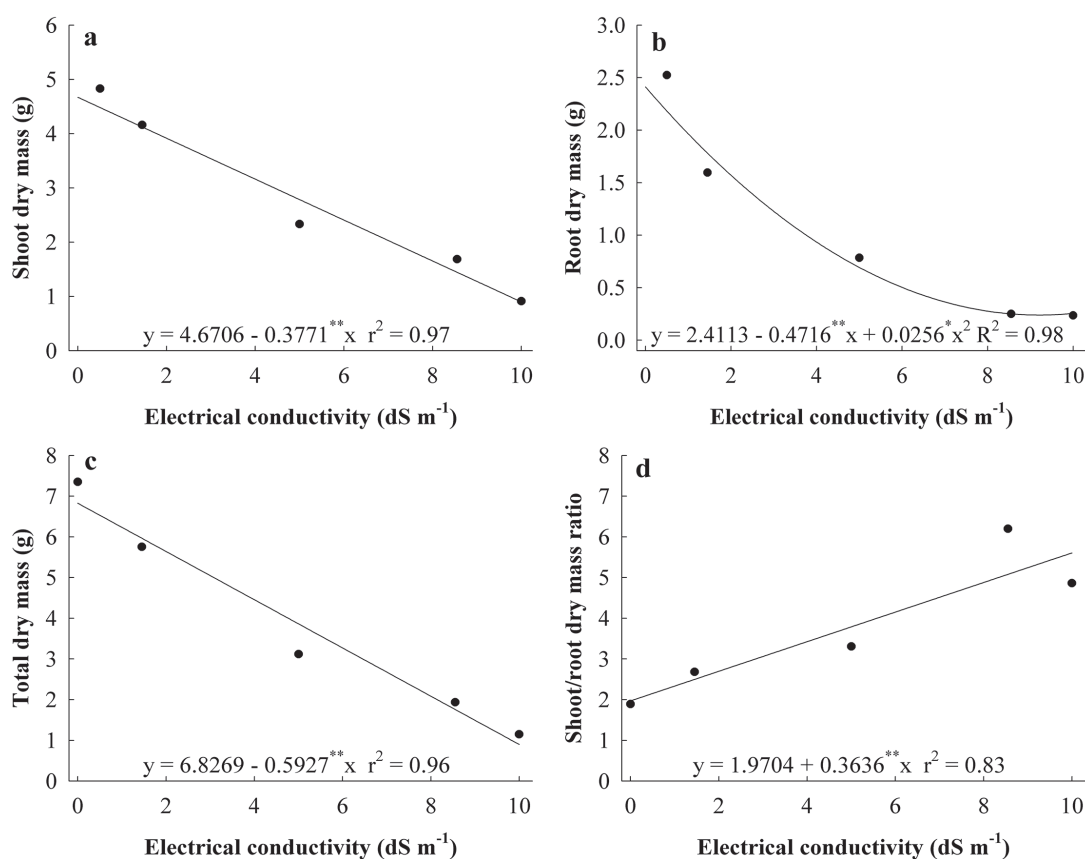
The contents of chlorophyll *a*, *b* and total, as well as the chlorophyll *a/b* ratio, were also determined on the fourth leaf, from apex to base, between 9:00 and 10:00 am, by a non-destructive method using a portable chlorophyllometer (ClorofiLOG®, model CFL 1030, Porto Alegre, RS). The values were expressed as the Falker chlorophyll index (FCI).

The data were submitted to analysis of variance by the F-test ( $P < 0.05$ ), and in the case of a significant effect, the data were submitted to regression analysis, using the statistical program SAS University (Cody 2015).

## Results

There was no significant interaction between the studied factors, seed osmoconditioning and salinity levels of irrigation water. For the shoot (SDM), root (RDM) and total (TDM) dry mass, a decreasing effect is observed, with reductions of 80.7%, 89.4% and 86.8%, respectively, at 10.00  $dS\ m^{-1}$  salinity level compared to the control treatment (Fig. 1a-c). However, the SDM/RDM ratio presented an inverse behavior to the above-mentioned variables, increasing linearly as a function of increases in salinity level of the irrigation water (Fig. 1d).

Regarding the gas exchange variables, a decreasing linear effect was observed for *A*. Plants irrigated with water at 10.00  $dS\ m^{-1}$  salinity level presented a reduction of 54.9% in *A* when compared to the control treatment (Fig. 2a). How-



**Fig. 1.** Shoot dry mass (a), root dry mass (b), total dry mass (c) and shoot/root dry mass ratio (d) of *Mesosphaerum suaveolens* subjected to electrical conductivities of irrigation water (0.50, 1.45, 5.00, 8.55 and 10.00 dS m<sup>-1</sup>).

ever, *C<sub>i</sub>* presented an increasing linear effect as a function of the increase in salinity level, with an increase of 11.3% (Fig. 2b). For *iCE* there was a 58.7% reduction in EC<sub>w</sub> at 10.0 dS m<sup>-1</sup> salinity level when compared to the control treatment (Fig. 2c). It was observed that increasing salinity levels in the irrigation water provided a linear reduction in the transpiration rate (*E*), decreasing by 33.8% at 10.0 dS m<sup>-1</sup> when compared to the control (Fig. 2d). The plants tended to use water less efficiently with increasing salinity levels, decreasing by 36.6% (Fig. 2e).

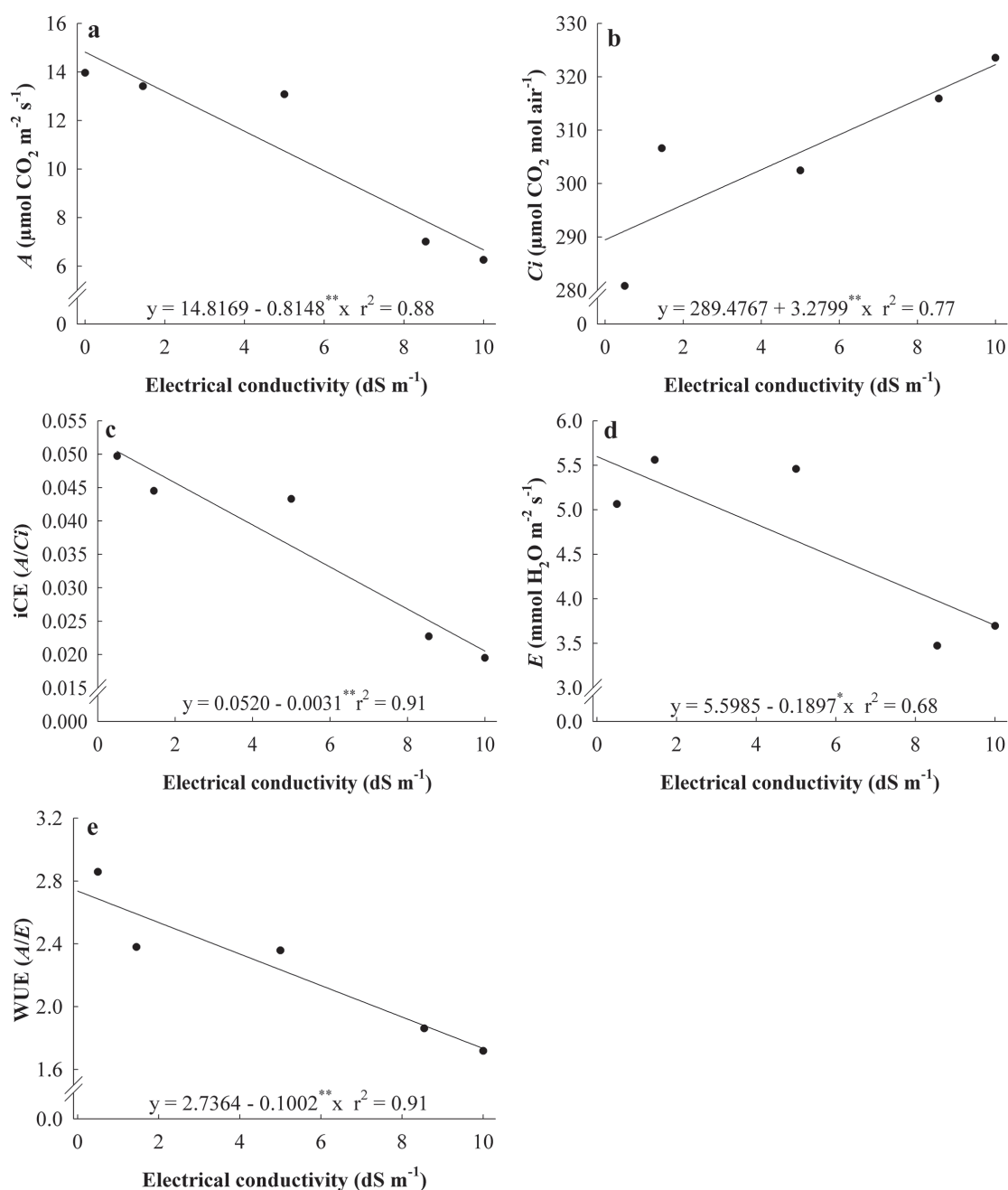
It can be observed that only from 3.2 dS m<sup>-1</sup> salinity level was there a reduction in the indexes of chlorophyll *a*, *b* and total, with decreases of 25.8%, 42.3% and 29.4%, respectively, at the highest salinity level when compared to the control treatment (Fig. 3A-C). Regarding the chlorophyll *a/b* ratio, the data show that there were increases in this variable from the EC<sub>w</sub> of 3.2 dS m<sup>-1</sup> (Fig. 3d).

Seed osmoconditioning with PEG did not provide significant differences for chlorophyll *a*. However, total chlorophyll and chlorophyll *b* were negatively affected by the increase in osmotic potential reduction of the solution, with decreases of 27.2 and 18.2%, respectively, when comparing the highest osmotic potential (-1.00 MPa) with the control (Fig. 4a,b). The reduction of the osmotic potential of the solution provided increases in the chlorophyll *a/b* ratio, with a 16.2% increase at the potential of -1.00 MPa (Fig. 4c).

## Discussion

The results for the dry mass may be related to the excessive absorption of ions such as Na<sup>+</sup> and Cl<sup>-</sup>, causing reductions in the accumulation of photoassimilates due to a lower photosynthetic rate. Also, there is an increase in energy expenditure by the plant due to the reduction of the osmotic potential, which makes it difficult to absorb water through the roots (Araújo et al. 2016). Similar results were verified by Bione et al. (2014) in salt-stressed basil plants (*Ocimum basilicum* L.) cultured in a hydroponic NFT system and Oliveira et al. (2016) in maize (*Zea mays* L.) submitted to saline stress and biostimulant treatment. These authors verified that dry mass production was reduced with the increase of salinity.

The reduction in *A* was possibly due to the damage caused by salts to the chloroplast, thus reducing CO<sub>2</sub> fixation by rubisco. High concentrations of Na<sup>+</sup> and Cl<sup>-</sup> cause toxicity at different levels and may alter several biochemical and physiological processes, among them the fixation of carbon dioxide during the photosynthesis process (Rouphael et al. 2012). The increase in *C<sub>i</sub>* can be directly related to the photosynthetic rate reduction, which results in a lower CO<sub>2</sub> consumption during the carboxylation process. Reductions in the photosynthetic rate lower the ATP and NADPH availability as well as the substrate for rubisco, which is associated with the reduction of intrinsic carboxylation effi-



**Fig. 2.** Net CO<sub>2</sub> assimilation rate – A (a), internal CO<sub>2</sub> concentration – Ci (b), instantaneous carboxylation efficiency – iCE (c), transpiration rate – E (d) and water use efficiency – WUE – A/E (e) of *Mesosphaerum suaveolens* subjected to electrical conductivities of irrigation water (0.50, 1.45, 5.00, 8.55 and 10.00 dS m<sup>-1</sup>).

ciency as a function of the increase in salinity (Silva et al. 2015). This decrease in *E* can usually be an adaptation mechanism in response to abiotic stresses, specifically saline and hydric, in order to reduce water loss.

Several authors have observed reductions in net CO<sub>2</sub> assimilation rate, transpiration rate and carboxylation efficiency as a function of saline stress, for example Huang et al. (2015) in *Boehmeria nivea* L., Yarami and Sepaskhah (2015) in *Crocus sativus* L., and Rouphael et al. (2016) in *Cucurbita pepo* L.

This reduction in chlorophyll indices may be related to several factors, such as: decreased chlorophyll *a* biosynthesis, increased chlorophyllase activity and instability of pro-

tein complexes caused by saline stress effects (Houimli et al. 2010). Melo et al. (2017), studying the effect of irrigation with saline water in bell pepper plants, obtained similar results, stating that chlorophyll *a* was the most sensitive variable to salinity. However, the increase in the chlorophyll *a/b* ratio, possibly, can be attributed to the greater reduction observed in chlorophyll *b* (42.3%) than in chlorophyll *a* (25.8%). Corroborating these results, Shimoda et al. (2012) report that the first step in the degradation of chlorophyll *b* is its conversion to chlorophyll *a*.

These decreases in total chlorophyll and chlorophyll *b* can reflect a biochemical adaptation of the plant in response to the osmotic conditioning and the action of oxidative deg-

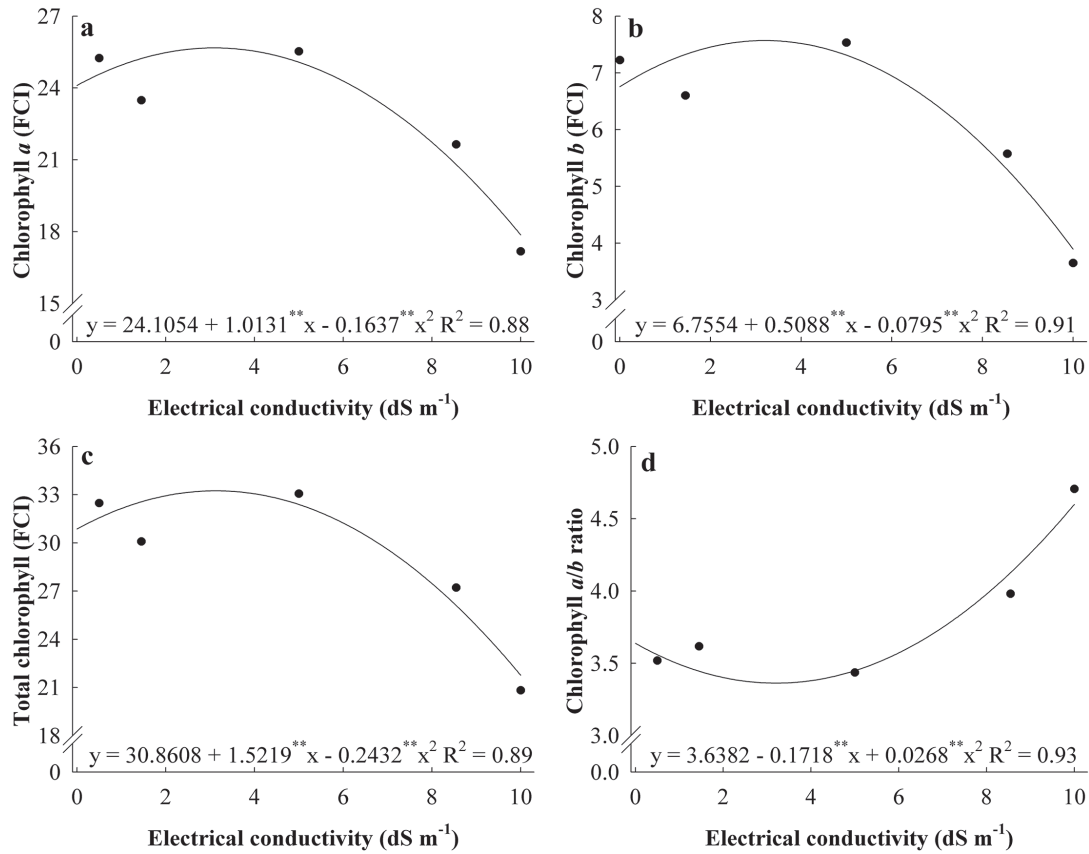


Fig 3. Chlorophyll *a* (a), chlorophyll *b* (b), total chlorophyll (c) and chlorophyll *a/b* ratio (d) of *Mesosphaerum suaveolens* subjected to electrical conductivities of irrigation water (0.50, 1.45, 5.00, 8.55 and 10.00 dS m<sup>-1</sup>).

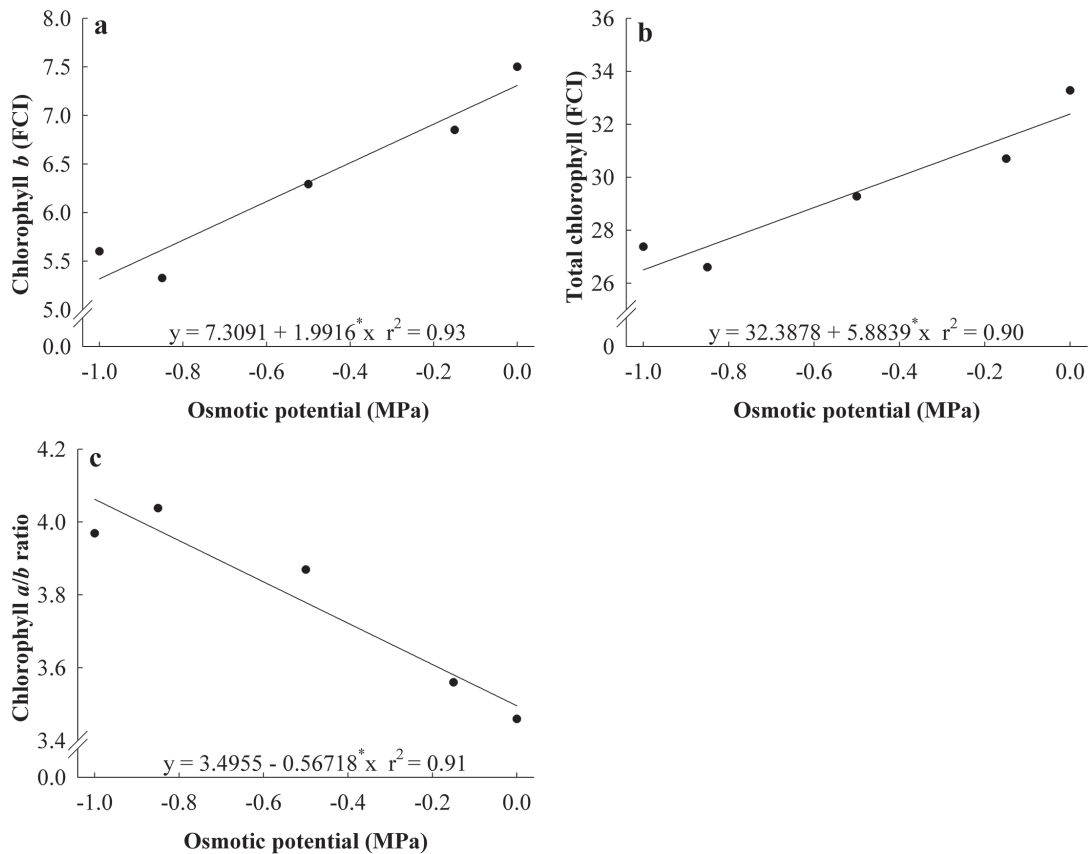


Fig. 4. Chlorophyll *b* (a), total chlorophyll (b) and chlorophyll *a/b* ratio (c) of *Mesosphaerum suaveolens* subjected to osmoconditioning with PEG (-1.00, -0.85, -0.50, -0.15 and 0.00 MPa).



radation agents (Cardoso et al. 2012). This increase in chlorophyll *a/b* ratio was possibly due to the reduction in chlorophyll *b* and unchanged chlorophyll *a* indices. However, in basil plants Santos et al. (2012) observed divergent results, where plants cultivated without water deficit showed a higher chlorophyll *a/b* ratio, attributing this behavior to the fact that chlorophyll *a* degradation by oxidative damage occurs faster when compared to chlorophyll *b*.

## Conclusions

Seed osmoconditioning did not attenuate the negative effects of saline stress on *M. suaveolens* plants. The salinity of irrigation water severely affected the dry biomass and the gas exchanges of *M. suaveolens*. Irrigation water with electrical conductivity above 3.2 dS m<sup>-1</sup> caused reductions in chlorophyll *a*, *b* and total contents in *M. suaveolens* plants.

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