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BIOLOGY

Ecophysiology of *Mesosphaerum suaveolens* (L.) Kuntze (*Lamiaceae*) under saline stress and salicylic acid

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ABSTRACT: *Mesosphaerum suaveolens (L.) Kuntze is a species widely used in folk medicine and has a high aromatic and therapeutic potential. However, its cultivation in semi-arid regions can be limited by salts in the irrigation water. The objective of this study was to evaluate the effect of salicylic acid (SA) as a mitigator of saline stress on the growth and gas exchange of M. suaveolens. The experimental design used was the randomized blocks in an incomplete factorial scheme (Central Composite Design), with five electrical conductivities water (ECw = 0.5, 1.45, 5.0, 8.55, and 10.0 dS m^{-1}) and five SA doses (0.0, 0.29, 1.0, 1.71, and 2.0 mM). Plant height, number of leaves, stem diameter, leaf area, root length, and height/diameter ratio, and gas exchange (stomatal conductance, net CO_2 assimilation rate, transpiration rate, instantaneous, and intrinsic water use efficiency, instantaneous carboxylation efficiency, and leaf temperature) were evaluated. The application of 0.9 mM SA attenuates the negative effect of saline stress on the number of leaves and leaf area of M. suaveolens. The application of SA stimulates the number of leaves, plant height, and root growth, but does not affect the gas exchange of M. suaveolens. ECw reduces the growth and gas exchange of M. suaveolens plants.*
Key words: *phytohormone, salinity, medicinal plant.*

Ecofisiologia de *Mesosphaerum suaveolens* (L.) Kuntze (*Lamiaceae*) sob estresse salino e ácido salicílico

RESUMO: *Mesosphaerum suaveolens (L.) Kuntze é uma espécie muito utilizada na medicina popular e que possui um elevado potencial aromático e terapêutico. No entanto, sua exploração em regiões semiáridas pode ser limitada em função do teor de sais na água de irrigação. O objetivo deste estudo foi avaliar o efeito do ácido salicílico (AS) como mitigador do estresse salino sobre o crescimento e trocas gasosas de M. suaveolens. O delineamento estatístico utilizado foi o de blocos casualizados, em esquema fatorial incompleto (Delineamento Composto Central), com cinco condutividades elétricas de água (CEa = 0,5, 1,45, 5,0, 8,55 e 10,0 dS m^{-1}) e cinco doses de AS (0,0, 0,29, 1,0, 1,71 e 2,0 mM). Foram avaliados a altura de plantas, número de folhas, diâmetro do caule, área foliar, comprimento de raiz e relação altura/diâmetro do caule e trocas gasosas (condutância estomática, taxa de assimilação líquida de CO_2, transpiração, eficiência instantânea e eficiência intrínseca no uso da água e de carboxilação e a temperatura foliar). A aplicação do AS na dose de 0,9 mM atenua o efeito do estresse salino sobre o número de folhas e área foliar. A aplicação de AS estimula o número de folhas e o crescimento em altura e da raiz, mas não afeta as trocas gasosas. A CEa reduz o crescimento e as trocas gasosas das plantas de M. suaveolens.*
Palavras-chave: *fitohôrmonio, salinidade, planta medicinal.*

INTRODUCTION

M. suaveolens (L.) Kuntze is a species belonging to the Lamiaceae family and commonly known as wild lavender, bamburral, erva de canudo and tapera velha. It is a species used in folk medicine in the Northeast region of Brazil, with medicinal and aromatic properties, their essential oil has substances that have antifungal, antibacterial, anticancer, antiseptic, insecticide and allelopathic activity (SILVA et al., 2017; ARRUDA et al., 2018; FIGUEIRÊDO et al., 2018).

The cultivation of *M. suaveolens* is affected by environmental conditions, such as saline stress caused by high levels of salts in the soil and in the water used for irrigation in arid and semi-arid regions, such as much of the Northeast of Brazil. A soil is considered salt affected when the electrical

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conductivity of the saturation extract (ECes) is greater than or equal to 4.0 dS m^{-1} and/or the percentage of exchangeable Na^+ greater than 15% (RICHARDS, 1954). Also according to the classification of Richards (1954), in relation to the quality of water for use in irrigation, water of classes C3 with electrical conductivity of 0.75-2.25 dS m^{-1} and C4 > 2.25 dS m^{-1}, present high to ver highrisk of salination for use in irrigation.

Excessive salts in soils and irrigation water, as is common in arid and semi-arid regions, can cause a series of disturbances in biochemical, physiological, morphological and molecular processes, compromising plant growth and development (JAHAN et al., 2020, LOFTI et al., 2020).

Strategies to mitigate the damage caused by saline stress are necessary. The use of phytohormones, such as salicylic acid (SA), is an alternative to mitigate the negative effects of saline stress on plants. SA is a phenolic compound that regulates several metabolic processes, such as the activation and expression of genes that act in the plant defense mechanisms and in the photosynthesis and of genes and proteins involved in homeostasis and in the production of osmoprotective substances, reducing the effect of salts excess (NAPOLEÃO et al., 2017, SHARMA et al., 2017, AHANGER et al., 2020).

Despite having great potential for exploration, little is known about the growth and physiology of *M. suaveolens* under salt stress, as well as the effect of the application of SA as a mitigation of the damage caused by salinity in this species. In this sense, the objective of present study was to evaluate the effect of SA as a salt stress mitigator on the growth and gas exchange of *M. suaveolens*.

MATERIALS AND METHODS

Experiment location

The experiment was carried out in a greenhouse of Departamento de Fitotecnia e Ciências Ambientais do Centro de Ciências Agrárias, Universidade Federal da Paraíba (CCA/UFPB), Campus II, Areia, Paraíba (6°58'1.45" S, 35°42'48.90" W, and 575 m). According to the Köppen classification, the predominant climate is is of the As' type, with dry and hot summer and winter rains, with precipitation of approximately 1.400 mm $year^{-1}$ (ALVAREZ et al., 2013). The temperature and relative air humidity were 28.4 °C and 54.8%, respectively, during the period of the experiment.

Preparation of salinities

The electrical conductivities of irrigation water (ECw) were prepared by adding sodium chloride (NaCl) in water from the supply system (ECw = 0.5 dS m^{-1}), up to the required electrical conductivities, the values being measured with a portable conductivimeter (Instrutherm®, model CD-860). Irrigations started 10 days after seedling emergence, being carried out daily and the amount of water applied determined by the drainage lysimetry method (ALVES et al., 2017). During the experiment the soil was kept at 80% of its field capacity.

Preparation of salicylic acid (SA)

Distilled water was used to prepare doses of SA. The seeds were placed in 200 mL of SA (priming) for 8 hours in plastic pot wrapped in aluminum foil and kept at room temperature and relative air humidity. After the priming, the seeds were washed with distilled water to remove excess acid.

Conditions and experimental design

The seeds were harvested from native plants of the Novo Horizonte settlement, municipality of Várzea, Paraíba, Brazil. The experiment was carried out in 1.2 dm^3 polyethylene bags. 10 seeds were sown per bag, thinning was done at 10 days after sowing, leaving only one plant per bag.

The substrate used was a mixture of soil (Latosol), washed sand and tanned bovine manure (3: 1: 1, v/v) (EMBRAPA, 2018). A chemical analysis of the substrate was performed: pH = 7.8; P = 85.5 mg kg^{-1}; K^+ = 693.6 mg kg^{-1}; Na^+ = 0.23 $cmol_c$ dm^{-3}; H^+Al^{+3} = 0.0 $cmol_c$ dm^{-3}; Ca^{+2} = 2.9 $cmol_c$ dm^{-3}; Mg^{+2} = 1.59 $cmol_c$ dm^{-3}; sum of bases = 6.5 $cmol_c$ dm^{-3}; cation exchange capacity = 6.5 $cmol_c$ dm^{-3}; organic matter = 22.2 g kg^{-1}, conductivity of the soil saturation extract = 2.0 dS m^{-1}.

The experimental design was in randomized blocks in an incomplete 5 × 5 factorial scheme (Central Composite Design), with nine treatments, four repetitions and four plants per repetition. The combination of five electrical conductivities of irrigation water and five doses of salicylic acid with minimum (-α) and maximum (α) values were 0.50 and 10.00 dS m^{-1} and 0.0 and 2.0 mM, respectively, totaling nine treatments (Table 1) (MATEUS et al., 2001).

Analyzed variables

The evaluations were carried out 45 days after the beginning of irrigation with saline water (DAI). Plant height, number of leaves, stem diameter, root length, height/diameter ratio were evaluated. The leaf area was calculated by measuring the width and

Table 1 - Combinations and factors used in the experiment. ECw – electrical conductivity of irrigation water; SA – salicylic acid doses. n = 4.

Treatments	------------------------Levels---------------------------		------------------------------Doses----------------------------	
	ECw	SA	ECw (dS m^{-1})	SA (mM)
1	-1	-1	1.45	0.29
2	-1	1	8.55	0.29
3	1	-1	1.45	1.71
4	1	1	8.55	1.71
5	- α	0	5.25	0.00
6	α	0	5.25	2.00
7	0	- α	10.00	1.00
8	0	α	0.50	1.00
9	0	0	5.25	1.00

length leaf using the formula: LA = 0.677871 x (L x W), where, LA = leaf area; L = leaf length; W = leaf width (RIBEIRO et al., 2020).

The gas exchanges were measured on the fourth leaf from the apex to the base, between 9 and 10 a.m. with the infrared gas analyzer - IRGA (model LI-6400xt, LI-COR®, Nebrasca, USA) with an air flow of 300 mL min^{-1} and relative air humidity between 50-60%, 400 µmol of CO_2 and a coupled light source of 1000 µmol m^{-2} s^{-1}. Stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), net CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-1}), intercellular CO_2 concentration (Ci - µmol CO_2 mol air^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), instantaneous water use efficiency (WUE = A/E), intrinsic water use efficiency (iWUE = A/gs), instantaneous carboxylation efficiency (iCE = A/Ci) and leaf temperature (TF) were evaluated.

Statistical analysis

The data were submitted to analysis of variance by the F test at 0.05 probability, applying polynomial regression analysis in cases of significance. The statistical program R (R CORE TEAM, 2020) was used.

RESULTS

The interaction between the factors, electrical conductivity of irrigation water (ECw) and doses of SA, was significant for the number of leaves and leaf area of *M. suaveolens* plants (Figure 1).

The number of leaves was higher (60 leaves) in plants of *M. suaveolens* submitted to the dose of 0.67 mM SA and irrigated with ECw of 0.5 dS m^{-1} (Figure 1A). The greatest increase in leaf area (14.77 cm^2) was observed in plants with seeds treated with 2.0 mM of SA and irrigated with ECw water of 0.5 dS m^{-1} (Figure 1B).

SA had an isolated effect on plant height, root length and stem height/diameter ratio (Figure 2). The highest plant height (28.1 cm) was observed at a dose of 0.9 mM, with increases of 36%, compared to the control (Figure 2A).

The root length was stimulated by the treatment of seeds with SA, with the maximum increase (30.4 cm) occurring in the plants submitted to the dose of 0.8 mM, with decreases as the SA concentration increased, with the lowest values (25.3 cm, equivalent to 16%) in the dose of 2.0 mM (Figure 2B). The highest height/diameter ratio (6.88) was observed in the dose of 0.9 mM of SA (Figure 2C).

Salinity drastically decreased the growth of *M. suaveolens* plants (Figure 3). The increase in ECw decreased the plant height, with a decrease of 33%, being about 10 cm when comparing plants under higher (10.00 dS m^{-1}) and lower salinity (0.50 dS m^{-1}) (Figure 3A).

The stem diameter was reduced with the increase in ECw with the highest values (4.25 mm) in the ECw of 0.5 dS m^{-1}, with decreases of 14% when compared to the largest ECw (Figure 3B). The root length was greater (31.8 cm) in the ECw of 0.5 dS m^{-1}, with a reduction of 7.9 cm (24%) in the ECw of 10 dS m^{-1} (Figure 3C.). The plant height was similar to the stem diameter, with the best results (6.93 cm) in the plants subjected to ECw of 0.5 dS m^{-1}, with

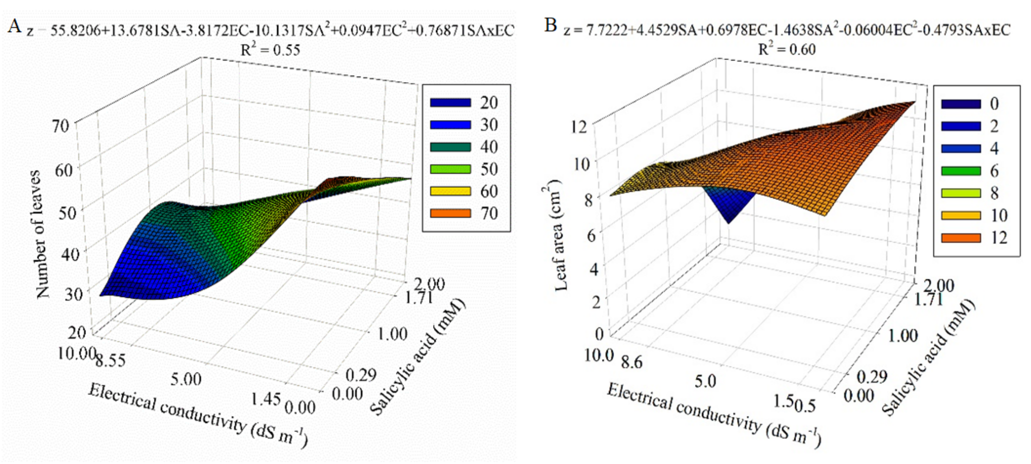

Figure 1 - Number of leaves (A) and leaf area (B) of *Mesosphaerum suaveolens* (L.) Kuntze plants submitted to seed treatment with salicylic acid and electrical conductivities of irrigation water. n = 4. P ≤ 0.05.

decreases as the ECw increased, being observed an decrease of 33.6% in the highest salinity (Figure 3D).

No significant effect was observed for the interaction between the ECw and the doses of SA, with a significant effect only of salinity on the physiology of *M. suaveolens* plants (Figure 4).

Stomatal conductance (gs) had a decreasing linear behavior, with the highest results (0.1363 mol H_2O m^{-2} s^{-1}) in plants irrigated with ECw of 0.5 dS m^{-1}, with severe reduction with increasing water salinity, with decreases 83% when comparing the values of the lowest and highest ECw (Figure 4A). The highest A (12.39 μmol CO_2 m^{-2} s^{-1}) was observed in the ECw of 0.5 dS m^{-1}, with severe reductions (77%) with the increase in ECw (Figure 4B). The highest internal CO_2 concentration - Ci (226.94 μmol CO_2 m^{-2} s^{-1}) was observed in plants submitted to ECw of 10 dS m^{-1}, different from the behavior observed in gs and A (Figure 4C).

The transpiration (E) was severely affected by ECw, with the highest rates (2.74 mmol H_2O m^{-2} s^{-1}) observed in the ECw of 0.5 dS m^{-1}, 74% higher than plants submitted to the highest ECw (Figure 4D). The intrinsic carboxylation efficiency (iCE) decreased by 83% when comparing the highest (10 dS m^{-1}) and lowest (0.5 dS m^{-1}) ECw (Figure 4E).

The greatest water use efficiency was observed in plants subjected to ECw of 0.5 dS m^{-1}, with decreases as the salinity increased, with losses of 33% (Figure 4F). On the other hand, the leaf temperature adjusted to the quadratic effect, with the maximum increase (31.7 ºC) observed in the ECw of 10 dS m^{-1} (Figure 4G).

DISCUSSION

The treatment of seeds with SA provided greater tolerance of plants to saline stress, promoting an increase on number of leaves and leaf area of *M. suaveolens* plants. SA regulates the production and accumulation of compatible solutes in the leaves, such as carbohydrates, phenolic compounds and proline and in the reduction of ions such as Na^+ and Cl^- (EL-ESAWI et al., 2017). Therefore, SA assists in the prevention of losses due to oxidative stress and in the maintenance of water content in plant tissues (MIMOUNI et al., 2016).

The plant height and root length of *M. suaveolens* plants were increased by SA, due to the role of this phytohormone in the expansion and elongation, in the regulation of the cycle and in the cell wall composition (NAPOLEÃO et al., 2017). In addition to promoting an increase in the production of osmoprotective compounds, such as proline, glycine betaine and sugars, enabling greater resistance to abiotic stress conditions, improving growth even under adverse conditions (AHANGER et al., 2020). The beneficial effect of SA on plants under stress conditions is reported by LORÍA & LARQUÉ-

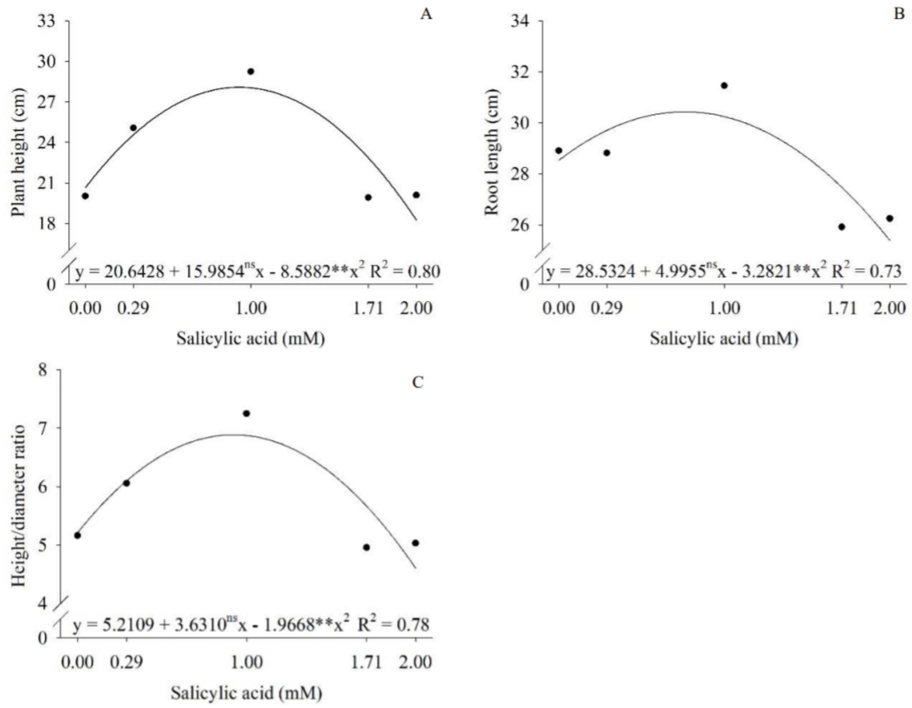

Figure 2 - Plant height (A), root length (B), height/diameter (C) ratio of *Mesosphaerum suaveolens* (L.) Kuntze plants submitted to seed treatment with salicylic acid. n = 4. ns = $P \geq 0.05$, ** = $P \leq 0.01$.

SAAVEDRA (2012) in Maya nut (*Brosimum alicastrum* Sw.), NAZAR et al. (2015) in mustard (*Brassica juncea* L.), NÓBREGA et al. (2018) in melon (*Cuccumis melo* L.) and SILVA et al. (2018a) on basil (*Ocimum basilicum* L.).

Salinity promoted damage to plant growth due to reduced water availability caused by increased soil osmotic stress and ion phytotoxicity, resulting in changes in physiological and biochemical processes, compromising plant growth (BEZERRA et al., 2018). The reduction in plant height, stem diameter and root length was due to damage caused by salinity in cell expansion and elongation. In this way, the plant reduces its vegetative development as an adaptive mechanism to saline stress to maintain minimum conditions for the execution of its vital processes (LIMA et al., 2018). Growth reduction by salinity in other species of the *Lamiacea* family was observed in *Plectranthus amboinicus* (Lour) Spreng (MESQUITA et al., 2014), *Rosmarinus officinalis* L. (EL-ESAWI et al., 2017), *Mentha piperita* L. (VERAS et al., 2017), *Ocimum basilicum* L. (CHOKAMI et al., 2019) and *Lavandula angustifolia* Mill (SZEKELY-VARGA et al., 2020).

Gas exchanges were severely affected by salt stress, with gs reduced by the increase in ECw, due to the high content of salts limiting water availability for the plant. Thus, the plant keeps the stomata closed to reduce water loss through the transpiration process as a defense mechanism (LOFTI et al., 2020). The transpiration of *M. suaveolens* plants was reduced through a mechanism developed from the stomata closure to prevent excessive water loss, stimulated by the high concentration of salts in the root zone (SILVA et al., 2018b). The decrease in stomatal conductance altered the CO_2 inflow and decreased the CO_2 assimilation rate, as observed *Salvia nemorosa* L. (SHARIFI & BIDABADI 2020), *Psidium guajava* L. (BEZERRA et al., 2018) and *Brassica juncea* L. (JAHAN et al., 2020).

The increase in Ci occurred due to the low gs presented in plants subjected to salinity, which is a mechanism developed by the plant to maintain the high concentration of internal carbon, as observed by

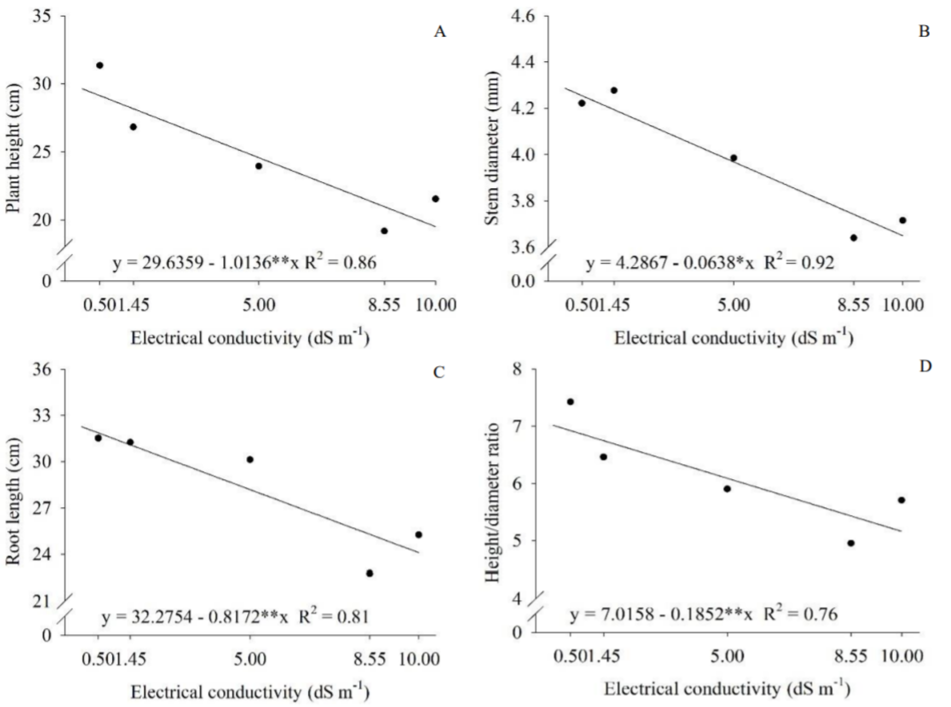

Figure 3 - Plant height (A), stem diameter (B), root length (C) and height/diameter ratio (D) of *Mesosphaerum suaveolens* (L.) Kuntze plants submitted to different electrical conductivities of irrigation water. n = 4. ns = P 0.05, * = P ≤ 0.05, ** = P ≤ 0.01.

NASCIMENTO et al. (2019) in *Hevea brasiliensis* Willd. Ex A. Juss. Müll. Arg, under water deficit.

Despite the increase in Ci with the elevation of ECw, carbon was not readily available during the carboxylation phase, as indicated by the reduction of iCE, where the efficiency of the photosynthetic apparatus, the carbon may remain within the substomatic cavity available for photosynthesis, but it cannot be synthesized during the carboxylation phase (MORAIS et al., 2018). As a consequence, there is a low activity and energy supply in the forms of ATP and NADPH for Rubisco, associated with the low availability of CO_2, resulting in low efficiency in carboxylation and limiting the photosynthetic process (SILVA et al., 2015).

Through the water use efficiency (WUE), it is possible to observe that the plants had an osmotic adjustment, where low water consumption (WUE) can be a mechanism developed by the plant to decrease the effect of saline stress, since the reduction of water consumption decreases the absorption of specific ions, which may be related to the exclusion of toxic ions by the roots (TAIZ et al., 2017).

The reduction in stomatal opening and transpiration provided an increase in leaf temperature due to the reduction in gas exchange between the plant and the environment. Such characteristics associated with low water availability due to decreased osmotic potential result in an increase in temperature, through stomatal closure, resulting in losses in photosynthetic rates promoted by heat stress (FIGUEIREDO et al., 2019).

CONCLUSION

The application of 0.9 mM SA attenuates the negative effect of saline stress on the number of leaves and leaf area of *M. suaveolens*. The application of SA stimulates the number of leaves, plant height and root growth, but does not affect the gas exchange of *M. suaveolens*. Salinity reduces the growth and gas exchange of *M. suaveolens* plants.

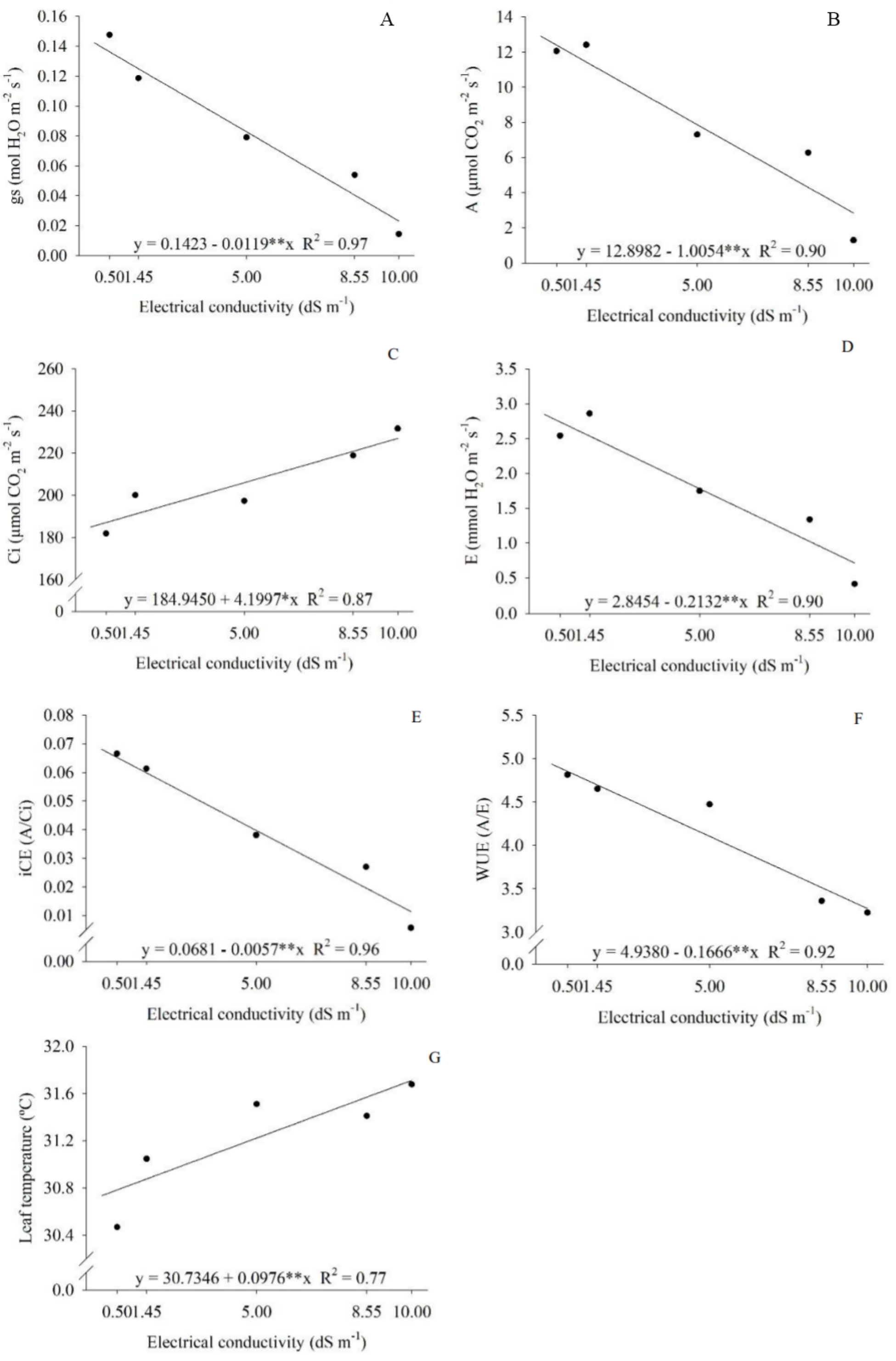

Figure 4 - Stomatal conductance - gs (A), CO2 assimilation rate - A (B), intercellular CO2 concentration - Ci (C), transpiration rate - E (D), intrinsic carboxylation efficiency - iCE (E), water use efficiency - WUE (F) and leaf temperature (G) of *Mesosphaerum suaveolens* (L.) Kuntze plants submitted to different electrical conductivities of irrigation water. n = 4. ns = $P \geq 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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