

Echophysiological aspects of *Ocimum basilicum* under saline stress and salicylic acid

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ABSTRACT: Basil (*Ocimum basilicum* L.) is one of the world's most widely cultivated medicinal herbs, but its physiological processes are affected by salinity. Therefore, mitigation strategies against the harmful effects of salt on plants have been increasingly on demand, including the use of salicylic acid. Thus, the effect of salicylic acid on the ecophysiology of basil plants grown under saline stress was evaluated in two different periods. The experimental design was the randomized complete block design using the Box Central Composite matrix with five irrigation water electrical conductivities (0.5, 1.3, 3.25, 5.2, 6.0 and dS m⁻¹) and five doses of salicylic acid (0.0, 0.29, 1.0, 1.71 and 2.0 mM), with five replicates. Gaseous exchanges, fluorescence and chlorophyll content were evaluated at 30 and 60 days after irrigation with saline water started. Data were evaluated by analysis of variance and regression and by correlation analysis. The water electrical conductivity negatively affected the analyzed variables, while the salicylic acid had a positive effect. The highest correlation between the variables was obtained at 30 days after the irrigation with saline water started. Salicylic acid treatment up to the dose of 1.0 mM has a beneficial effect on basil plants.

Key words: basil; gas exchange; photosynthesis; salinity

Aspectos ecofisiológicos de *Ocimum basilicum* sob estresse salino e ácido salicílico

RESUMO: Manjeriçao (*Ocimum basilicum* L.) é uma das ervas condimentares e medicinais mais cultivadas no mundo, porém, seus processos fisiológicos podem ser afetados pela salinidade. Contudo, é uma demanda crescente a busca por estratégias que mitiguem os efeitos nocivos dos sais às plantas, e dentre estas, está o ácido salicílico. Com isso, avaliou-se o efeito mitigador do ácido salicílico na ecofisiologia do manjeriçao submetido ao estresse salino em dois períodos de avaliação. O delineamento utilizado de blocos casualizados, utilizando a matriz composto central de Box com cinco condutividades elétricas da água de irrigação (0,5; 1,3; 3,25; 5,2; 6,0 e dS m⁻¹) e cinco doses de ácido salicílico (0,0; 0,29; 1,0; 1,71 e 2,0 mM), com cinco repetições. Foram avaliadas as trocas gasosas, fluorescências e índices de clorofilas aos 30 e 60 dias após a irrigação com água salina. Os dados foram avaliados por análise de variância e de regressão e por análise de correlação. A condutividade elétrica da água afetou negativamente, enquanto o ácido salicílico afetou positivamente as variáveis analisadas. A maior correlação entre as variáveis foi obtida 30 dias após o início da aplicação das águas salinas. O ácido salicílico possui efeito benéfico em plantas de manjeriçao até a dose de 1,0 mM.

Palavras-chave: manjeriçao; trocas gasosas; fotossíntese; salinidade

Introduction

Basil (*Ocimum basilicum* L.) is an aromatic and medicinal plant cultivated commercially around the world (Jakovljević et al., 2017). The basil essential oil, which is extracted mainly from the leaves, is rich in linalool, substance widely used in the pharmaceutical, chemical, cosmetic and flavoring industries. Basil is cultivated in several regions where salinity stress is common (Tarchoune et al., 2012).

Salinity is one of the major factors limiting plant growth and development. It is estimated that more than 900 million hectares of the world's arable land are affected by salinity (Mancarella et al., 2016). Salinity is caused mostly by weathering processes on rocks, irrigation with high soluble ions concentration water, and fertilizer applications. The latter two are of greater concern in modern agriculture.

Salinity affects many crops, especially those grown in water scarce regions, as in the northeastern region of Brazil. Saline stress can affect plants in many ways, often limiting their growth and yield, as well as damaging photosynthetic processes, mineral composition and nutrient's absorption, leading up to imbalanced nutrition or ionic toxicity (Bekhradi et al., 2015).

Taking into account the harmful implications of soil salinity to plants, there has been a great interest in developing strategies to minimize the damage caused by this stress condition. The use of salicylic acid, among others acids, is one of those strategies. Salicylic acid plays an important role in abiotic stress tolerance, increasing tolerance to oxidative stress and minimizing osmotic stress. It also influences fruit productivity, gas exchange, water composition and resistance development to plant diseases. However, the salicylic acid effect will depend on genetic and environmental factors, as well as the application method and dosage (El-Esawi et al., 2017).

Several studies have investigated the salicylic acid effect on salt stressed basil plants (Parizi et al., 2011; Shekoofeh et al., 2012; Mohammadzadeh et al., 2013; Angooti & Nourafcan, 2015). However, information using confidence bands are scarce. The aim of this study was to evaluate the salicylic acid effect on the physiology of basil (*Ocimum basilicum* L.

cv. Cinnamon) submitted to salinity stress, in two different evaluation periods, using confidence bands.

Material and Methods

The experiment was carried out in a greenhouse at the Agricultural Sciences Center of the Federal University of Paraíba, located in the city of Areia, Paraíba, Brazil (6° 51' 47" S, 35° 34' 13" W; 575 m). The soil used was classified as Planosol (Embrapa, 2014), sandy-loam texture, with the following physical characteristics: sand (g kg⁻¹): 756.9; silt (g kg⁻¹): 59.1; clay (g kg⁻¹): 184.0; bulk density (kg dm⁻³): 1.38; particle density (kg dm⁻³): 2.67; total porosity (%): 48; field capacity (g kg⁻¹): 78; permanent wilting point (g kg⁻¹): 43.

The basil seeds (*Ocimum basilicum* L. cv. Cinnamon) were provided by the Federal University of Sergipe. Basil seeds were sown in polyethylene trays containing 162 cells with commercial organic compound as substrate (1:1 v/v). Twenty-five days after sowing (DAS) the seedlings were transplanted to soil containing pots, previously moistened to their field capacity. The experimental units were composed of polyethylene pots with volume capacity of 5 dm³ filled with soil and 100 g of poultry manure. A sample of the soil and manure mixture was collected for fertility analysis. Table 1 presents some of the sample characteristics.

Irrigation was performed by drainage lysimeter as needed. The water with the desired electrical conductivities (EC_w) were prepared weekly. The following salts: NaCl, CaCl₂·2H₂O and MgCl₂·6H₂O (7: 2: 1, respectively) were added on water (0,5 dS m⁻¹) and stored in buckets with a capacity of 60 dm³ (Medeiros, 1992). Salicylic acid (SA) solutions were prepared using distilled water and 0.05% of Tween 80, a nonionic surfactant for better absorption effect. The control treatment was prepared with distilled water and Tween 80. The basil plants were sprayed weekly with the above described solutions until they were completely wet, during twenty-one days. The water salinity analysis is presented below (Table 2).

The evaluations were performed at 30 and 60 days after the irrigation with saline water started (DAI). The gas exchanges were measured with an open-system infrared gas analyzer (IRGA: LI-6400xt, LI-COR®, Nebraska, USA) during the

Table 1. Chemical analysis of the substrate.

pH	P	K ⁺	Na ⁺	H ⁺ + Al ³⁺	Al ³⁺	Ca ⁺²	Mg ⁺²	SB	CEC	V	OM
	(mg dm ⁻³)			(cmol _c dm ⁻³)				(%)	(g dm ⁻³)		
6.9	11.71	87.43	0.24	1.60	0.00	4.65	0.39	7.52	9.12	82.45	22.73

Table 2. Chemical analysis of salt water used for irrigation.

pH	EC _w ^a	SO ₄ ⁻²	K ⁺	Na ⁺	Ca ⁺² +Mg ⁺²	Ca ⁺²	Mg ⁺²	CO ₃ ⁻²	HCO ₃ ⁻	Cl ⁻	SAR ^b	ESP ^c	Classification	
	(dS m ⁻¹)	(mg L ⁻¹)	(mmol _c dm ⁻³)											
7.7	0.50	3.13	0.26	2.28	2.01	1.08	0.93	0.00	2.67	4.17	2.27	2.05	Normal	C ₂ S ₁
7.6	1.30	3.66	0.19	9.37	1.83	0.98	0.85	0.00	2.50	12.50	9.79	11.65	Saline	C ₃ S ₃
7.9	3.25	4.22	0.22	25.44	1.93	0.86	1.07	0.00	2.50	32.83	25.90	26.98	S. sodic	C ₄ S ₄
6.3	5.20	4.26	0.20	40.62	1.99	0.84	1.15	0.00	2.83	54.00	40.72	37.03	S. sodic	C ₄ S ₄
7.8	6.00	4.60	0.20	49.56	2.00	0.90	1.10	0.00	2.67	63.83	49.56	41.81	S. sodic	C ₄ S ₄

^aEC_w = Electrical conductivities of irrigation water; ^bSAR = Sodium adsorption ratio; ^cESP = Exchangeable sodium percentage.

morning, between 9 and 10 o'clock. The variables measured were: net photosynthesis rate (A) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (gs) ($\text{mol m}^{-2} \text{ s}^{-1}$), internal carbon dioxide concentration (Ci) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) transpiration rate (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), vapor pressure deficit (VPD), water use efficiency (WUE = A/E), instantaneous water use efficiency (iWUE = A/gs) intrinsic carboxylation efficiency (EiC = A/Ci).

Chlorophyll fluorescence measurements were obtained by a modulated fluorometer (Sciences Inc. - Model OS-30p, Hudson, USA). Foliar tweezers were placed and after a period of 30 minutes of dark adaptation the fluorescence parameters were measured. The initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence ($F_v = F_m - F_0$) and maximum quantum yield of photosystem II (F_v/F_m) were measured. Chlorophyll a, chlorophyll b and total chlorophyll levels were determined by a non-destructive method using a portable Chlorophyll Meter (ClorofiLOG®, model CFL 1030, Porto Alegre, RS) and expressed as the Falker chlorophyll index (FCI).

A randomized complete block design was used with five irrigation water electrical conductivities (ECw - 0.5, 1.3, 3.25, 5.2 and 6.0 dS m^{-1}) and five doses of salicylic acid (SA - 0, 0.29, 1.0, 1.71 and 2.0 mM), with five replicates. The incomplete factorial scheme was used, generated nine combinations through of the Box Central Composite matrix (Table 3).

Irrigation was performed by drainage lisimetry as needed. For this, the pots with the plants were placed in other pots and water was added until the water began to drain. The drained water was quantified and then the difference between the amount of applied water and the drained was calculated and, thus, the amount of water to be applied was obtained. For this, four plants submitted to 0.5 dS m^{-1} and 0.0 mM of SA were used as standards. Statistical analyzes were performed with their confidence interval to compare the two evaluation dates and correlation analysis between the variables using the statistical program R (R Core Team, 2017).

Table 3. Combinations (treatments) generated through the Box Central Composite matrix.

Levels		Doses	
ECw	SA	ECw	SA
-1	-1	1.30	0.29
-1	1	1.30	1.71
1	-1	5.20	0.29
1	1	5.20	1.71
$-\alpha$	0	0.50	1.00
α	0	6.00	1.00
0	α	3.25	2.00
0	$-\alpha$	3.25	0.00
0	0	3.25	1.00

ECw = Electrical conductivities of irrigation water; SA = salicylic acid.

Results and Discussion

The irrigation water electric conductivity (ECw) affected the net photosynthesis (A), stomatal conductance (gs), internal carbon dioxide concentration (Ci) and the transpiration rate (E) of basil plants at 30 and 60 days after the irrigation with

saline water started (DAI) (Figure 1). It was observed that the net photosynthesis (A) showed significant difference up to the ECw of 5.2 dS m^{-1} . The mean values obtained when ECw was superior to 5.2 dS m^{-1} , were very close in both evaluation dates, 30 and 60 DAI. This observation might be a response to the high salts concentrations in the plants root zone.

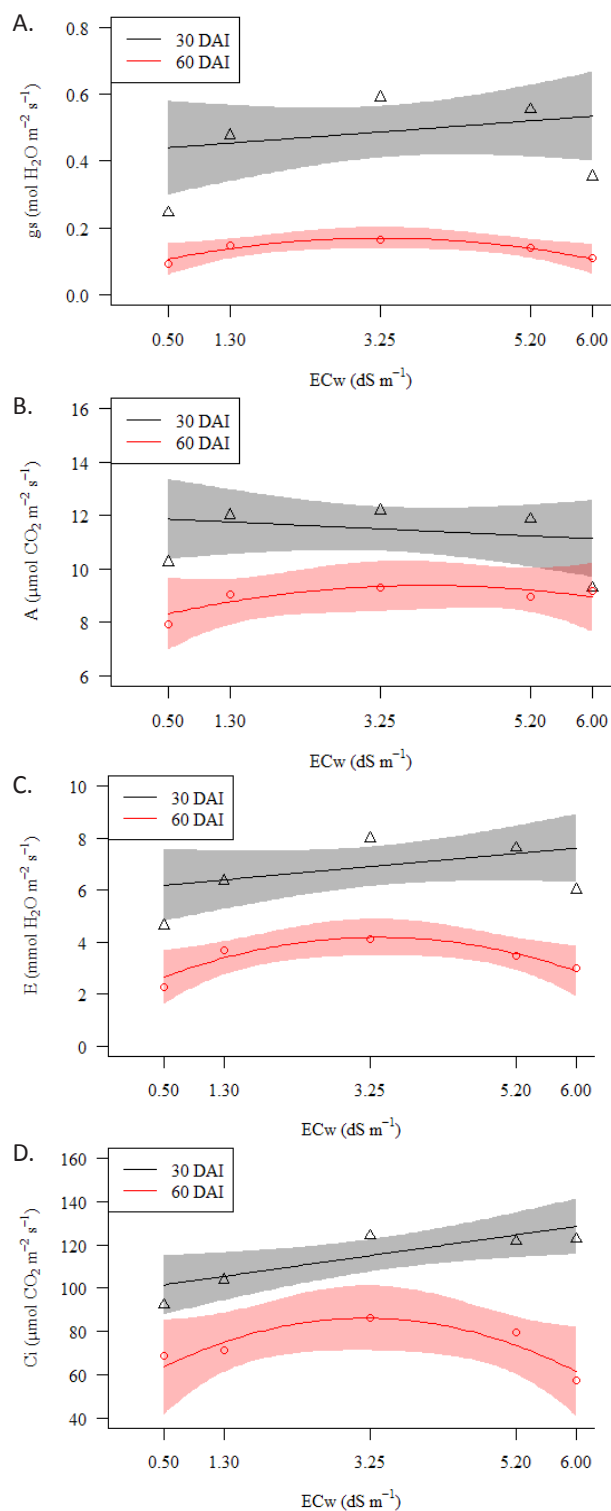


Figure 1. Stomatal conductance (gs) (A), net photosynthesis (A) (B), transpiration (E) (C) and internal carbon concentration (Ci) (D) of basil plants submitted to saline stress (ECw) at 30 and 60 days after the beginning of irrigation with saline water (DAI).

The stomatal conductance (g_s), internal carbon dioxide concentration (C_i) and transpiration rate (E) presented differences for all the ECw values in both evaluation periods. In both evaluation periods, the mean values of the above mentioned variables increased up to the ECw of 3.25 dS m^{-1} , and then started to drop. The increase in salinity reduces stomatal conductance and intercellular CO_2 pressure, affecting CO_2 assimilation. Plants under saline stress close their stomata as an acclimation response mechanism, which causes reduction in the leaf transpiration, attenuating the salts toxic effect (Bosco et al., 2009). Regarding the salicylic acid treatment, differences were also observed, as presented in Figure 2.

For net photosynthesis, it was observed that the SA molarities values of 0.0 and 2.0 mM showed no significant differences during both evaluation periods. However, there were significant differences for the intermediate SA molarities (0.29, 1.0 and 1.71 mM) (Figure 2). The same pattern was observed for the internal carbon dioxide concentration (C_i) values. The stomatal conductance (g_s) presented differences for all the SA molarities tested. The transpiration rate (E) also presented differences for all the SA molarities tested, in both evaluated periods. Salinity immediately affects plants stomatal conductance and consequently causes disturbances in water relations and decrease in abscisic acid (ABA) synthesis. Salt stressed plants also have a decrease in their photosynthetic rate (Munns & Tester, 2008).

The vapor pressure deficit (VPD), water use efficiency (WUE), and instantaneous water use efficiency (iWUE) showed differences in both assessment periods. However, the intrinsic carboxylation efficiency (EiC) did not present any differences, as can be observed in Figure 3.

The VPD and iWUE presented differences in all ECw tested while WUE did not show any difference only for the ECw of 3.25 dS m^{-1} . It was observed that the biggest variation occurred at 60 DAI, and the mean values at 30 DAI remained without large oscillations. Regarding the SA treatment, differences in variables followed similar behavior to that occurred for ECw (Figure 4).

No differences were observed for WUE and iWUE when the basil plants were treated with 0.0 and 2.0 mM of salicylic acid. However, differences were observed for intermediate molarities. Regarding the EiC, no differences were observed for any salicylic acid molarities tested, while VPD presented differences for all salicylic acid molarities tested. At 60 DAI, it was noted that VPD, WUE and iWUE values increased up to the salicylic acid molarity of 1 mM. From 1 mM of salicylic acid on, its mean values started to decrease. This behavior might be related to the fact that salicylic acid concentrations higher than 1.0 mM can be toxic to basil plants. The exogenous application of SA can increase A, C_i , WUE, g_s and E in some species such as *Brassica juncea*, *Zea mays* and *Glycine max* (Hayat et al., 2010).

Basil plants submitted to different ECw had its initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum quantum yield of PSII (F_v/F_m) influenced, at 30 and 60 DAI (Figure 5).

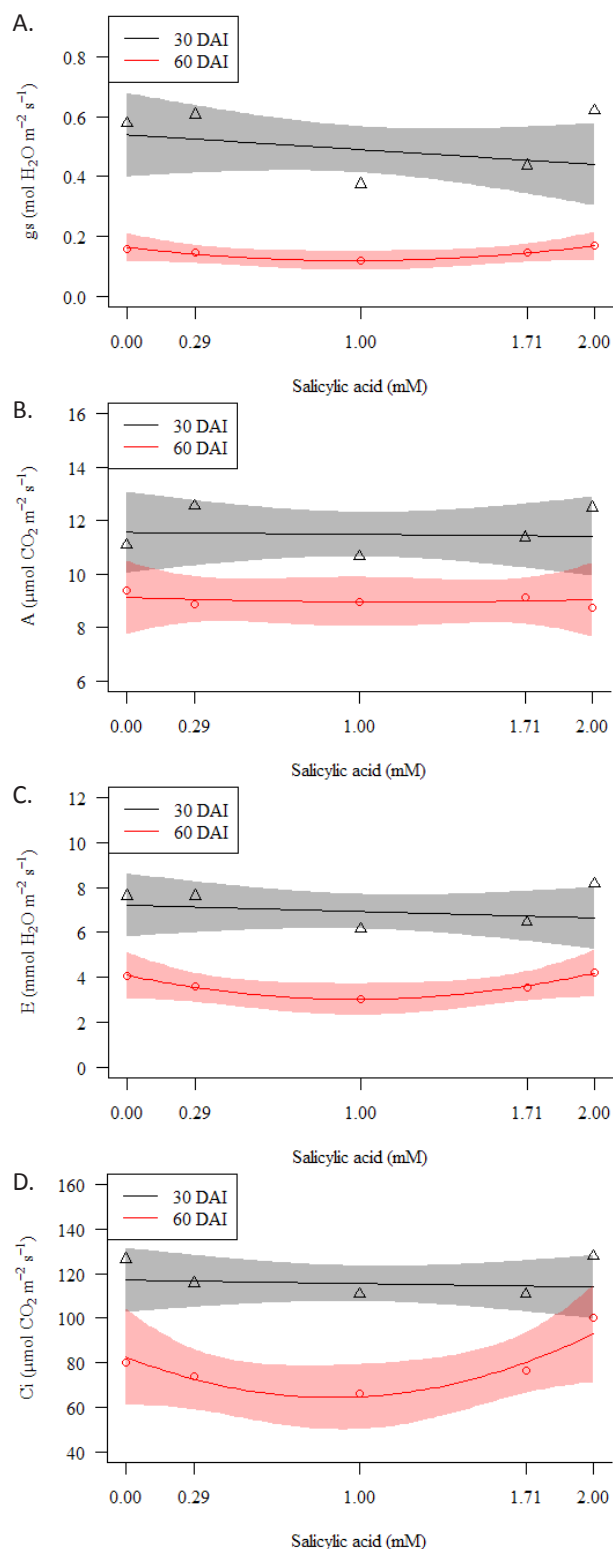


Figure 2. Stomatal conductance (A), net photosynthesis (A) (B), transpiration (E) (C) and internal carbon concentration (C_i) (D) of basil plants submitted to salicylic acid at 30 and 60 days after the beginning of day after irrigation (DAI).

Differences were observed for F_0 , F_v and F_v/F_m in all ECw tested and for both assessment periods, while F_m did not show any difference when submitted to the ECw of 6.0 dS m^{-1} . At 60 DAI, it was observed that as the ECw increased, there was also

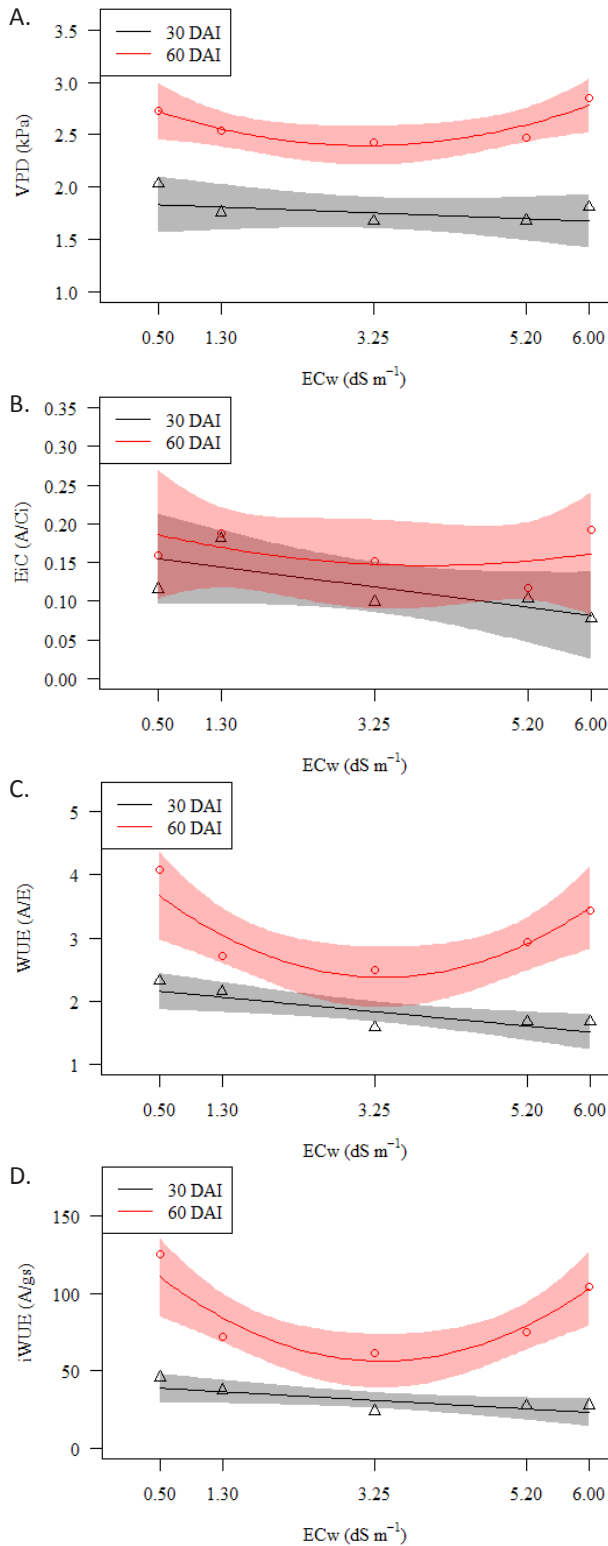


Figure 3. Vapor pressure deficit (A), intrinsic carboxylation efficiency (Eic) (B), water use efficiency (WUE) (C) instantaneous water use efficiency (iWUE) (D) of basil plants submitted to saline stress (ECw) at 30 and 60 days after the beginning of day after irrigation (DAI).

an increase in the F_m , F_v and F_m/F_v variables. At 30 DAI, no large mean variations were observed as the ECw increased, for all above mentioned variables.

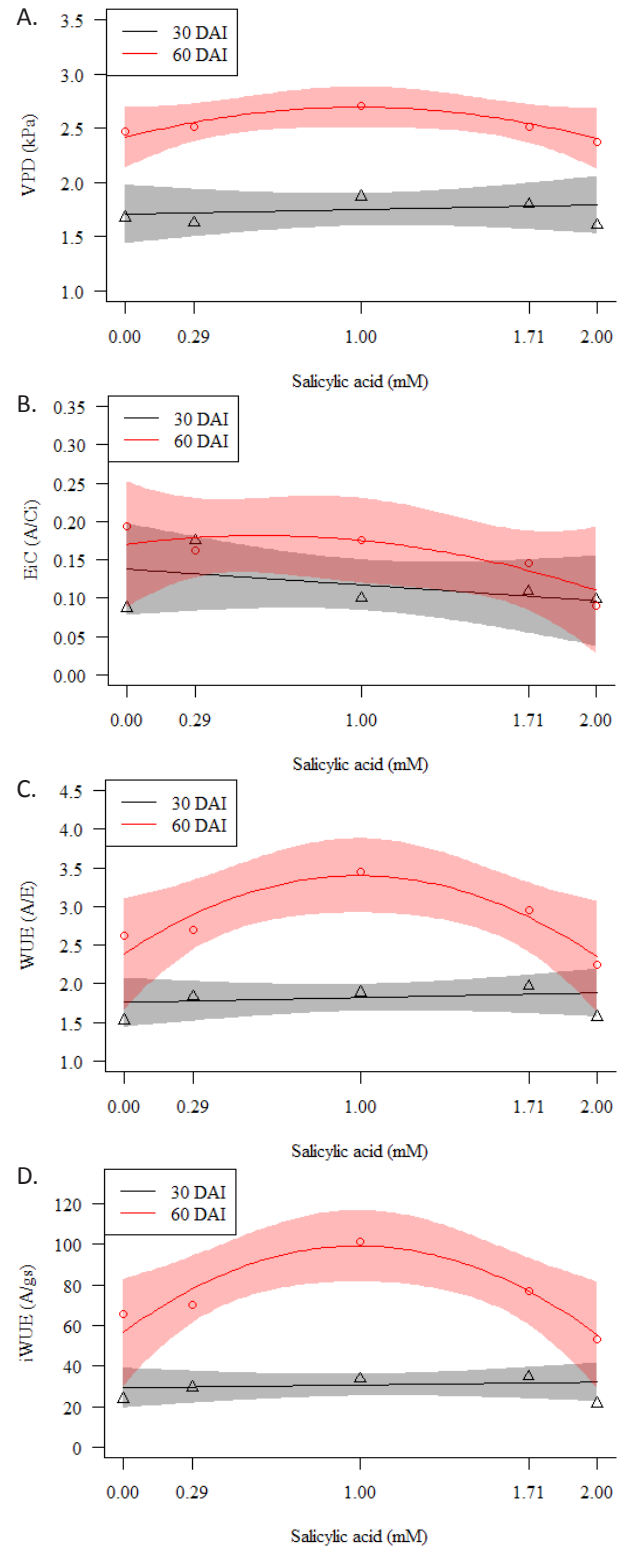


Figure 4. Vapor pressure deficit (A), intrinsic carboxylation efficiency (Eic) (B), water use efficiency (WUE) (C) instantaneous water use efficiency (iWUE) of basil plants submitted to salicylic acid at 30 and 60 days after the beginning of day after irrigation (DAI).

Photosystem II (PSII) is relatively sensitive to salt stress. The reduction in photosynthetic rates of plants submitted to saline stress is associated with several factors, such as: cell

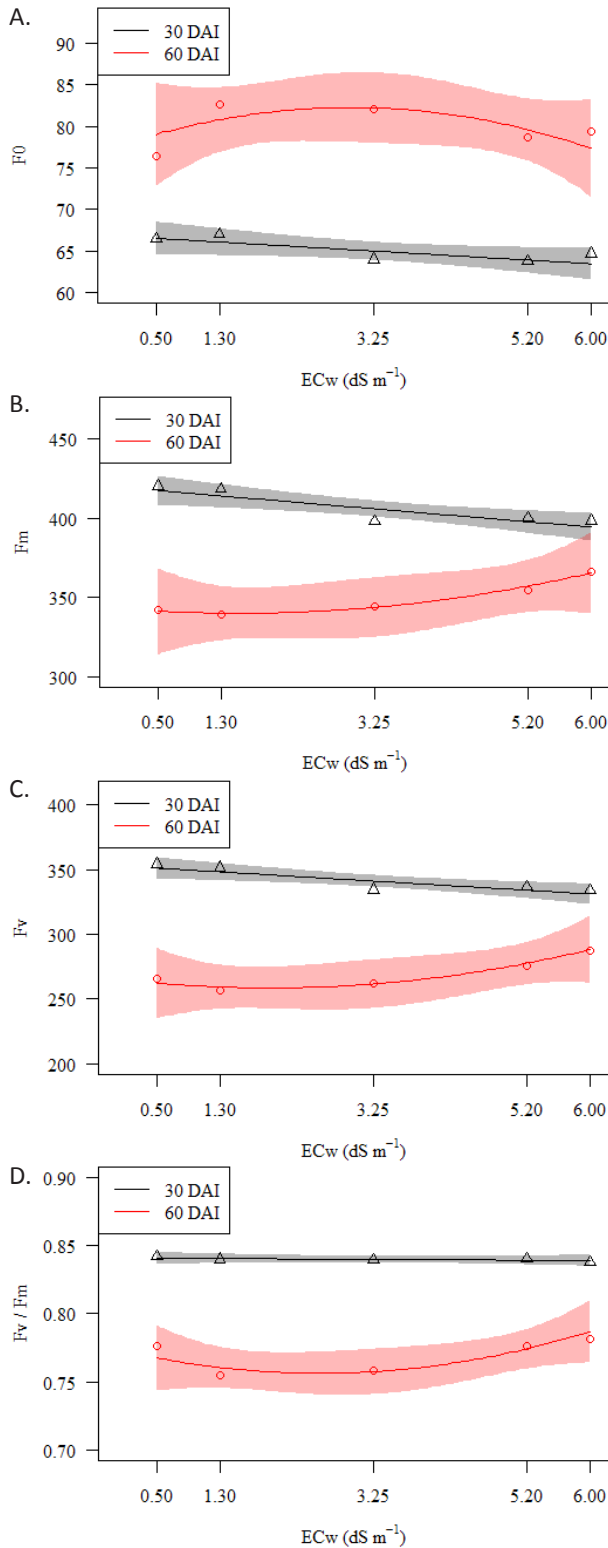


Figure 5. Fluorescence initial (F_0) (A), maximum fluorescence (F_m) (B), variable fluorescence (F_v) (C) and maximum quantum yield of PSII (F_v/F_m) of basil plants submitted to saline stress (ECw) at 30 and 60 days after the beginning of day after irrigation.

membrane dehydration, salt toxicity, increased senescence, increased enzymatic activity due to altered cytoplasmic structure, among others (Parihar et al., 2014).

The SA treatment influenced the F_0 , F_m , F_v and F_v/F_m of basil plants at 30 and 60 DAI (Figure 6). At 30 DAI, no large oscillations were observed as the SA concentration increased. At 60 DAI, it was observed that from the SA concentration of

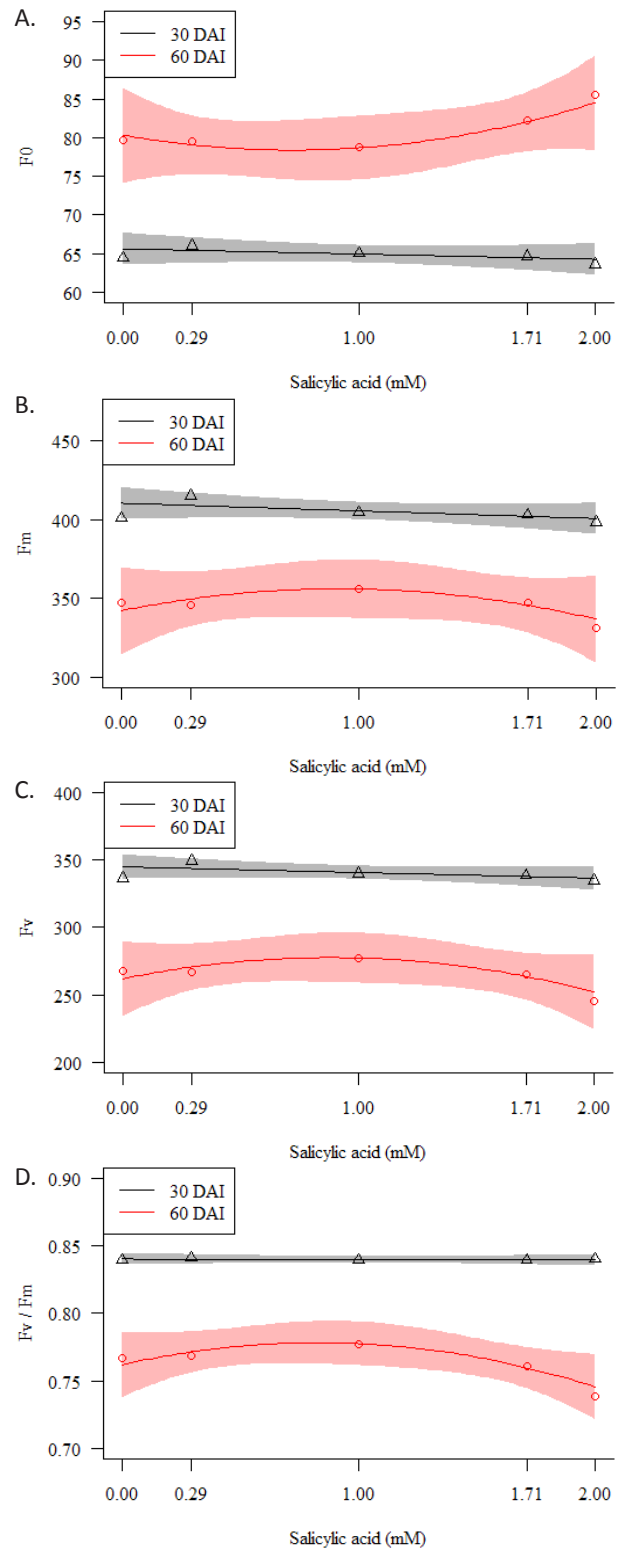


Figure 6. Initial fluorescence (F_0) (B), variable fluorescence (F_v) (C) and maximum quantum yield of PSII (F_v/F_m) (D) of basil plants subjected to salicylic acid at 30 and 60 days after the beginning of day after irrigation (DAI).

1.0 mM on, there was a decrease in F_m , F_v and F_v/F_m and an increase in F_0 .

As the ECw values increased, chlorophyll a, b and total, presented differences, at 30 and 60 DAI, as can be seen in Figure 7. The reduction in chlorophyll content of plants submitted to saline stress can be attributed to the degradation of the photosynthetic pigments and the instability of the protein complex, in addition to interference in the protein synthesis and in the structure of the chlorophyll components (Hussein et al., 2012).

At 30 DAI, it was observed that as the ECw values increased, there was an increase in chlorophylls a, b and total. However, at 60 DAI, there was an increase in these variables until the ECw of 5.2 dS m⁻¹ and above that value there was a decrease. This behavior might be related to the higher accumulation of toxic ions (mainly Na⁺ and Cl⁻) in the root zone, which may have damaged the photosynthetic apparatus. Bagherifard et

al. (2015) verified that artichoke plants (*Cynara scolymus* L.) submitted to saline stress and SA treatment had its chlorophyll content reduced.

Regarding the SA treatment, there were differences for a, b and total chlorophylls in both assessment periods, as can be observed on Figure 8. El-Esawi et al. (2017) verified that the SA treatment on salt-stressed rosemary plants (*Rosmarinus officinalis* L.) mitigated the toxic effect on chlorophyll content.

At 30 DAI, chlorophylls b and total increased up to the SA molarity of 1.71 mM, and had a decrease in the molarity of 2.0 mM. At 60 DAI, chlorophyll a decreased up to 1.71 mM of SA. Chlorophylls b and total showed no differences under the SA molarities of 0.0 and 2.0 mM, while chlorophyll a presented differences for all tested SA molarities. Karlidag et al. (2009) verified that salt-stressed strawberry plants (*Fragaria chiloensis*) had its chlorophyll content affected and that the SA treatment attenuated this effect. Correlations of the above

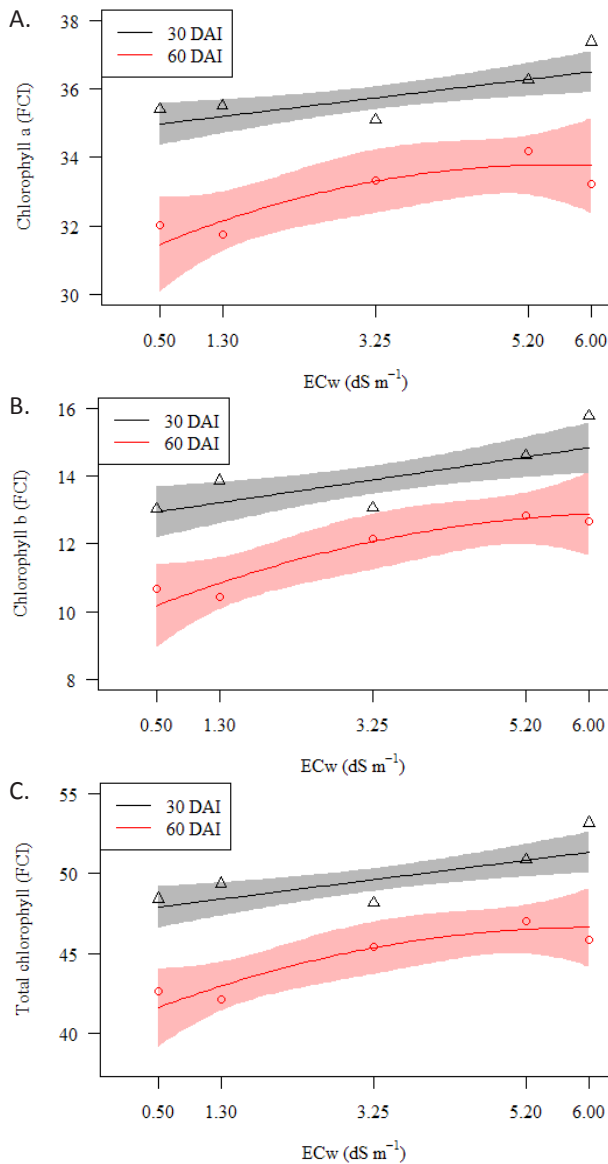


Figure 7. Chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) of basil plants submitted to saline stress (ECw) at 30 and 60 days after the beginning of day after irrigation.

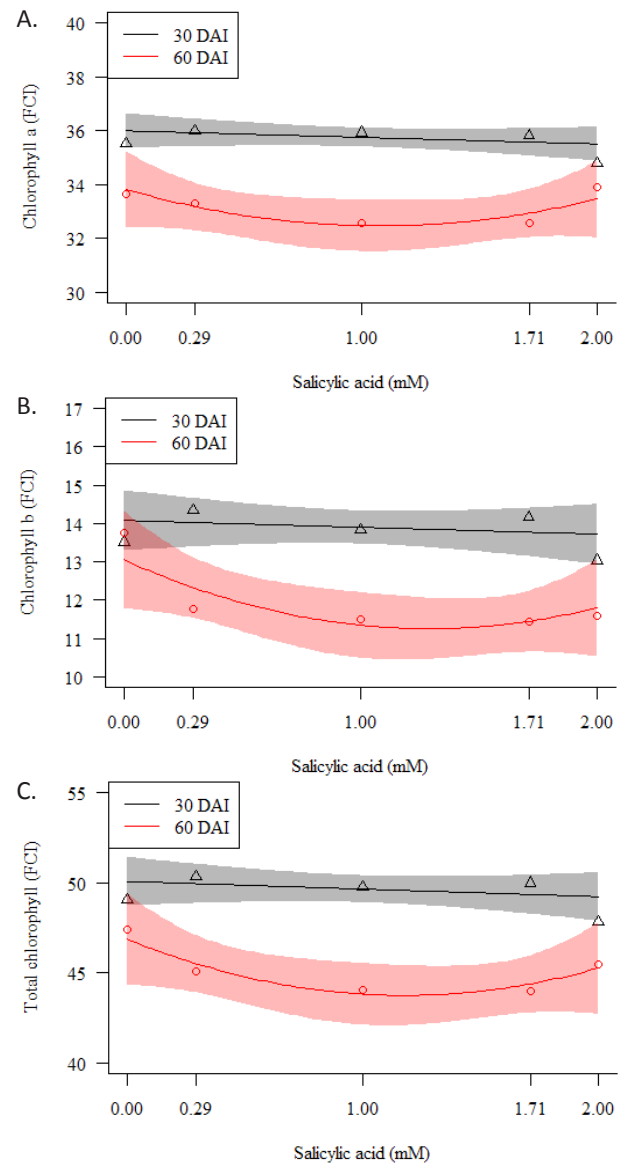


Figure 8. Chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) of basil plants submitted to salicylic acid at 30 and 60 days after the beginning of day after irrigation.

mentioned variables with ECw and SA at 30 and 60 DAI were realized (Figure 9).

There was a positive correlation between A and g_s (0.68) and E (0.72), between g_s and E (0.76) and Ci (0.70), between iWUE and WUE (0.73), EIC (0.69) and VPD (0.68). The highest negative correlation occurred between iWUE and Ci (0.98). There was a positive correlation between chlorophyll a, b and total – being the correlation between the last two the largest (0.96). There was also a positive correlation between F_0' , F_m and F_v – being the correlation between F_v and F_m the largest (0.99). Melo et al. (2017) when studying giant saltbush plants (*Atriplex nummularia*) under saline and water stress, emphasized that, generally, the decrease in the quantum yield of PSII can be associated to chlorophyll degradation, mainly chlorophyll a.

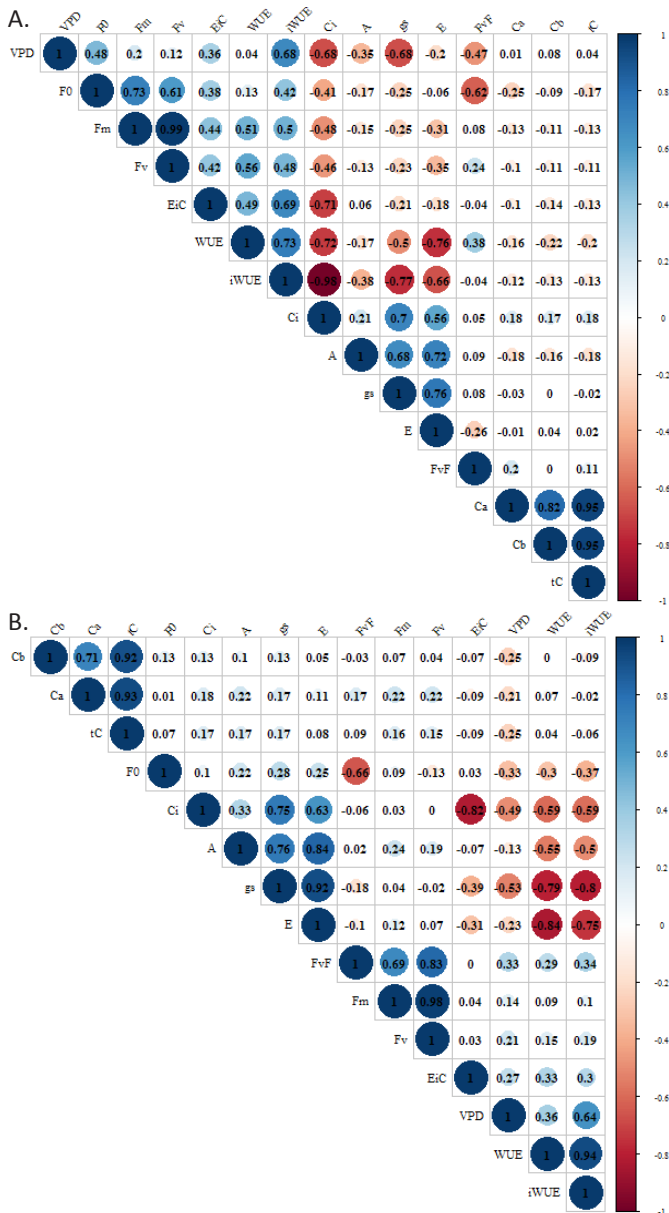


Figure 9. Correlation among gas exchanges (A, g_s , Ci, E, VPD, EIC, WUE and iWUE) (A), fluorescence and chlorophyll indices (F_0' , F_m , F_v and F_v/F_m , Ca, Cb and tC) at 30 (A) and 60 (B) days after irrigation (DAI).

When subjected to saline stress, plants can significantly decrease their photosynthetic process, which may be related to the enzymes activity disorganization as well as the reduction of CO_2 partial pressure within leaves, as a consequence of stomata closing. Further, saline stress damage photosystems activities, as a consequence of the laminar system disorganization and chloroplasts integrity loss (Bybordi, 2012).

At 60 DAI, the VPD presented a positive correlation with WUE (0.36), EIC (0.27) and iWUE (0.64). Also, g_s presented positive correlation with A (0.76), Ci (0.75) and E (0.92), showing negative correlation with VPD (0.53), EIC (0.39), WUE (0, 79) and iWUE (0.80). When analyzing the correlation between the chlorophyll content variables, it was observed that the highest positive correlation was obtained between F_m and F_v (0.98) and the negative between F_0 and F_v (0.66). In accordance to Mancarella et al. (2016), by measuring the chlorophyll fluorescence it is possible to diagnose the state in which the plant is located, besides obtaining information for a clarification of a cause and effect relationship between the mechanisms controlling the water balance and plant growth.

Conclusions

The greatest effect of electrical conductivity of irrigation water in the physiology of basil plants (*Ocimum basilicum* cv. Cinnamon) was observed at 60 days after irrigation with saline water (DAI). At 60 DAI, salicylic acid treatment mitigated the saline stress effects, for most of the analyzed variables up to the dose of 1.0 mM, while at 30 DAI, salicylic acid treatment showed low activity.

Acknowledgments

The authors thank the Scientific and Technological Development Council (CNPq) for the financial support as a form of a master's degree scholarship to the first author of this work.

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