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ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE | BOTÁNICA

ECOPHYSIOLOGY AND GROWTH OF BASIL (*Ocimum basilicum*) UNDER SALINE STRESS AND SALICYLIC ACID

Ecofisiología y crecimiento de albahaca (*Ocimum basilicum*) bajo estrés salino y ácido salicílico

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ABSTRACT

Salinity is one of the major problems of modern agriculture, affecting physiological, growth and plant production. Basil (*Ocimum basilicum*) is a plant widely used in cooking, and in the pharmaceutical and cosmetics industries. Salicylic acid can be a strategy to mitigate the harmful effects of saline stress on basil plant. The present study aimed to evaluate plants with, gas exchange, chlorophyll a fluorescence and chlorophyll indices of basil (cv. Cinnamon) plants under saline stress and salicylic acid. The experimental design was a randomized block design in a 5x5 incomplete factorial scheme generated through the central composite design. The factors we five electrical conductivities of irrigation water (ECw– 0.5, 1.3, 3.25, 5.2 and 6.0 dS m^{-1}) and five doses of salicylic acid (SA– 0.0, 0.29, 1.0, 1.71 and 2.0 mM), with five replications and two plants per replicate. Growth, gas exchange, chlorophyll a fluorescence and chlorophyll indices of *O. basilicum* cv. Cinnamon were evaluated. Canonical variables analysis and confidence ellipses ($p \leq 0.01$) were performed to study the interrelationship between variables and factors. Salicylic acid alleviated the deleterious effects of salt stress on growth, gas exchange, chlorophyll fluorescence and chlorophyll indices of basil.

Keywords: photosynthesis, salinity, gas exchange

RESUMEN

La salinidad es uno de los mayores problemas de la agricultura moderna, afectando la fisiología, el crecimiento y la producción vegetal. La albahaca (*Ocimum basilicum*) es una planta muy utilizada en la cocina y en las industrias farmacéutica y cosmética. El ácido salicílico puede ser una estrategia para mitigar los efectos nocivos del estrés salino en las plantas de albahaca. El objetivo del presente estudio fue evaluar el crecimiento, intercambio de gases, fluorescencia de clorofila a e índices de clorofila de plantas de albahaca (cv. Cinnamon) bajo estrés salino y ácido salicílico. El diseño experimental fue un diseño de bloques al azar en un esquema factorial incompleto de 5x5 generado a través del diseño compuesto central. Los factores fueron cinco conductividades eléctricas del agua de riego (ECw– 0,5, 1,3, 3,25, 5.2 y 6,0 dS m^{-1}) y cinco dosis de ácido salicílico (SA– 0,0, 0,29, 1,0, 1.71 y 2,0 mM), con cinco repeticiones y dos plantas por réplica. Crecimiento, intercambio de gases, fluorescencia de clorofila a e índices de clorofila de *O. basilicum* cv. Cinnamon fue evaluado. Se realizaron análisis de variables canónicas y elipses de confianza (p< 0.01) para estudiar la interrelación entre variables y factores. El ácido salicílico alivió los efectos nocivos del estrés salino sobre el crecimiento, el intercambio de gases, la fluorescencia de la clorofila y los índices de clorofila de la albahaca.

Palabras clave: fotosíntesis, salinidad, intercambio de gases

INTRODUCTION

Salinization due to the use of irrigation water with a high salt content affects more than 20 % of irrigated areas and reduces crop yield (Sako *et al.* 2018). Increasing demand and freshwater scarcity intensify the use of saline water to irrigate agricultural crops in many regions of the world, especially in arid and semi-arid regions (Batista *et al.* 2019; Dastranj and Sepaskhah, 2019).

Salinity is one of the main factors responsible for reducing agricultural production (El-Nasharty *et al.*, 2019; Li *et al.*, 2019; Astaneh *et al.*, 2019). The adverse effect of salinity is mainly due to the accumulation of toxic ions (Na^+ and Cl^-) causing oxidative stress and decreasing osmotic potential in plants (Shams *et al.*, 2019).

Initially, plants exposed to salinity undergo osmotic stress, with inhibition of cell division and limiting gas exchange and, if continued, may undergo ionic stress, causing nutritional imbalance, oxidative stress and inhibition of protein synthesis and enzymatic activity, reducing plant growth, development and survival (Bekhradi *et al.*, 2015; Cirillo *et al.*, 2019). The photosynthetic capacity of plants grown under saline conditions is lower depending on the severity and/or duration of stress, genotype and plant age (Sarabi *et al.*, 2019).

Saline stress can disrupt the electron transport chain, reducing photosystem II efficiency and increasing fluorescence emission (Melo *et al.*, 2017). Processes in and around PSII reaction centers change receptor redox balance and quantum energy yield and affect chlorophyll fluorescence (Bordenave *et al.*, 2019).

Salicylic acid (SA) influences various physiological and biochemical functions in plants and has diverse effects on the tolerance to biotic and abiotic stress (Li *et al.*, 2014; Silva *et al.*, 2022). Exogenous SA can improve plant adaptation to salinity by various mechanisms, such as ameliorating photosynthetic capacity, increasing antioxidative protection, inhibiting Na^+ and Cl^- accumulation, accumulating soluble carbohydrates, stimulating ABA accumulation, improving mineral nutrient, enhancing N and S assimilation etc. (Nazar *et al.*, 2011; Poór *et al.*, 2011; Hao *et al.*, 2012). SA alleviated the adverse effects of saline stress in mungbean (*Vigna radiata* – Fabaceae) through the improvement of plant photosynthesis, and growth and enhancing the antioxidant system (Khan *et al.*, 2014).

Basil (*Ocimum basilicum* L. – Lamiaceae), native to India, Africa, and Southern Asia (Tavallali *et al.*, 2019), an annual or perennial herbaceous plant depending on where it is grown (Blank *et al.*, 2015), has medicinal, seasoning, aromatic or ornamental use in pots and gardens (Blank *et al.*, 2012). This plant is used as a condiment, and its essential oil, composed of a complex mixture of volatile and semi-volatile compounds that determine its specific aroma and the flavor of the condiment (Lung *et al.*, 2016), can be used in the chemical, pharmaceutical and cosmetics industries (Jakovljević *et al.*, 2017). The aim of the present study was to evaluate growth, gas exchange, chlorophyll a fluorescence and chlorophyll indices of basil (cv. Cinnamon) plants under saline stress and salicylic acid.

MATERIAL AND METHODS

Experimental conditions and plant material

This experiment was carried out in a protected environment at the Center for Agricultural Sciences of the Universidade Federal da Paraíba in the municipality of Areia, Paraíba state, Brazil. The soil used was classified as Planosols, of a sandy loam texture, whose physical characteristics were sand: 756.9 g kg^{-1}; silt: 59.1 g kg^{-1}; clay: 184.0 g kg^{-1}; bulk density: 1.38 kg dm^{-3}; particle density: 2.67 kg dm^{-3}; total porosity: 48 %; field capacity: 78 g kg^{-1}; and permanent withering point: 43 g kg^{-1}. The salinity of the water used was analyzed (Table 1).

Table 1. Analysis of saline water with conductivities, from 0.50 to 6.00, used for irrigation of basil cv. Cinnamon

	Conductivities				
	0.50	1.30	3.25	5.20	6.00
pH	7.70	7.60	7.90	6.30	7.80
ECw	0.50	1.30	3.25	5.20	6.00
SO_4^{-2}	3.13	3.66	4.22	4.26	4.60
K^+	0.26	0.19	0.22	0.20	0.20
Na^+	2.28	9.37	25.44	40.62	49.56
Ca^{+2}	2.01	1.83	1.93	1.99	0.90
Mg^{+2}	1.08	0.98	0.86	0.84	0.90
CO_3^{-2}	0.93	0.85	1.07	1.15	1.10
HCO_3^-	2.67	2.50	2.50	2.83	2.67
Cl^-	4.17	12.50	32.83	54.00	63.83
SAR	2.27	9.79	25.90	40.72	49.56
ESP	2.05	11.65	26.98	37.03	41.81
Class	N - C_2S_1	S - C_3S_3	SS - C_4S_4	SS - C_4S_4	SS - C_4S_4

Class= classification; N= normal; S= saline; SS= sodium saline; SAR= sodium adsorption ratio; ESP= exchangeable sodium percentage.

The basil seedlings were produced in 162-cell polyethylene trays and, after 25 days of planting, transplanted into 5.0 dm^3 pots with 100 g poultry manure. The chemical analysis of this mixture was: pH: 6.9; P: 11.71 mg dm^{-3}; K^+: 873.43 mg dm^{-3}; Na^+: 0.24 cmol dm^{-3}; H^++Al^{+3}: 1.6 cmol dm^{-3}; Al^{+3}: 0.00 cmol dm^{-3}; Ca^{+2}: 4.65 cmol dm^{-3}; Mg^{+2}: 0.39 cmol dm^{-3}; sum of bases: 7.52 cmol dm^{-3}; cation exchange capacity: 9.12 cmol dm^{-3}; base saturation: 82.45 %; and organic matter: 22.73 g dm^{-3}.

The basil plants were irrigated with saline water after transplantation. The irrigation water with the desired electrical conductivity (ECw) was a mixture of NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ (7:2:1) salts in non-chlorinated water (0.5 dS m^{-1}) obtained at the experiment site. The total water used for irrigation was defined as four plants in the soil with ECw of 0.5 dS m^{-1}.

EXPERIMENTAL DESIGN

The experimental design was a randomized block design in a 5x5 incomplete factorial scheme generated through the central composite design. The factors were five electrical conductivities of irrigation water (ECw– 0.5, 1.3, 3.25, 5.2, and 6.0 dS m^{-1}) and five doses of salicylic acid (SA– 0.0, 0.29, 1.0, 1.71 and 2.0 mM), with five replications and two plants per replicate.

GROWTH MEASUREMENT

Plants were evaluated 60 days after the application of salt stress. Plant height (cm), number of branches, number of leaves, stem diameter (mm), inflorescence dry mass (g), leaf dry mass (g), stem dry mass (g), leaf area (cm^2), leaf area ratio (cm^2 g^{-1}), specific leaf area (cm^2 g^{-1}), stem mass ratio (g g^{-1}), root mass ratio (g g^{-1}), robustness quotient, sclerophilia index and root/shoot ratio were evaluated. The plants were divided into roots, stem, and leaves and dried in an oven with forced air circulation at 65 °C for 72 hours. Then, the plant parts were weighed on a precision scale (0.001 g).

The leaf area ratio was obtained using the formula: leaf area (LA)/total dry mass (TDM). The specific leaf area was obtained using the formula: LA/leaf dry mass (LDM). The stem mass ratio was obtained using the formula: stem dry mass/TDM. The root mass ratio was obtained using the formula: root dry mass/TDM. The robustness quotient was obtained using the formula: plant height/stem diameter. The sclerophilia index was obtained using the formula: LDM/LA.

GAS EXCHANGES MEASUREMENT

Gas exchange was determined at 60 days from the beginning of saline irrigation (DAI) in an infrared gas analyzer (LI-COR® – model LI-6400XT, Nebraska, USA), with measurements made between 9 and 10 a.m. Stomatic conductance (*gs*– mol H_2O m^{-2} s^{-1}), transpiration (*E*– mmol H_2O m^{-2} s^{-1}), net photosynthesis (*A*– µmol CO_2 m^{-2} s^{-1}), internal carbon concentration (*Ci*– µmol CO_2 mol air^{-1}), vapor pressure deficit (VPD), instantaneous water use efficiency (WUE– *A*/*E*), intrinsic water use efficiency (iWUE– *A*/*gs*) and intrinsic carboxylation efficiency (iCE– *A*/*Ci*) were then measured gas exchanges.

Chlorophyll a fluorescence measurement

Chlorophyll a fluorescence was evaluated at 60 DAI on the third leaf, using a non-destructive method, with a fluorometer (Opti-Sciences Inc.-Model OS-30p, Hudson, USA) with leaf clamps placed for 30 minutes before readings for adaptation of the leaves in the dark. The initial fluorescence (F_0), maximum fluorescence (F_m), and quantum yield of photosystem II (PSII= F_v/F_m) was the chlorophyll fluorescence variable evaluated.

Chlorophyll indices measurement

The chlorophyll a and b indices were evaluated on the third leaf with a portable electronic chlorophyll meter (ClorofiLOG®, model CFL 1030, Porto Alegre, RS).

Data analysis

Canonical variables analysis and confidence ellipses ($p \leq 0.01$) were performed to study the interrelationship between variables and factors using the candisc package (Friendly and Fox, 2017). The statistical program R (R Core Team, 2020) was used to perform the statistical analysis.

RESULTS

The harmful effects of saline stress up to 1.3 dS m^{-1} on the inflorescence dry mass (idm), plant height (ph), stem diameter (sd), leaves dry mass (ldm), root dry mass (rdm), stem dry mass (sdm) and a number of leaves (nl) were attenuated with the application of 0.29- and 1.71-mM salicylic acid (Fig. 1a). The harmful effects of irrigation water salinity above 1.3 dS m^{-1} on basil growth were not mitigated by the application of salicylic acid.

The harmful effects of saline stress up to 1.3 dS m^{-1} on the stem mass ratio (smr), leaf area (la), Dickson's quality index (dqi), sclerophilia index (si), and root mass ratio (rmr) was attenuated with the application of 0.29 and 1.71 mM of salicylic acid (Fig. 1b). The exogenous application of 1.0 and 1.71 mM of salicylic acid attenuated the harmful effects of salinity on the leaf area ratio (lar), specific leaf area (sla) and robustness quotient (rq).

The harmful effects of saline stress up to 5.2 dS m^{-1} on *Ci*, *gs*, *E,* and *A* were mitigated by the application of salicylic acid above 0.29 mM (Fig. 2a). The exogenous application of 1.0 mM of salicylic acid attenuated the harmful effects of saline stress on WUE, VPD, and iWUE.

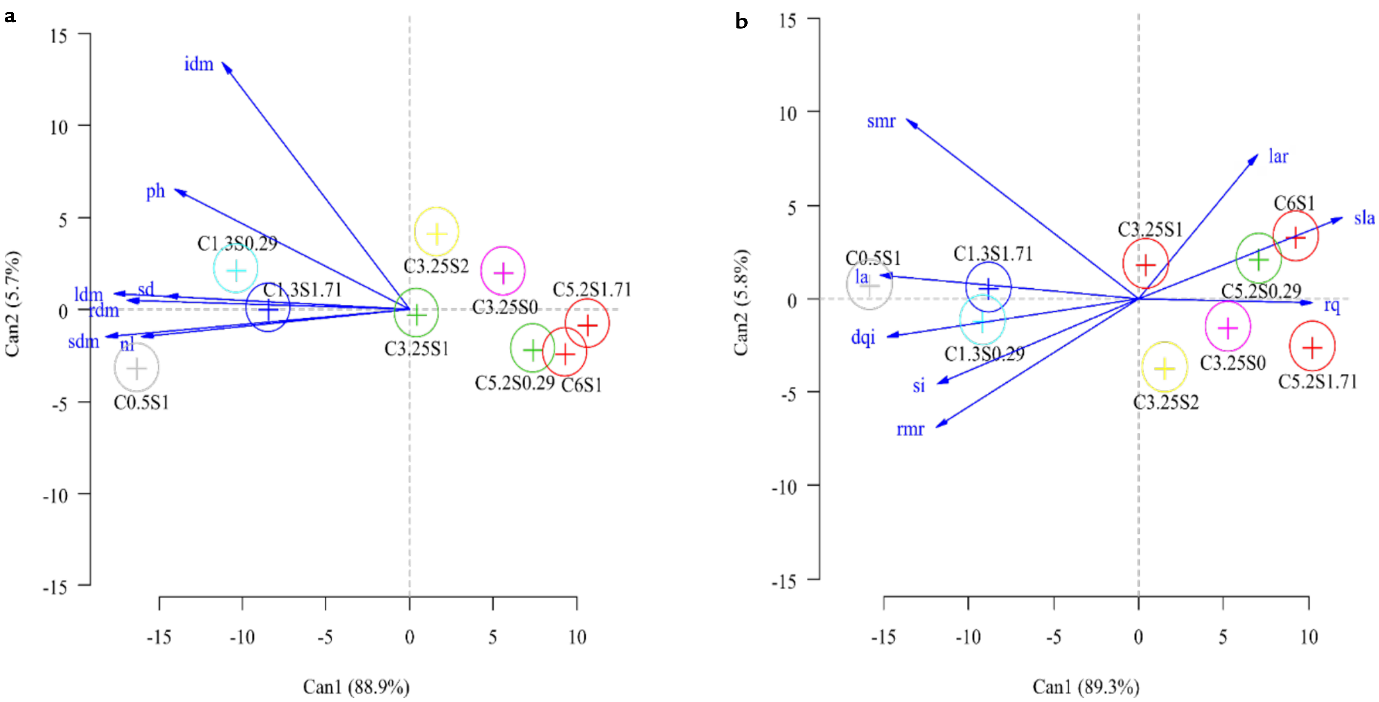

Figure 1. Canonical variables analysis and confidence ellipses between growth variables **(a and b)** of *Ocimum basilicum* under saline stress and salicylic acid. C= electrical conductivities of irrigation water; S= salicylic acid; idm= inflorescence dry mass; ph= plant height; tdm= total dry mass; sd= stem diameter; ldm= leaf dry mass; sdm= stem dry mass; nl= number of leaves; smr= stem mass ratio; la= leaf area; dqi= Dickson's quality index; si= sclerophilia index; rmr= root mass ratio; lar= leaf area ratio; sla= specific leaf area; rq= robustness quotient.

The application of salicylic acid up to 1.71 mM attenuated the harmful effects of saline stress on maximum fluorescence (F_m) and quantum yield of photosystem II (F_v/F_m), chlorophylls a and b of basil plant under ECw of 5.2 and 6.0 dS m^{-1} (Fig. 2b). Saline stress up to 3.25 dS m^{-1} favored the highest chlorophyll indices (a and b).

DISCUSSION

Salicylic acid (SA) attenuated the deleterious effects of moderate saline stress (ECw 1.3 dS m^{-1}) on the growth of basil plants because this phytohormone increases stimulation in physiological and biochemical processes (Jini and Joseph, 2017), in addition to increasing the K^+ content and decreasing Na^+ accumulation in the shoot of plants (Jayakannan *et al.*, 2013). SA decreased the harmful effects of saline stress in rice plants (*Oriza sativa* – Poaceae) (Jini and Joseph, 2017) and periwinkle (*Catharanthus roseus* – Apocynaceae) (Idress *et al.*, 2011).

SA relieved the damaging effects of salt stress (up to 5.2 dS m^{-1}) on *Ci*, *gs*, *A*, and *E* due to its effect on increasing photosynthetic rate, carbon fixation, transpiration, stomatal conductance, and antioxidant activity (Jayakannan *et al.*, 2015). In addition, the positive effect of SA on *A* may be due to its stimulatory effects on the pigment contents and Rubisco enzyme activity (Li *et al.*, 2014). The reduction of gas exchange by saline water irrigation with high ECws is due to the high concentration of Na^+ and/or Cl^- accumulated in chloroplasts (Shahbaz *et al.*, 2016). These ions, when accumulated in chloroplasts, reduce stomatal opening and, consequently, photosynthesis, with lower CO_2 diffusion in the substomatic chamber (Bybordi, 2012; Silva *et al.*, 2015), as reported for two basil cultivars (Attia *et al.*, 2011). In addition, excess salts in the transpiratory stream (Bekhradi *et al.*, 2015) accumulate reactive oxygen species and cause severe oxidative damage (El-Esawi *et al.*, 2018). The lack of effect of ECw on net photosynthesis (*A*) may be associated with mechanisms of restriction of Na^+ uptake and recycling in the xylem stream to the root system and sequestration of this ion in vacuoles and exportation outside cells (El-Esawi *et al.*, 2018).

The increase in the F_v/F_m under high ECw is related to the tolerance of this Cinnamon cultivar to water salinity stress with adjustment mechanisms in electron transport and entrapment in the PSII reaction center (Kalaji *et al.*, 2016). Stress response may vary according to the species' adaptive capacity (Rocha *et al.*, 2016). These may also be related to net photosynthesis (Li *et al.*, 2019) and plant physiological responses to salt stress that depend on the phenotypic plasticity and genotypic characteristics of each plant (Batista *et al.*, 2019). The average F_v/F_m of 0.78 indicates the lack of damage to the PSII, as the plant F_v/F_m under stress is lower than 0.75 (Silva *et al.*, 2015). Plants, under saline conditions, adapt by osmotic adjustment, osmoprotectant

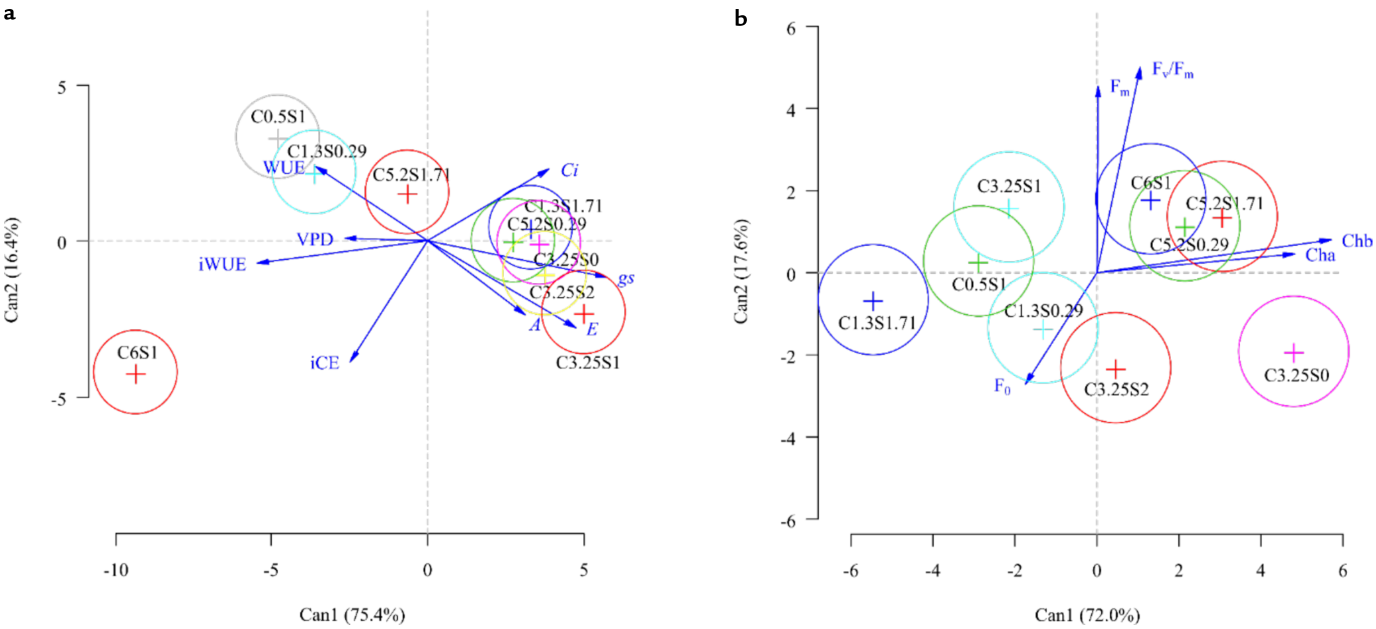

Figure 2. Canonical variables analysis and confidence ellipses between gas exchange variables **(a)** and chlorophyll variables **(b)** of *Ocimum basilicum* under saline stress and salicylic acid. C= electrical conductivities of irrigation water; S= salicylic acid; *gs*= stomatal conductance; *A*= net photosynthesis; *E*= transpiration; *Ci*= internal carbon concentration; WUE= instantaneous water use efficiency; iWUE= intrinsic water use efficiency; iCE= intrinsic carboxylation efficiency; F_0= initial fluorescence; F_m= maximum fluorescence; F_v/F_m= quantum yield of photosystem II.

accumulation, and modifications in ion transport to prevent ionic toxicity and enzyme activation (Javed *et al.*, 2019).

The increase in chloroplast pigments under the SA treatment might be due to the ability of SA to increase the activity of certain enzymes, thereby stimulating chlorophyll biosynthesis or reducing chlorophyll degradation, leading to increased net photosynthesis under saline stress tolerance (Li *et al.*, 2014). In addition, the positive effect of SA on photosynthetic pigments could be attributed to its stimulatory effects on RuBisCO activity and the rate of photosynthesis (Idrees *et al.*, 2012). The increase in chlorophyll a, b, and total levels with ECw are due to the intensity/duration of salt stress on photosynthetic pigment biosynthesis, chlorophyllase activity, and protein complex instability, and the maintenance of chlorophyll accompanied by the conservation of photochemical reactions, like those of the PSII (Huang *et al.*, 2015; Elhindi *et al.*, 2016). The decrease in dry mass by ECw is due to Na^+ accumulation and reduction in the K^+/Na^+ ratio (Kaushal and Wani, 2016) and reduced water absorption capacity due to osmotic stress in basil plants (Morales *et al.*, 2012).

CONCLUSIONS

Exogenous application of salicylic acid alleviated the deleterious effects of salt stress on growth, gas exchange, chlorophyll fluorescence and chlorophyll indices of basil (*Ocimum basilicum* cv. Cinnamon).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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