



## Salicylic acid relieves salt stress damage on basil growth

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### ABSTRACT

Salinity is one of the main challenges in agriculture, especially in arid and semi-arid regions. Salicylic acid application may be a strategy to mitigate the harmful effects of salt stress on plant growth. Basil (*Ocimum basilicum*) is a medicinal, spice, and ornamental plant grown around the world that has its growth reduced by salt stress. Therefore, the objective was to evaluate salicylic acid in mitigating the damage caused by salt stress in 'Cinnamon' basil. Growth was evaluated at 90 days after the start of irrigation with saline water. Salicylic acid application attenuates the harmful effects of salt stress on the number of leaves, number of inflorescences, leaf dry mass, inflorescence dry mass, root dry mass, seed mass, leaf area, leaf area ratio, specific leaf area, leaf mass ratio, root mass ratio, inflorescence mass ratio, Dickson quality index, sclerophilia index, robustness quotient and shoot root ratio. Salt stress decreased plant height, stem diameter, stem dry mass, and stem mass ratio of basil 'Cinnamon'. Salicylic acid can be used to lessen the harmful effects of salt stress on basil 'Cinnamon'.

**Keywords:** *Ocimum basilicum*; salinity of irrigation water; phytohormone

### INTRODUCTION

Basil (*Ocimum basilicum* L. - Lamiaceae) is a species native to tropical Asia, introduced to Brazil by Italian immigrants (Oliveira *et al.*, 2020). Basil is used in cosmetics and medicine industries, cooking, folk medicine, and essential oil extraction, mainly due to its high content of linalool (Vilanova *et al.*, 2018; Chokami *et al.*, 2019; Silva *et al.*, 2019). Basil cultivation in Northeast Brazil is mainly done by small family farmers. However, this region is characterized by low rainfall, causing producers to use low-quality irrigation water, most of the time with high levels of salts (Silva *et al.*, 2018).

The high salts content in irrigation water is a limiting factor for plant development, which can compromise the growth and physiology of plants (Nóbrega *et al.*, 2021).

The decrease in plant growth under salt stress is caused by the osmotic effect that reduces the water and essential elements absorption, causing nutritional imbalance, oxidative stress, and specific ions toxicity (Ahammed *et al.*, 2018; Silva *et al.*, 2019; Naveed *et al.*, 2020).

The use of techniques to mitigate the harmful effects of salinity in plants is necessary. Salicylic acid can alleviate the harmful effects of salt stress by adjusting physiological and biochemical mechanisms (Kim *et al.*, 2018). Foliar application of salicylic acid decreased lipid peroxidation caused by excess NaCl and improved plant growth. This behavior is related to a decrease in NaCl and an increase in Ca and Mg in the plant, in addition to the increase in the activity of the antioxidant system enzymes (SOD, CAT,

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GPX, etc.) (Joseph *et al.*, 2010). Salicylic acid is used in the formulation of some biostimulants used in agriculture, as a growth promoter and plant resistance inducer. In addition, this phytohormone acts as an effective inducer of genes related to stress-linked proteins (Angooti & Nourafcan, 2015).

Several studies are performed with basil plants under salt stress and salicylic acid (Elhindi *et al.*, 2017; Silva *et al.*, 2019; Kahveci *et al.*, 2021); however, few studies have been performed about such effects on the growth of ‘Cinnamon’ basil. Therefore, the objective was to evaluate salicylic acid in mitigating the damage caused by salt stress in ‘Cinnamon’ basil.

## MATERIAL AND METHODS

The experiment was performed in a greenhouse at the Universidade Federal da Paraíba, Areia, Brazil. The experimental design was in randomized blocks, using an incomplete factorial scheme generated through the central composite design, with five electrical conductivities of irrigation water (ECw – 0.5, 1.3, 3.25, 5.2, and 6.0 dS m<sup>-1</sup>) and five concentrations of salicylic acid (SA – 0, 0.29, 1.0, 1.71, and 2.0 mM – Silva *et al.*, 2019). Totaling nine combinations (Table 1), with five repetitions and two plants per plot, with 90 plants analyzed at the end of the experiment.

Basil seeds were sowed in 162-cell polyethylene trays that were filled with soil and commercial organic compost (1:1, v:v). Basil seeds were obtained from the basil germplasm bank of the Universidade Federal de Sergipe. Seedlings were transplanted 25 days after sowing into 5 dm<sup>3</sup> pots with soil and 100 g of poultry manure. Saline waters

were prepared by adding of NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O, and Mg-Cl<sub>2</sub>.6H<sub>2</sub>O (7:2:1, v:v), respectively, to water (0.5 dS m<sup>-1</sup>). Salicylic acid concentrations were prepared with 30% ethyl alcohol and Tween 80 (0.05%). Distilled water and Tween 80 (0.05%) were used as control. The plants were sprayed with 10 mL per plant of each treatment, every week for 21 days. Salt stress was applied 30 days after sowing along with salicylic acid applications.

Basil growth was evaluated 90 days after the start of saline water irrigation (DAI). A graduated ruler was used to measure plant height (PH); calipers were used to measure stem diameter (Ds); the number of leaves (NL) was measured manually. The plants were divided into root, stem, leaves and inflorescences and dried in an oven with forced air circulation at 65 °C. Then, the plant parts were weighed on a precision scale (0.001 g). Seeds were separated from the inflorescences and weighed on a precision scale (0.001 g) to obtain the seed mass.

Leaf area (LA) was obtained by averaging the product of length and width of 20 leaves and the leaf area form factor (0.6775). Leaf area ratio (LAR) was obtained using the formula: leaf area (LA)/total dry mass (TDM). The specific leaf area (SLA) was obtained using the formula: LA/leaf dry mass (LDM). The leaf mass ratio (LMR) was obtained using the formula: leaf dry mass/TDM. The stem mass ratio was obtained using the formula: stem dry mass/TDM. The root mass ratio (RMR) was obtained using the formula: root dry mass/TDM. The inflorescence mass ratio (IMR) was obtained using the formula: inflorescence dry mass/TDM. The sclerophilia index was obtained using the formula: LDM/LA. The robustness quotient was obtained

**Table 1:** Combinations generated by the central composite design

Levels		Dose	
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
$\alpha$	0	6.00	1.00
0	$\alpha$	3.25	2.00
0	- $\alpha$	3.25	0.00
0	0	3.25	1.00

Electrical conductivity of irrigation water (ECw); salicylic acid (SA).

using the formula: plant height/stem diameter. Dickson quality index was obtained using the formula:  $TDM/((PH/Ds) + (\text{shoot dry mass}/\text{root dry mass}))$ . The root/shoot ratio was obtained using the formula: root dry mass/shoot dry mass.

Data were subjected to the normality test (Shapiro-Wilk) and homogeneity of variances (Bartlett) and to the regression analysis and, when the interaction between the factors was significant, response surface graphs were created, with the equations being generated by the rsm package (Lenth, 2009) and by the GA package (Scrucca, 2013). A canonical variables analysis with confidence ellipses ( $p \leq 0.01$ ) were performed to study the interrelationship between variables and factors using the candisc package (Friendly & Fox, 2021). The statistical program R (R Core Team, 2021) was used to perform the statistical analysis.

## RESULTS AND DISCUSSION

The summary of the analysis of variance (ANOVA) is shown in Table 2. The mean values per treatment for each variable are shown in Table 3.

The increase in salt stress reduced the plant height and stem diameter, with losses of 71.90 and 63.22%, respectively, when comparing the lowest and highest ECw (Figures 1A and 1C). Increasing the dose of salicylic acid increased plant height and stem diameter, with an increase of 109.02 and 89.87%, respectively, when comparing the lowest and highest doses (Figures 1B and 1D). Salicylic acid reduced the harmful effects of salt stress on the number of leaves and number of inflorescences, with the highest values (671 and 104) observed at ECws 0.52 and 0.91  $\text{dS m}^{-1}$  and 1.98 and 1.99 mM of salicylic acid, respectively (Figures 1E and F).

The negative effect of salt stress on plant height and stem diameter is related to the decrease in  $\text{CO}_2$  assimilation due to stomatal limitation and/or impairment in metabolic processes, which can lead to inhibition of cell division and expansion, as observed in pistachio (*Pistacia vera* L.) (Rahneshan *et al.*, 2018). Furthermore, this behavior may be related to the negative effects of high salinity that can cause hyperionic and hyperosmotic stress, causing reduced plant growth and even death (Wang *et al.*, 2018a). This action was also observed by Silva *et al.* (2022) in basil plants grown under salt stress. The beneficial effect of salicylic acid on plant height and stem diameter and attenuating salt stress on the number of leaves and number of inflorescences is related to the effect of this phytohormone in the regulation of several defense mechanisms, especially the

maintenance of ionic balance and photosynthetic activity and detoxification of reactive oxygen species (ROS), as observed in maize (*Zea mays* L.) (Tahjib-Ul-Arif *et al.*, 2018).

The increase in salinity of the irrigation water decreased the stem dry mass with losses of 33.05% when comparing the lowest and highest ECw (Figure 2A). Salicylic acid reduced this variable up to the dose of 1.71 mM, with an increase in the dose of 2.0 mM (Figure 2B). Salicylic acid mitigated the harmful effects of salt stress on leaf dry mass and inflorescence dry mass, with the highest values (17.40 and 40.40 g) observed in ECws 0.53 and 0.52  $\text{dS m}^{-1}$  and at doses of 0.0015 and 1.99 mM salicylic acid, respectively (Figures 2C and 2D). In addition, salicylic acid application reduces the harmful effects of salt stress on root dry mass and seed mass, with the highest values (6.07 and 8.86 g) observed at ECws 0.51 and 0.50  $\text{dS m}^{-1}$  and at doses of 0.013 and 0.774 mM of salicylic acid, in that order (Figures 2E and 2F). The attenuating effect of salicylic acid at low doses may be related to the signaling cascade effect that this phytohormone can cause in plants under stress.

Stem dry mass was reduced by increased salt stress due to membrane degradation, lipid peroxidation, and several other physiological and biochemical changes caused by stress, as observed in *Capsicum annum* L. (Wang *et al.*, 2018b). The beneficial effect of salicylic acid on stem dry mass and mitigating salt stress on leaf dry mass, inflorescence dry mass, root dry mass, and seed mass is related to the action of this phytohormone in accelerating plant growth under stress, improving physiological processes in leaves and reducing oxidative damage, as observed in *Cucumis sativus* L. (Miao *et al.*, 2020). In addition to stimulating the production of antioxidants, osmotic substances, phytohormones, and secondary metabolites to protect photosynthetic organs and alleviate cellular damage (Miao *et al.*, 2020).

Salicylic acid attenuated the harmful effects of salt stress on leaf area and leaf area ratio, with the highest values (2657.57  $\text{cm}^2$  and 34.87  $\text{cm}^2 \text{g}^{-1}$ ) observed at ECws 0.54 and 5.99  $\text{dS m}^{-1}$  and 1.98 and 0.25 mM of salicylic acid, respectively (Figures 3A and 3B). Salicylic acid also mitigated the harmful effects of salt stress on specific leaf area and leaf mass ratio, with the highest values (328.98  $\text{cm}^2 \text{g}^{-1}$  and 0.23  $\text{g g}^{-1}$ ) observed in ECws 5.99 and 0.51  $\text{dS m}^{-1}$  and 0.86 and 0.0069 mM of salicylic acid, respectively (Figures 3C and D). The highest stem mass ratio (0.33  $\text{g g}^{-1}$ ) was observed with ECw of 2.64  $\text{dS m}^{-1}$  (Figure 3E) and the lowest (0.29  $\text{g g}^{-1}$ ) was observed with 1.17 mM

**Table 2:** Summary of analysis of variance

SV	DF	Mean square				
		PH	Ds	NL	Ninf	SDM
Block	1	2.3000 <sup>ns</sup>	0.0120 <sup>ns</sup>	59.0000 <sup>ns</sup>	29.4700 <sup>ns</sup>	2.68 <sup>ns</sup>
ECw	1	3411.0000 <sup>**</sup>	47.3860 <sup>**</sup>	571920 <sup>**</sup>	1826.91 <sup>**</sup>	1251.85 <sup>**</sup>
SA	1	191.4000 <sup>**</sup>	0.2290 <sup>**</sup>	3865 <sup>**</sup>	347.84 <sup>**</sup>	12.65 <sup>**</sup>
ECw x SA	1	0.5000 <sup>ns</sup>	0.0060 <sup>ns</sup>	13468 <sup>**</sup>	369.80 <sup>**</sup>	2.24 <sup>ns</sup>
Residuals	35	8.80	0.0160	226.00	46.34	1.68
SV	DF	LDM	IDM	RDM	SM	LA
Block	1	0.000001 <sup>ns</sup>	2.26 <sup>ns</sup>	1.3460 <sup>ns</sup>	0.1900 <sup>ns</sup>	936 <sup>ns</sup>
ECw	1	521.36 <sup>**</sup>	1245.49 <sup>**</sup>	92.8270 <sup>**</sup>	157.8240 <sup>**</sup>	9043512 <sup>**</sup>
SA	1	16.11 <sup>**</sup>	19.65 <sup>**</sup>	0.6170 <sup>ns</sup>	12.7970 <sup>**</sup>	61121 <sup>**</sup>
ECw x SA	1	6.35 <sup>**</sup>	43.01 <sup>**</sup>	6.0720 <sup>**</sup>	2.4740 <sup>**</sup>	212964 <sup>**</sup>
Residuals	35	0.48	2.44	0.57	0.35	3577
SV	DF	LAR	SLA	LMR	SMR	RMR
Block	1	1.972 <sup>ns</sup>	262 <sup>ns</sup>	0.0002 <sup>ns</sup>	0.000013 <sup>ns</sup>	0.0002 <sup>ns</sup>
ECw	1	23.1720 <sup>**</sup>	80866 <sup>**</sup>	0.0224 <sup>**</sup>	0.0118 <sup>**</sup>	0.0059 <sup>**</sup>
SA	1	13.4350 <sup>*</sup>	9063 <sup>**</sup>	0.0037 <sup>**</sup>	0.0036 <sup>**</sup>	0.0004 <sup>*</sup>
ECw x SA	1	45.6060 <sup>**</sup>	10514 <sup>**</sup>	0.0013 <sup>**</sup>	0.0003 <sup>ns</sup>	0.0014 <sup>**</sup>
Residuals	35	2.3560	510.00	0.0001	0.0003	9.9E-5
SV	DF	IMR	DQI	SI	RQ	RSR
Block	1	0.000015 <sup>ns</sup>	0.1670 <sup>ns</sup>	5.10E-8 <sup>ns</sup>	0.0225 <sup>ns</sup>	0.0002 <sup>ns</sup>
ECw	1	0.1126 <sup>**</sup>	27.9627 <sup>**</sup>	3.09E-5 <sup>**</sup>	14.1225 <sup>**</sup>	0.0075 <sup>**</sup>
SA	1	0.0047 <sup>**</sup>	0.0569 <sup>ns</sup>	6.33E-6 <sup>**</sup>	7.6866 <sup>**</sup>	0.0004 <sup>ns</sup>
ECw x SA	1	0.0082 <sup>**</sup>	1.1110 <sup>**</sup>	4.23E-6 <sup>**</sup>	0.4143 <sup>ns</sup>	0.0018 <sup>**</sup>
Residuals	35	0.0003	0.0670	1.66E-7	0.2734	0.0001

Source of variation (SV); degree of freedom (DF); electrical conductivity of irrigation water (ECw); salicylic acid (SA); plant height (PH); stem diameter (Ds); number of leaves (NL); number of inflorescences (Ninf); stem dry mass (SDM); leaf dry mass (LDM); inflorescence dry mass (IDM); root dry mass (RDM); seed mass (SM); leaf area (LA); leaf area ratio (LAR); specific leaf area (SLA); leaf mass ratio (LMR); root mass ratio (RMR); inflorescence mass ratio (IMR); Dickson quality index (DQI); sclerophilia index (SI); robustness quotient (RQ); root/shoot ratio (RSR). The sources of

of salicylic acid (Figure 3F), above this dose, there was an increase for this variable. Salicylic acid reduced the harmful effects of salt stress on root mass ratio and inflorescence mass ratio, with the highest values (0.075 and 0.939 g g<sup>-1</sup>) observed with ECws 0.53 and 5.99 dS m<sup>-1</sup> and 0.025 and 1.99 mM of salicylic acid, respectively (Figures 3G and 3H).

Salicylic acid alleviated the deleterious effects of salt stress on leaf area, leaf area ratio, specific leaf area, leaf mass ratio, root mass ratio, and inflorescence mass ratio of basil plants, possibly because this phytohormone promotes the plant growth under salt stress, as observed

in *C. sativus* (Miao *et al.*, 2020). In addition, this phytohormone may increase the osmotic adjustment capacity of plants, improving their stress defense mechanisms, in addition to increasing the production of phytohormones to adjust physiological processes and reduce the harmful effects of stress (Khan *et al.*, 2018; Malik *et al.*, 2021). Thus, this phytohormone allows biomass accumulation in plants, even under stress. Salicylic acid increased the absorption of water, nutrients, root growth, and photosynthesis, contributing to the increase in the dry mass of plants under stress in a study performed with cucumber seedlings (Miao *et al.*, 2020).

**Table 3:** Mean values per treatment of *Ocimum basilicum* 'Cinnamon' under salt stress (ECw) and treated with salicylic acid. Mean  $\pm$  standard deviation

ECw (dS m <sup>-1</sup> )	Salicylic acid (mM)				
	0.00	0.29	1.00	1.71	2.00
<b>Plant height (cm)</b>					
0.50			102.26 $\pm$ 2.27		
1.30		89.08 $\pm$ 1.37		92.62 $\pm$ 2.62	
3.25	83.92 $\pm$ 0.92		84.48 $\pm$ 1.34		91.76 $\pm$ 0.79
5.20		74.82 $\pm$ 1.61		77.7 $\pm$ 4.06	
6.00			70.64 $\pm$ 5.08		
<b>Stem diameter (mm)</b>					
0.50			8.42 $\pm$ 0.07		
1.30		8.79 $\pm$ 0.15		7.762 $\pm$ 0.14	
3.25	7.054 $\pm$ 0.11		7.112 $\pm$ 0.12		8.037 $\pm$ 0.19
5.20		6.686 $\pm$ 0.14		5.726 $\pm$ 0.07	
6.00			5.19 $\pm$ 0.11		
<b>Number of leaves</b>					
0.50			576.8 $\pm$ 18.13		
1.30		545.4 $\pm$ 13.04		636.4 $\pm$ 7.23	
3.25	431.0 $\pm$ 10.95		445.2 $\pm$ 22.49		431.2 $\pm$ 12.93
5.20		307.8 $\pm$ 4.58		295.0 $\pm$ 12.93	
6.00			310.0 $\pm$ 14.25		
<b>Number of inflorescences</b>					
0.50			81.4 $\pm$ 6.56		
1.30		90.2 $\pm$ 5.19		99.6 $\pm$ 3.72	
3.25	89.4 $\pm$ 6.18		85.4 $\pm$ 7.39		91.7 $\pm$ 3.57
5.20		87.6 $\pm$ 6.05		79.8 $\pm$ 8.84	
6.00			59.0 $\pm$ 5.09		
<b>Stem dry mass (g)</b>					
0.50			23.42 $\pm$ 0.44		
1.30		24.13 $\pm$ 0.76		22.21 $\pm$ 2.24	
3.25	18.18 $\pm$ 1.48		20.35 $\pm$ 1.78		18.08 $\pm$ 0.53
5.20		12.19 $\pm$ 0.43		11.61 $\pm$ 0.61	
6.00			7.72 $\pm$ 0.59		
<b>Leaf dry mass (g)</b>					
0.50			15.02 $\pm$ 0.84		
1.30		11.97 $\pm$ 0.81		9.69 $\pm$ 0.89	
3.25	10.88 $\pm$ 0.51		6.79 $\pm$ 0.52		8.91 $\pm$ 0.61
5.20		4.41 $\pm$ 0.40		4.39 $\pm$ 0.38	
6.00					
<b>Inflorescence dry mass (g)</b>					
0.50					
1.30		31.39 $\pm$ 1.26		36.52 $\pm$ 2.01	
3.25	28.47 $\pm$ 1.81		28.48 $\pm$ 1.71		29.33 $\pm$ 1.29
5.20		23.36 $\pm$ 0.85		22.622 $\pm$ 1.32	
6.00			20.63 $\pm$ 1.09		

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ECw (dS m <sup>-1</sup> )	Salicylic acid (mM)				
	0.00	0.29	1.00	1.71	2.00
<b>Root dry mass (g)</b>					
0.50			5.50 ± 0.28		
1.30		4.84 ± 0.25		3.53 ± 0.31	
3.25	3.12 ± 1.23		3.33 ± 0.41		4.38 ± 0.24
5.20		1.28 ± 0.14		1.57 ± 0.27	
6.00			1.21 ± 0.13		
<b>Seed mass (g)</b>					
0.50			11.89 ± 0.32		
1.30		8.44 ± 0.85		8.25 ± 1.04	
3.25	7.79 ± 0.37		8.49 ± 0.28		6.42 ± 0.20
5.20		4.69 ± 0.25		5.90 ± 0.35	
6.00			4.96 ± 0.40		
<b>Leaf area (cm<sup>2</sup>)</b>					
0.50			2293.64 ± 72.08		
1.30		2168.78 ± 51.88		2530.64 ± 28.74	
3.25	1713.87 ± 43.56		1770.34 ± 89.43		1714.67 ± 20.65
5.20		1223.97 ± 18.21		1173.07 ± 51.42	
6.00			1232.72 ± 56.68		
<b>Leaf area ratio (cm<sup>2</sup> g<sup>-1</sup>)</b>					
0.50			28.45 ± 0.89		
1.30		29.98 ± 1.48		35.29 ± 2.22	
3.25	28.35 ± 1.59		30.05 ± 1.58		28.28 ± 1.06
5.20		29.69 ± 0.62		29.20 ± 1.19	
6.00			37.12 ± 1.97		
<b>Specific leaf area (cm<sup>2</sup> g<sup>-1</sup>)</b>					
0.50			153.01 ± 6.71		
1.30		182.27 ± 16.20		263.18 ± 23.58	
3.25	157.98 ± 10.13		262.88 ± 32.43		193.53 ± 15.75
5.20		279.65 ± 25.07		268.85 ± 22.86	
6.00			334.65 ± 14.36		
<b>Leaf mass ratio (g g<sup>-1</sup>)</b>					
0.50			0.186 ± 0.009		
1.30		0.165 ± 0.011		0.134 ± 0.009	
3.25	0.179 ± 0.011		0.116 ± 0.011		0.147 ± 0.006
5.20		0.107 ± 0.008		0.109 ± 0.011	
6.00			0.111 ± 0.002		
<b>Stem mass ratio (g g<sup>-1</sup>)</b>					
0.50			0.29 ± 0.005		
1.30		0.333 ± 0.011		0.308 ± 0.022	
3.25	0.299 ± 0.009		0.345 ± 0.024		0.298 ± 0.015
5.20		0.295 ± 0.013		0.289 ± 0.017	
6.00			0.232 ± 0.015		

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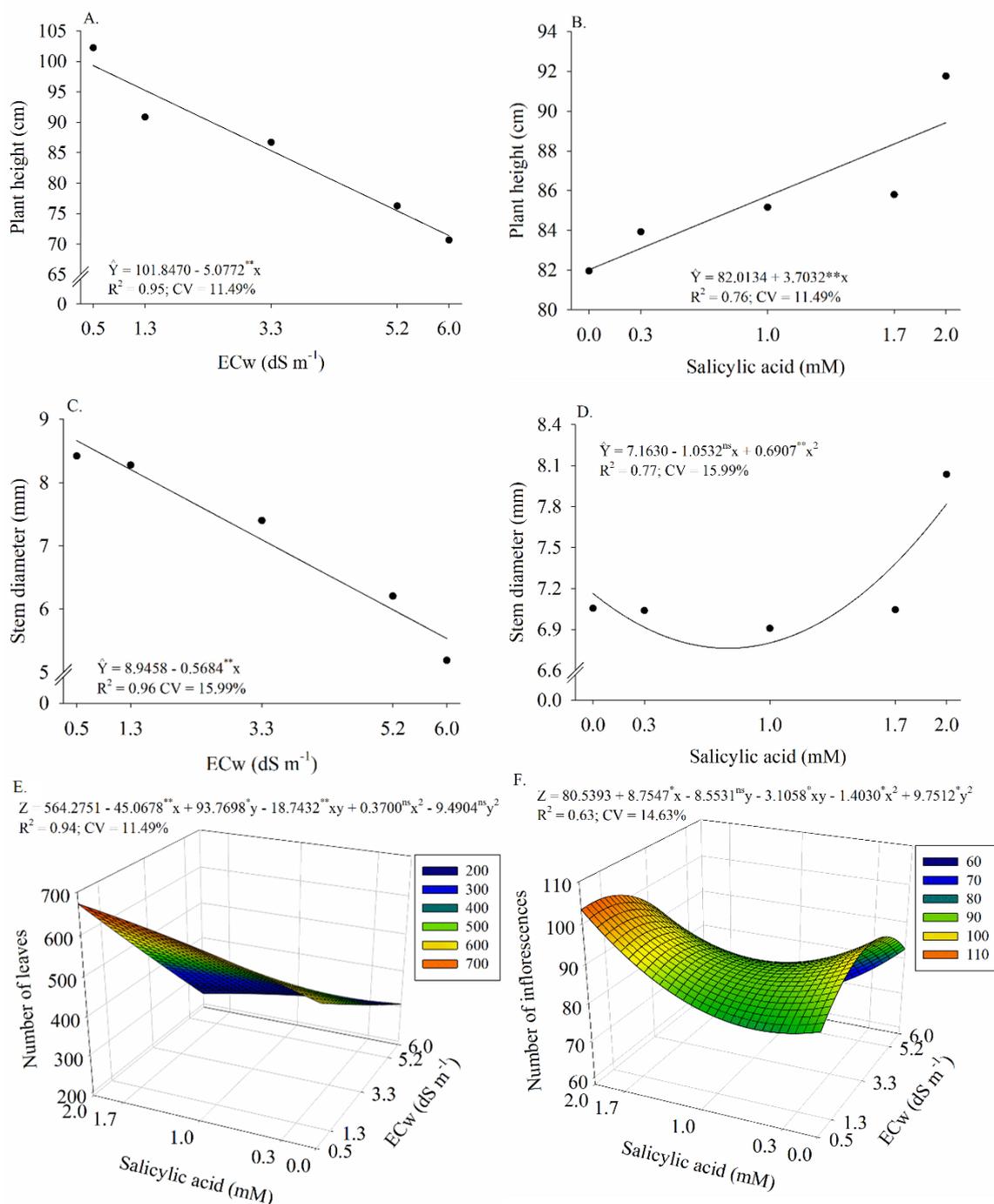
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ECw (dS m <sup>-1</sup> )	Salicylic acid (mM)				
	0.00	0.29	1.00	1.71	2.00
<b>Root mass ratio (g g<sup>-1</sup>)</b>					
0.50			0.068 ± 0.003		
1.30		0.067 ± 0.004		0.049 ± 0.002	
3.25	0.051 ± 0.016		0.056 ± 0.006		0.072 ± 0.002
5.20		0.031 ± 0.003		0.039 ± 0.006	
6.00			0.036 ± 0.005		
<b>Inflorescence mass ratio (g g<sup>-1</sup>)</b>					
0.50			0.455 ± 0.008		
1.30		0.434 ± 0.013		0.508 ± 0.015	
3.25	0.469 ± 0.023		0.483 ± 0.016		0.483 ± 0.008
5.20		0.566 ± 0.015		0.563 ± 0.022	
6.00			0.620 ± 0.014		
<b>Dickson quality index</b>					
0.50			3.124 ± 0.112		
1.30		3.002 ± 0.109		2.295 ± 0.172	
3.25	1.955 ± 0.498		2.054 ± 0.170		2.498 ± 0.118
5.20		0.969 ± 0.083		1.047 ± 0.135	
6.00			0.825 ± 0.050		
<b>Sclerophilia index</b>					
0.50			0.0065 ± 0.0003		
1.30		0.0055 ± 0.0004		0.0038 ± 0.0003	
3.25	0.0063 ± 0.0004		0.0038 ± 0.0005		0.00512 ± 0.0004
5.20		0.0036 ± 0.0003		0.0037 ± 0.0003	
6.00			0.0029 ± 0.0001		
<b>Robustness quotient</b>					
0.50			12.14 ± 0.24		
1.30		10.14 ± 0.31		11.94 ± 0.52	
3.25	11.90 ± 0.31		11.88 ± 0.22		11.42 ± 0.19
5.20		11.19 ± 0.11		13.57 ± 0.66	
6.00			13.61 ± 0.93		
<b>Root/shoot ratio</b>					
0.50			0.0732 ± 0.0039		
1.30		0.0723 ± 0.0043		0.0514 ± 0.0027	
3.25	0.0537 ± 0.0189		0.0598 ± 0.0071		0.0776 ± 0.0027
5.20		0.0319 ± 0.0032		0.0405 ± 0.0063	
6.00			0.0380 ± 0.0058		

Source of variation (SV); degree of freedom (DF); electrical conductivity of irrigation water (ECw); salicylic acid (SA); plant height (PH); stem diameter (Ds); number of leaves (NL); number of inflorescences (Ninf); stem dry mass (SDM); leaf dry mass (LDM); inflorescence dry mass (IDM); root dry mass (RDM); seed mass (SM); leaf area (LA); leaf area ratio (LAR); specific leaf area (SLA); leaf mass ratio (LMR); root mass ratio (RMR); inflorescence mass ratio (IMR); Dickson quality index (DQI); sclerophilia index (SI); robustness quotient (RQ); root/shoot ratio (RSR). The sources of variations intermediate that complete the ANOVA for the central composite design have been removed, keeping only the main ones.

Salicylic acid reduced the harmful effects of salt stress on the Dickson quality index and sclerophilia index, with the highest values (3.43 and 0.0082) observed in ECws 0.53 and 0.51 dS m<sup>-1</sup> and with 0.0152 and 0.0013 mM of

salicylic acid, respectively (Figures 4A and 4B). The lowest robustness quotient (11.34) was observed with the ECw of 2.44 dS m<sup>-1</sup> (Figure 4C) and the highest (12.55) was observed with 1.28 mM of salicylic acid (Figure 3D). In

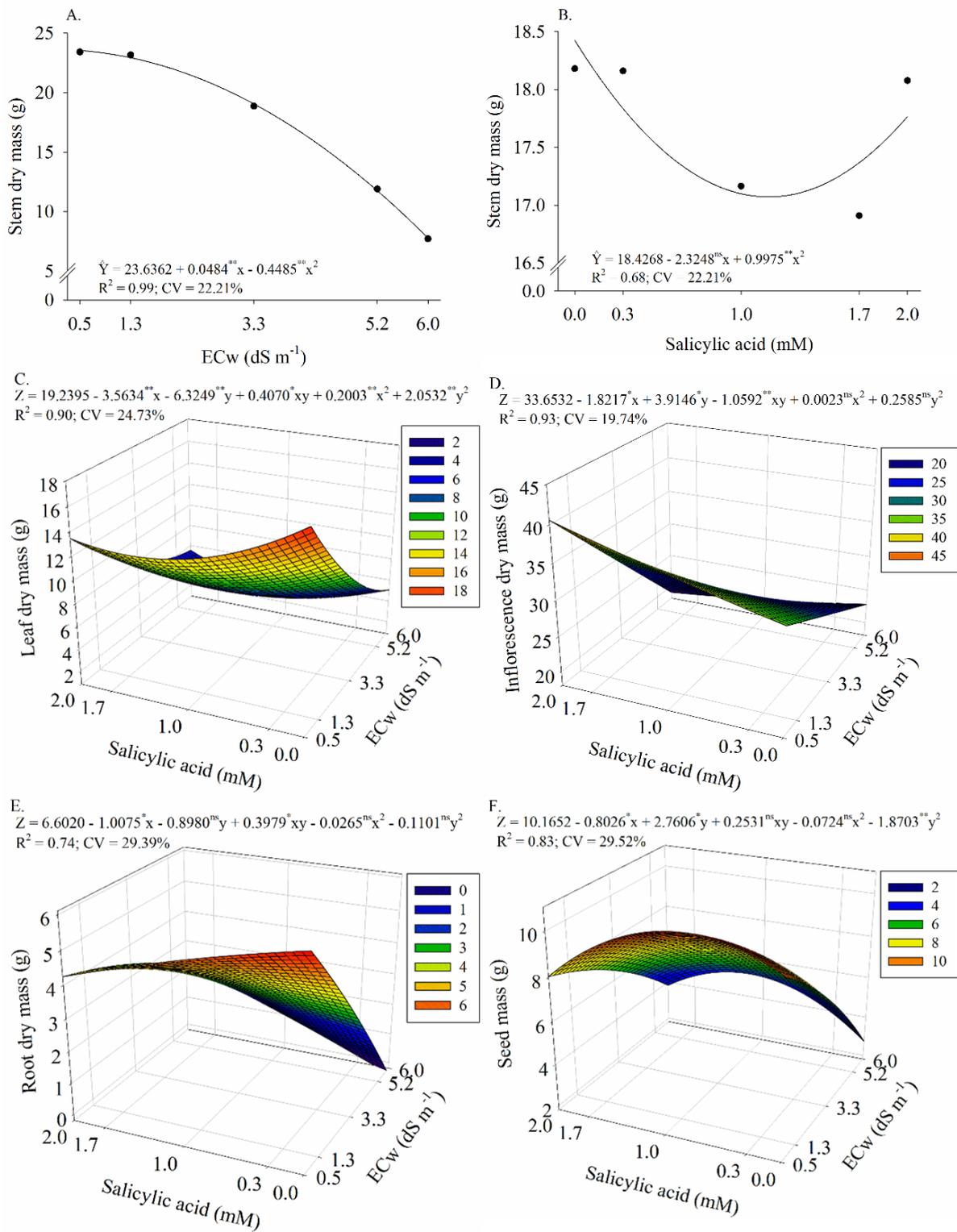


**Figure 1:** Plant height (A and B), stem diameter (C and D), number of leaves (E) and number of inflorescences (F) of *Ocimum basilicum* 'Cinnamon' under salt stress (ECw) and treated with salicylic acid. ns, \*, \*\* - Not significant, significant at  $p \leq 0.05$  and at  $p \leq 0.01$  by F test, respectively.

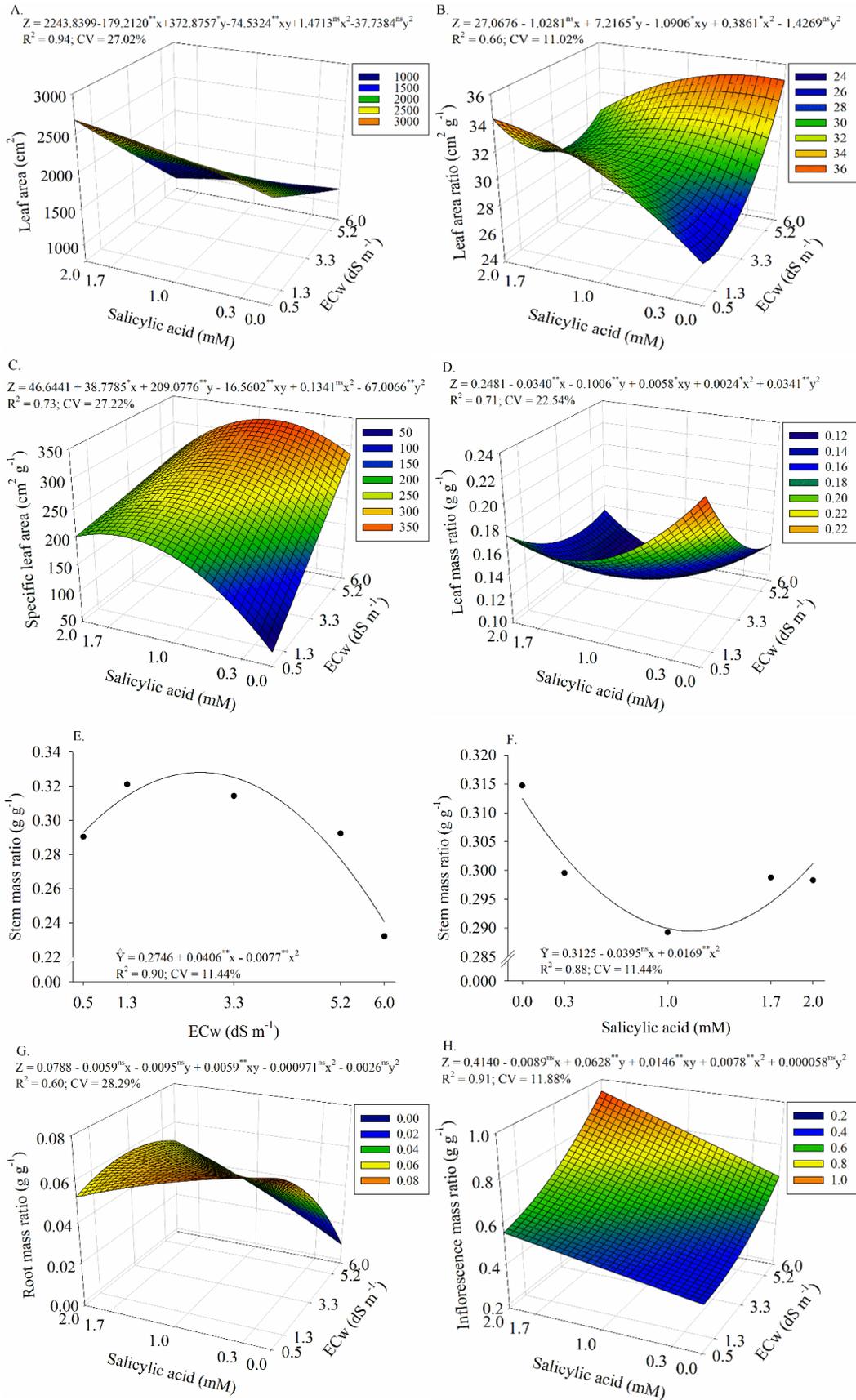
addition, salicylic acid attenuated the harmful effects of salt stress on root/shoot ratio, with the highest value (0.082) observed with ECw 0.56 dS m<sup>-1</sup> and 0.0059 mM of salicylic acid (Figure 4E).

The harmful effects of salt stress on the Dickson quality index, sclerophilia index, robustness quotient, and root/shoot ratio were mitigated by the salicylic acid. This

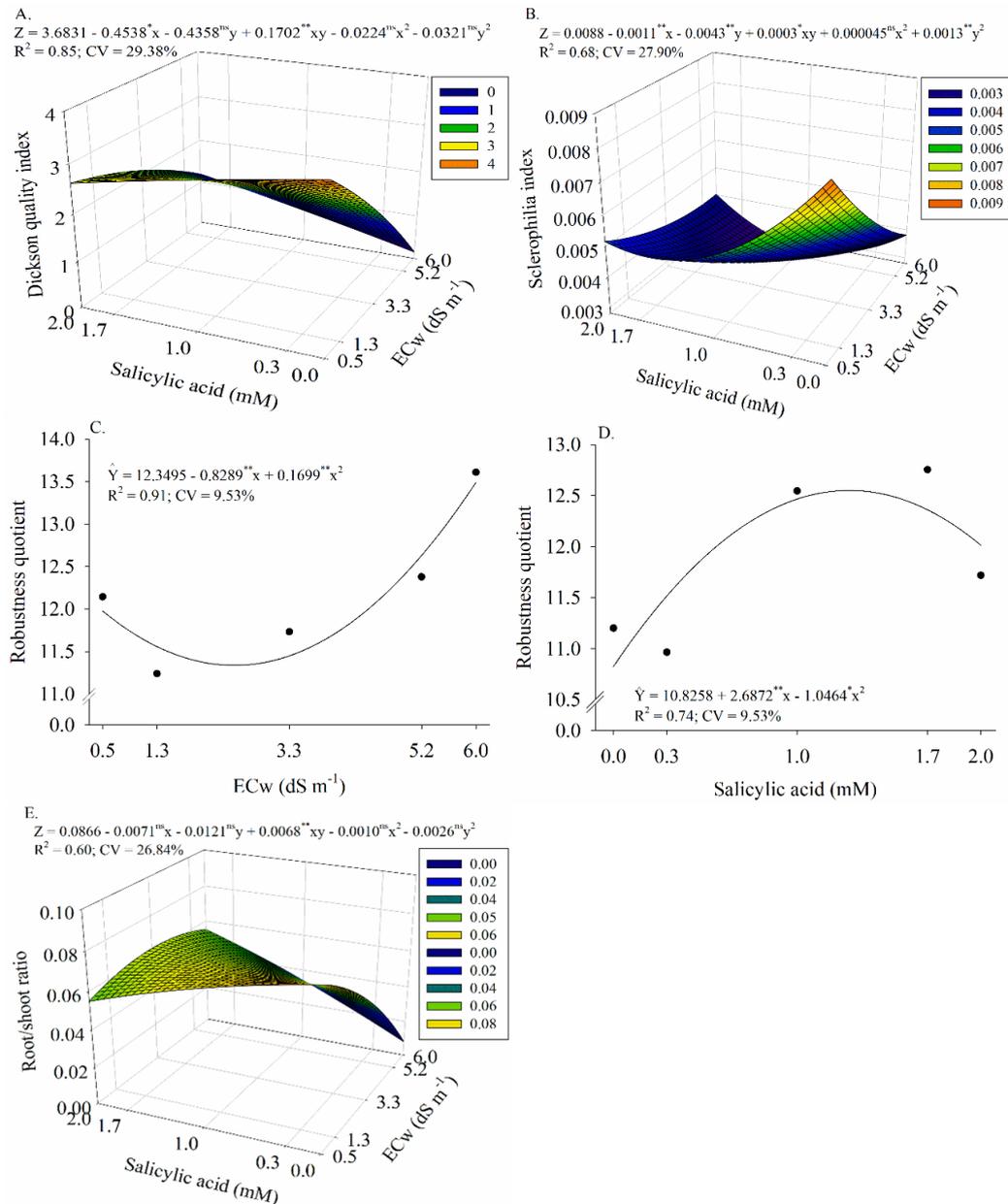
behavior may be related to the action of salicylic acid as a regulator of the synthesis and osmolytes of the enzyme that is linked to growth and photosynthesis, acting mainly against the deleterious effects of salinity (Khanam & Mohammad, 2018). In addition, phytohormones act on gene signaling and expression, increasing plant tolerance to the effects of salt stress (Arif *et al.*, 2020).



**Figure 2:** Stem dry mass (A and B), leaf dry mass (C), inflorescence dry mass (D), root dry mass (E) and seed mass (F) of *Ocimum basilicum* ‘Cinnamon’ under salt stress (ECw) and treated with salicylic acid. ns, \*, \*\* - Not significant, significant at  $p \leq 0.05$  and at  $p \leq 0.01$  by F test, respectively.



**Figure 3:** Leaf area (A), leaf area ratio (B), specific leaf area (C), leaf mass ratio (D), stem mass ratio (E and F), root mass ratio (G) and inflorescence mass ratio (H) of *Ocimum basilicum* ‘Cinnamon’ under salt stress (ECw) and treated with salicylic acid. ns, \*, \*\* - Not significant, significant at  $p \leq 0.05$  and at  $p \leq 0.01$  by F test, respectively.

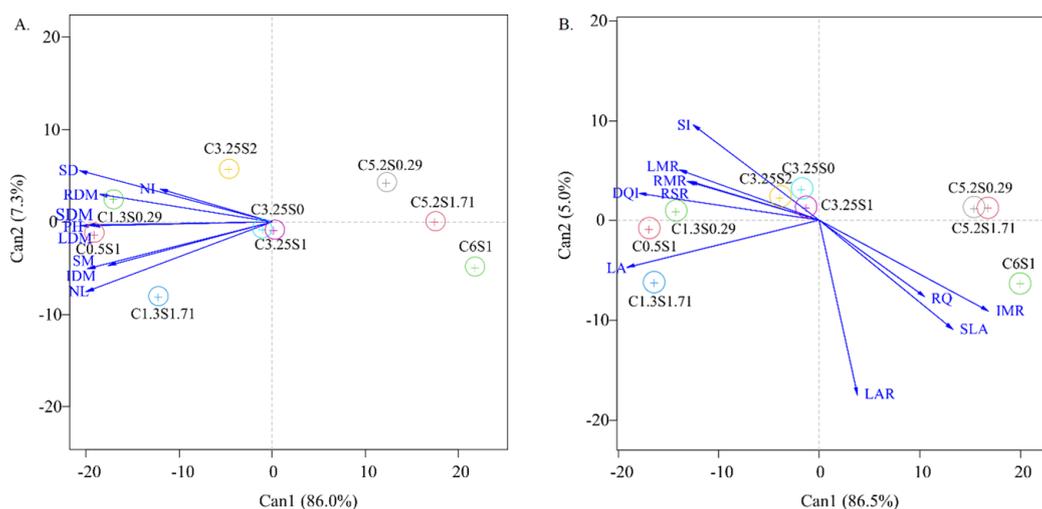


**Figure 4:** Dickson quality index (A), sclerophyllia index (B), robustness quotient (C and D) and root/shoot ratio (E) of *Ocimum basilicum* 'Cinnamon' under salt stress (ECw) and treated with salicylic acid. ns, \*, \*\* - Not significant, significant at  $p \leq 0.05$  and at  $p \leq 0.01$  by F test, respectively.

A canonical variables analysis with confidence ellipses were performed to assess the interrelationships between factors and variables. Stem diameter (Ds), root dry mass (RDM), stem dry mass (SDM), plant height (PH), leaf dry mass (LDM), seed mass (SM), inflorescence dry mass (IDM), and the number of leaves (NL) were more related to ECws 0.50 and 1.30  $\text{dS m}^{-1}$  and 0.29 and 1.0 mM of salicylic acid (Figure 5A). Sclerophyllia index (SI), leaf mass ratio (LMR), root mass ratio (RMR), root/shoot ratio (RSR), Dickson quality index (DQI), and leaf area (LA) had the highest relationship, with ECws 0.50 and 1.30  $\text{dS}$

$\text{m}^{-1}$  and 0.29, 1.0 and 1.71 mM salicylic acid (Figure 5B). Robustness quotient (RQ), inflorescence mass ratio (IMR), specific leaf area (SLA) were more closely related to ECw 6.0  $\text{dS m}^{-1}$  and 1.0 mM of salicylic acid (Figure 5B).

The growth variables of basil plants were benefited from the salicylic acid application, indicating that the presence of this phytohormone mitigated the harmful effects of salt stress. This behavior may have occurred because this phytohormone increases plant tolerance under stress through changes in gene expression (Khan *et al.*, 2020). The reduction in growth due to increased salt stress may be



**Figure 5:** Canonical variables analysis with confidence ellipses between growth variables (A and B) of *Ocimum basilicum* ‘Cinnamon’ under salt stress (C) and salicylic acid (S). Stem diameter (SD); root dry mass (RDM); stem dry mass (SDM); plant height (PH); leaf dry mass (LDM); seed mass (SM); inflorescence dry mass (IMD);, number of leaves (NL); sclerophyllia index (SI); leaf mass ratio (LMR); root mass ratio (RMR); root/shoot ratio (RSR); Dickson quality index (DQI); leaf area (LA); robustness quotient (RQ); inflorescence mass ratio (IMR); specific leaf area (SLA).

related to the harmful effects of salts on physiological and biochemical aspects, such as water absorption, photosynthesis, and oxidative stress (Tahjib-Ul-Arif *et al.*, 2018).

## CONCLUSIONS

Salicylic acid attenuates the harmful effects of salt stress in the basil growth. Salt stress decreased plant height, stem diameter, stem dry mass, and stem mass ratio of basil ‘Cinnamon’. Salicylic acid can be used to lessen the harmful effects of salt stress on basil ‘Cinnamon’.

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