# Water salinity and salicylic acid on tomato plants growth

Salinidade da água e ácido salicílico no crescimento de plantas de tomate

Salinidad del agua y ácido salicílico en el crecimiento de las plantas de tomate

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# Abstract

The tomato is one of the most important vegetables in Brazilian market. The production of this vegetable can be limited by the excess of salts in the water used for irrigation. The use of phytohormones, such as salicylic acid (SA), is used to minimize the negative effects of excess salts on plants. The objective of this paper was to evaluate the attenuating effect of salicylic acid on tomato plants growth irrigated with saline water. The experimental design was the randomized blocks in an incomplete factorial scheme 5 (SA doses: 0.0, 0.29, 1.0, 1.71 and 2.0 mM) x 5 (electrical conductivities of irrigation water - ECw: 0.5, 1.3, 3.25, 5.2 and 6 dS m<sup>-1</sup>), combined according to the experimental matrix Central Compound of Box, with four replicates and two plants per experimental plot. Growth evaluations were performed 45 days after the beginning of irrigation with saline water. Plant height, number of leaves, stem diameter, absolute and relative growth rate for plant height, root dry mass, shoot dry mass, total dry mass, Dickson's quality index, leaf area, specific leaf area and specific leaf weight were evaluated. The tomato plants growth was reduced by the increase in ECw. Salicylic acid, applied exogenously up to 2.0 mM, did not promote attenuating effect of salinity on tomato plants.

Keywords: Solanum lycopersicon L; Saline stress; Phytohormone.

#### Resumo

A tomate é uma das hortaliças mais importantes do mercado brasileiro. A produção dessa hortaliça pode ser limitada pelo excesso de sais na água utilizada para irrigação. O uso de fitormônios, como o ácido salicílico (AS), é utilizado para minimizar os efeitos negativos do excesso de sais nas plantas. O objetivo deste trabalho foi avaliar o efeito

atenuante do ácido salicílico no crescimento de tomateiro irrigado com água salina. O delineamento experimental foi o de blocos ao acaso em esquema fatorial incompleto 5 (doses de AS: 0,0, 0,29, 1,0, 1,71 e 2,0 mM) x 5 (condutividades elétricas da água de irrigação - CEa: 0,5, 1,3, 3,25, 5,2 e 6 dS m<sup>-1</sup>), combinados de acordo com a matriz experimental Composto Central de Box, com quatro repetições e duas plantas por parcela experimental. As avaliações de crescimento foram realizadas 45 dias após o início da irrigação com água salina. Foram avaliados a altura da planta, número de folhas, diâmetro do caule, taxa de crescimento absoluto e relativo para altura da planta, massa seca da raiz, massa seca da parte aérea, massa seca total, índice de qualidade de Dickson, área foliar, área foliar específica e peso específico da folha. O crescimento do tomateiro foi reduzido pelo aumento da CEa. O ácido salicílico, aplicado exogenamente até 2,0 mM, não promoveu efeito atenuante da salinidade em tomateiro. **Palavras-chave:** *Solanum lycopersicon* L; Estresse salino; Fitohôrmonio.

### Resumen

El tomate es una de las hortalizas más importantes del mercado brasileño. La producción de esta hortaliza puede verse limitada por el exceso de sales en el agua utilizada para el riego. El uso de fitohormonas, como el ácido salicílico (AS), se utiliza para minimizar los efectos negativos del exceso de sales en las plantas. El objetivo de este trabajo fue evaluar el efecto atenuante del ácido salicílico sobre el crecimiento del tomate regado con agua salina. El diseño experimental fue de bloques al azar en un esquema factorial incompleto 5 (dosis de AS: 0.0, 0.29, 1.0, 1.71 y 2.0 mM) x 5 (conductividades eléctricas del agua de riego - CEa: 0.5, 1.3, 3.25, 5.2 y 6 dS m<sup>-1</sup>), combinados según la matriz experimental del Compuesto Central de Caja, con cuatro repeticiones y dos plantas por parcela experimental. Las evaluaciones de crecimiento se realizaron 45 días después del inicio del riego con agua salina. Se evaluó altura de planta, número de hojas, diámetro de tallo, tasa de crecimiento absoluto y relativo para altura de planta, peso de raíz seca, masa seca de parte aérea, masa seca total, índice de calidad de Dickson, área foliar, área foliar específica y peso foliar específico. El crecimiento del tomate se vio frenado por el aumento de CEa. El ácido salicílico, aplicado exógenamente hasta 2,0 mM, no promovió el efecto de atenuación de la salinidad en tomate. **Palabras-clave:** *Solanum lycopersicon* L; Estrés salino; Fitohormona.

# 1. Introduction

Tomato (*Solanum lycopersicon* L. - Solanaceae) is one of the main vegetables produced and consumed around the world, being a source of vitamins and minerals (Akbar et al., 2018). The productivity of this vegetable can be influenced by rainfall, cultivar used, temperature, fertilization, in addition to the quality of the water used for irrigation (Rodriguez-Ortega et al., 2019).

Semi-arid regions are characterized by low rainfall and water scarcity, making it necessary to use waters that have high levels of salts (Carbonell et al., 2020), which can compromise or limit the growth and productivity of cultivated plants (Song et al., 2017; Win et al., 2018). Tomato is a species moderately sensitive to salinity, with tolerance up to the electrical conductivity of irrigation water of 3.0 dS m<sup>-1</sup>, above this, growth and production is limited (Rosaldi et al., 2014). The accumulation of salts in the soil, resulting from inadequate anthropic management, increases the salts content in the plant's organs, promoting serious negative effects on the metabolic and physiological processes, such as reduced water absorption capacity by plants, toxicity by specific ions (Na<sup>+</sup> and Cl<sup>-</sup>), causing disorders and injuries in the vegetative and reproductive phases (Acosta-Motos et al., 2017; Lofti et al., 2018; Li et al., 2019).

The exogenous application of salicylic acid (SA) is an alternative to minimize the deleterious effects of salt stress on plants (Silva et al., 2018; Riaz et al., 2019). This phytohormone regulates plant growth, acting on several physiological processes, as responses to the damage caused by abiotic stresses (Farhangi-Abriz & Ghassemi-Golezani, 2018; Kaya et al., 2020). In addition to being involved in the modulation of enzymes that act in physiological and biochemical processes, such as germination, photosynthesis and glycolysis (Nazar et al., 2015; Abdelaal et al., 2020). The beneficial effect of SA on plants under saline stress conditions was found in tomato plants (Gharbi et al., 2018), peppers (Kaya et al., 2020) and melons (Nóbrega et al., 2018).

In view of the need for techniques that reduce the damage caused by salt stress in cultivated plants, the objective of this paper was to evaluate the attenuating effect of salicylic acid on the tomato (*Solanum lycopersicon*) plants growth irrigated with saline water.

# 2. Methodology

The research was carried out in a greenhouse at the Department of Plant Science and Environmental Sciences of the Agricultural Sciences Center of the Federal University of Paraíba (CCA/UFPB), municipaly of Areia, Paraíba, Brazil. Tomato seedlings Santa Cruz Kada (Paulista - Isla<sup>®</sup>) were produced in 128-cells polyethylene trays with commercial substrate (Baseplant<sup>®</sup>), sowing five seeds per cell. Plant thinning was performed after emergence, leaving one plant per cell.

The transplanting of the seedlings to polyethylene pots with a capacity of 5 dm<sup>3</sup> was carried out at 25 days after sowing. The substrate used was soil, sand and cattle manure (3:1:1 v/v), with the chemical characteristics:  $P = 85.55 \text{ mg kg}^{-3}$ ;  $K+ = 693.60 \text{ mg kg}^{-3}$ ;  $Na^+ = 0.23 \text{ cmol}_c \text{ dm}^{-3}$ ;  $H^++Al^{+3} = 0.00 \text{ cmol}_c \text{ dm}^{-3}$ ;  $Al^{+3} = 0.00 \text{ cmol}_c \text{ dm}^{-3}$ ;  $Ca^{+2} = 2.91 \text{ cmol}_c \text{ dm}^{-3}$ ;  $Mg^{+2} = 1.59 \text{ cmol}_c \text{ dm}^{-3}$ ; pH = 7.8;  $BS = 6.50 \text{ cmol}_c \text{ dm}^{-3}$ ; CEC = 6.50;  $OM = 22.21 \text{ g kg}^{-1}$ .

Irrigation was performed daily, with irrigation control established through drainage lysimetry. The saline waters were prepared by adding NaCl to the water with less electrical conductivity (0.5 dS m<sup>-1</sup>) from the UFPB supply system. A microprocessed portable conductivimeter (Instrutherm<sup>®</sup>, model CD-860) was used to measure the ECw.

Distilled water heated to  $\pm$  90 °C was used to prepare the doses of salicylic acid (SA), which were applied with a manual spray. SA applications were performed every 15 days, with three applications (with about 35 mL of solution per plant). The plants were watered completely until the leaf solution started to drain.

The evaluations were carried out at 45 days after the beginning of the irrigation with saline water (day after irrigation - DAI), being measured the variables: number of leaves (obtained from counting the number of fully formed leaves); plant height (established by measuring with a graduated ruler of the shoot of the plant, considering the part above the neck to the stem apex, with the results expressed in cm); stem diameter (determined with the a digital caliper, with results expressed in mm); root dry mass and shoot dry mass (after the separation of the parts, they were packed in Kraft paper bags and placed to dry in a oven at 65 °C until constant weight). The material weighing was carried out on a precision scale (0.001 g), the results being expressed in g plant<sup>-1</sup>); total dry mass (obtained from the sum of the dry mass values of the root and shoot, with the results in g plant); Dickson's quality index (Dickson et al., 1960) (DQI = [TDM/(PH/SD)/(SDM/RDM)]), being PH (plant height), SD (stem diameter), SDM, RDM, TDM (shoot dry mass, root dry mass and total dry mass, respectively); leaf area (Blanco and Folegatti, 2003), in cm<sup>2</sup>, LA = 0.347x (LW)-10.7; specific leaf area (obtained by the ratio between leaf area (LA) and leaf dry mass (LDM), expressed in cm<sup>2</sup> g<sup>-1</sup> (Benincasa, 2003); specific leaf weight (determined by the ratio between LDM and LA, expressed in g cm<sup>2</sup> (Benincasa, 2003); relative and absolute growth rates for plant height were performed according to the equations: TCAap = (PH2-PH1/t2-t1) and TCRap (lnPH2-lnPH1/t2-t1) (Benincasa, 2003).

The experimental design was the randomized blocks in an incomplete factorial scheme 5 (SA doses: 0.0, 0.29, 1.0, 1.71 and 2.0 mM) x 5 (electrical conductivities of irrigation water - ECw: 0.5, 1.3, 3.25, 5.2 and 6 dS m<sup>-1</sup>), combined according to the experimental matrix Central Compound of Box, with four replicates and two plants per experimental plot. The data were submitted to analysis of variance by the F test and, as they are not significant by this parametric method, standard errors were estimated for the graphs production. The statistical program used was the R (R Core Team, 2018).

# 3. Results

## Effect of salinity on tomato plants growth

The electrical conductivities of irrigation water (ECw) negatively affected the tomato plants growth. The highest plant height (68.2 cm) was observed in plants submitted to ECw of 0.5 dS  $m^{-1}$ . A 33.3% reduction was observed when comparing the largest with the smallest ECw. The increase in ECw reduced the plant height by 5.88% per unit increase of salinity (Figure 1A).





The stem diameter decreased with the increase in ECw, with decreases of 13.5% when subjected to the highest salinity when compared to the lowest ECw (Figure 1B). A 2.43% reduction per unit increment of ECw was observed. The increase in ECw decreased the number of leaves. The highest leaf emission (11 leaves) was in the ECw of 0.5 dS m<sup>-1</sup>, with a

reduction of 26.5% in plants submitted to the highest ECw (Figure 1C). A 4.53% reduction per unit increment of ECw was observed. The leaf area of tomato plants was reduced with the increase in ECw, with a decrease of 6.57% by unit increase in salinity and reductions of 37.4% in ECw of 6 dS m<sup>-1</sup> (Figure 1D). The largest specific leaf area (160.96 cm<sup>2</sup> g<sup>-1</sup>) and specific leaf weight (0.0080 g cm<sup>2</sup>) were obtained in plants submitted to ECw of 1.3 dS m<sup>-1</sup> and 3.25 dS m<sup>-1</sup>, respectively (Figure 1E and 1F).

The relative growth rate (Figure 2A) and absolute growth rate (Figure 2B) were negatively influenced by salinity, with severe decreases as the ECw increased, with decreases of 29.2 and 46.1% and a unit decrease per unit increment of ECw of 5.22 and 8.03%, respectively, when comparing the values of the lowest and highest salinity.

**Figure 2.** Absolute growth rate of height (A), relative growth rate of height (B), root dry mass (C), shoot dry mass (D), total dry mass (E) and Dickson's quality index (F) of tomato plants submitted to different electrical conductivities of irrigation water.





The dry biomass accumulation from tomato plants was reduced by the negative effects of saline stress, with reductions in root dry mass, shoot dry mass and total dry mass, with losses of 59.3, 38.5 and 43.4%, respectively, when comparing plants submitted to higher and lower ECw (Figure 2C, 2D and 2E). The reductions were 10.24, 6.76 and 7.61% per unit increment of ECw, respectively. The best seedlings quality, evaluated by the Dickson's quality index, was observed in plants grown under ECw of 0.5 dS m<sup>-1</sup>, with a superiority of 51.2% in relation to seedlings produced under ECw of 6.0 dS m<sup>-1</sup> (Figure 2F). A decrease of 8.90% per unit increment of ECw was observed.

# Effect of salicylic acid on tomato plants growth

The salicylic acid (SA) application up to 2.0 mM did not influence the growth of tomato plants subjected to saline stress (Figure 3).





The largest (59.2 cm) and smallest (54.45 cm) mean for the plant height was observed in the doses of 1.71 and 0.29 mM SA, respectively (Figure 3A). The largest (6.74 mm) and smallest (6.41 mm) stem diameter was observed in plants

submitted to doses of 0.29 and 2.0 mM of SA, respectively (Figure 3B). The largest (10 leaves) and smallest (9 leaves) number of leaves was observed in plants under 1.0 and 0.0 mM SA, respectively (Figure 3C). The largest (645.18 cm<sup>2</sup>) and smallest (570.83 cm<sup>2</sup>) leaf area was observed in plants subjected to the application of 0.29 and 0.0 mM SA, respectively (Figure 3D). The highest (160.06 cm<sup>2</sup> g<sup>-1</sup>) and lowest (118.15 cm<sup>2</sup> g<sup>-1</sup>) mean for the specific leaf area were observed in plants submitted to a dose of 1.71 and 0.0 mM AS, respectively (Figure 3E). The opposite behavior was observed for the specific leaf weight, with the highest value (0.0089 g cm<sup>2</sup>) obtained in the plants of the control treatment (0.0 mM), and the lowest (0.0074 g cm<sup>2</sup>) at the dose of 1.71 mM SA (Figure 3F).

The rates of absolute and relative growth for plant height, root dry mass, shoot dry mass, total dry mass and Dickson's quality index of tomatoes were not influenced by the SA application (Figure 4). The highest averages (2.31 cm day<sup>-1</sup> and 0.062 cm cm day<sup>-1</sup>, respectively) and the lowest (2.0 cm day<sup>-1</sup> and 0.051 cm cm day<sup>-1</sup>, respectively) for the absolute and relative growth rates of height of tomato plants were obtained at doses of 1.71 and 0.0 mM SA, respectively (Figure 4A and 4B).

**Figure 4.** Absolute growth rate of height (A), relative growth rate of height (B), root dry mass (C), shoot dry mass (D), total dry mass (E) and Dickson's quality index (F) of tomato plants submitted to salicylic acid.



The highest (2.12 g plant) and smallest (1.93 g plant) accumulation of dry root biomass was observed in plants submitted to doses of 0.29 and 0.0 mM SA, respectively (Figure 3D). The highest averages (8.17 and 10.10 g plant,

respectively) for the shoot dry mass and total dry mass were obtained in the control (0.0 mM) and the lowest averages (6.37 and 8.0 g plant), respectively, were observed in the application of 1.71 mM SA (Figure 3E and 3F). The highest (0.34) and lowest (0.24) mean for Dickson's quality index was observed in plants submitted to a dose of 0.29 and 1.71 mM of SA, respectively (Figure 4F).

# 4. Discussion

The salinity of the irrigation water reduced the growth of tomato plants. The reduction in plant growth is associated with damage caused by saline stress, causing a decrease in turgor pressure by reducing the availability and absorption of water, leading to restriction in cell expansion and plant growth (Wang et al., 2019). Changes in cellular metabolic activities caused by saline stress limit cell elongation and cell wall elasticity (Charfeddine et al., 2018), negatively affecting plant growth and development (Song et al., 2017; Carbonell et al., 2020).

The greatest reduction in plant height, stem diameter and number of leaves were observed in the highest concentrations of NaCl (higher ECw), due to the toxicity of these specific ions, nutritional imbalance and decreased metabolic activities of the plant (Alvares-Acosta et al., 2019; Li et al., 2019). The reduction in leaf area, specific leaf area and specific leaf weight occurs due to acclimative morphological changes of the plant to reduce the saline stress damages, since the saline ions accumulation reduces the turgor in leaves, decreases gas exchange, limiting photosynthesis and, consequently, leaf expansion (He et al., 2019). The negative effect of saline stress on the growth of tomato plants has been observed by other authors (Rosadi et al., 2014; Win et al., 2018; Khalid et al., 2020).

The increase in ECw reduced the biomass accumulation in tomato plants. The decrease in biomass accumulation is associated with ions phytotoxicity (Na<sup>+</sup> and Cl<sup>-</sup>, mainly), nutritional imbalance, oxidative stress caused by increased production of reactive oxygen species (ROS) and biochemical and metabolic changes (Acosta-Motos et al., 2017; Li et al., 2019). The dry mass production is negatively affected by the increase in the levels of salts in the cellular compartments that cause water restriction, osmotic stress, reducing growth due to the effects on the assimilation and diffusion of CO<sub>2</sub> in the stomata, photosynthesis and accumulation of cellular reserves (Khalid et al., 2020). These effects were seen in tomatoes (El Arroussi et al., 2018) and peppers - *Capsicum annum* L. (Kaya et al., 2020).

The tomato plants quality, as evidenced by the absolute and relative growth rates for plant height and Dickson's quality index, demonstrate the damage caused by saline stress, resulting from osmotic stress and water reduction, nutritional imbalance, result in physiological and morphological changes, affecting the yield and vigor of plants (Rodriguez-Ortega et al., 2019).

The plants height, number of leaves, leaf area, specific leaf area and root dry mass were stimulated by the SA application, although the increments provided were not very representative. SA is a phytohormium that can act directly or indirectly in the plant growth regulation, acting in several processes involved in plant growth, acting in the division, elongation and cell death (Nóbrega et al., 2018).

The absolute and relative growth rates for the plants height that were stimulated by the SA application, even if in small proportions. This phytohormone can exert systemic resistance in response to salt stress, because it is a hypersensitive molecule, acting against the ionic restriction imposed by the high Na<sup>+</sup> concentration and the osmotic restriction by reducing the osmotic and water potential, reducing the water availability for the plant (Gharbi et al., 2018). The response to resistance induction was proven in rosemary - *Rosmarinus officinallis* L. (El-Esawi et al., 2017), basil seedlings - *Ocimum basilicum* L. (Silva et al., 2018), and sweet pepper - *Capsicum annum* L. (Abdelaal et al., 2020).

SA did not promote increases in stem diameter, specific leaf weight, shoot dry mass and total dry mass, decreasing with the application of this phytohormone. The exogenous application of salicylic acid will not always promote effects on plant growth, and the effects may vary according to the species (El-Esawi et al., 2017; Farhangi-Abriz & Ghassemi-Golezani, 2018).

The values obtained for the Dickson's quality index are considered low and indicate that, even with the application of salicylic acid, the tomato plants were not very vigorous. Possibly, the SA applied via leaf did not manage to accumulate and be metabolized by the plant, since after its application it is necessary around 12 hours for the maximum concentration to accumulate and to be able to signal and activate enzymes that act in the antioxidant system of plant (Poór et al., 2017).

# 5. Conclusion

The salinity of the irrigation water negatively affected the growth of tomato plants, with the most severe effects on the highest electrical conductivities. The exogenous application of salicylic acid is not efficient to mitigate the osmotic and toxic effects of saline stress in tomato plants.

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