









# Combined fertilization with nitrogen-potassium to mitigate salt stress in okra

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## Abstract

Okra (*Abelmoschus esculentus*) is sensitive to the salinity of water and soil, a common abiotic stress in the semi-arid region of northeastern Brazil; however, such sensitivity is variable with water management in the soil-plant-atmosphere system. Thus, the objective was to evaluate the fertilization with nitrogen-potassium combinations in okra cultivated under salt stress. Photosynthetic pigments, gas exchange, growth and fruit quality were evaluated. Fertilization with N1:K1 (40% N + 40% K<sub>2</sub>O) reduced the damage caused by salt stress and increased the chlorophyll contents, while the application of N4:K4 (130% N + 100% K<sub>2</sub>O) decreased the damage caused by salt stress and increased the carotenoid contents. The combination of N:K fertilization did not affect gas exchange, however, salt stress decreased these variables. Fertilization with N2:K2 (70% N + 60% K<sub>2</sub>O) reduced the damage caused by salt stress and increased the leaf area of okra. In addition, salt stress decreased the number of fruits and increased the fresh mass of okra fruits. The N:K fertilization also reduced the damage caused by salt stress and in the soluble solids content, titratable acidity, SS/TA ratio, and ascorbic acid content. Thus, N:K fertilization can be seen as an important agronomic strategy to improve the performance of okra quality subjected to saline conditions.

**Keywords:** *Abelmoschus esculentus*, fruit quality, growth, photosynthesis

## Introduction

Brazil stands out for the edaphoclimatic conditions favorable to most cultivated plants, including okra (*Abelmoschus esculentus* L.) due to its rusticity, highlighting the tolerance to heat and the non-requirement of advanced technologies for its cultivation (Oliveira et al., 2007). Okra is a source of important nutrients for human health, such as vitamins, potassium, calcium, carbohydrates and unsaturated fatty acids (linolenic and oleic acids) (Elshaikh et al., 2018).

However, to produce satisfactorily in the semi-arid region of northeastern Brazil, the okra crop requires the use of supplemental irrigation due to the agroclimatic imbalance. The low rainfall levels hamper the development of this crop; however, most of the time the waters used for irrigation, from wells and dams, are considered to be of low quality because they have an average salinity of 2.5 dS m<sup>-1</sup>. Salinity is one of the main

limiting abiotic factors of the environment for plant growth and development, due to the reduction in the osmotic potential of the soil solution, so the plant needs to spend more energy to absorb water and nutrients (Leonardo et al., 2008).

The increase of fertilizers applied in a salinity-sensitive crop may increase the concentrations of N and K in leaves and, therefore, promote an increase in the tolerance of the crop to salinity (Andrade Júnior et al., 2011). Due to the importance of nitrogen for plant growth and development, for being a structural element and because it is part of numerous organic compounds that are vital to the plant such as amino acids, proteins, chlorophyll, nucleic acids (Taiz et al., 2017). The proper management of this nutrient contributes to vegetative growth and photosynthetic expansion and increases crop yield under conditions of salt stress (Souza et al., 2017). Nitrogen nutrition plays an important role in plant

tolerance to salt stress, therefore, adequate supply of nitrogen can compensate and correct nutritional imbalance in plants under salt stress (Yao et al., 2021). The relationship between salinity and N metabolism depends on the degree and duration of salt stress, plant species, plant growth stage, amount, type and form of N in the rhizosphere (Ashraf et al., 2018).

Similarly, potassium also has great importance in the plant, being a constituent with several functions, such as control of tissue turgor, activation of many enzymes involved in respiration and photosynthesis, opening and closing of stomata, transport of carbohydrates, transpiration, and resistance to drought and salinity. These organic compounds can increase the capacity of plants to adjust to salinity, corroborating the resistance to salt stress. Upregulation of K decreases the generation of reactive oxygen species (ROS) in plants by reducing the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and retaining photosynthetic electron transport activity (Hasanuzzaman et al., 2018).

Thus, given the relevance of the crop for the economy and diet of Brazilians and the sensitivity that this species to salinity, the objective was to evaluate the fertilization with nitrogen-potassium combinations in okra cultivated under salt stress.

## Material and Methods

The study was performed in the experimental area of the Center for Science and Agri-Food Technology of the Universidade Federal de Campina Grande (CCTA/UFCEG), Pombal, PB, Brazil (6°46'13" S and 37°48'06" W, altitude of 184 m). The predominant climate in the region according to Köppen's classification is BSh, that is, hot semi-arid, with annual rainfall of 750 mm and rains concentrated from December to April.

The experimental design used was randomized blocks, in a 5 x 5 factorial scheme with three replications, composed of the following factors: levels of irrigation water salinity – ECw (0.3; 1.0; 1.7; 2.4 and 3.1 dS m<sup>-1</sup>) and nitrogen-potassium fertilization combinations (N1:K1 = 40% N + 40% K<sub>2</sub>O; N2:K2 = 70% N + 60% K<sub>2</sub>O; N3:K3 = 100%

N + 80% K<sub>2</sub>O; N4:K4 = 130% N + 100% K<sub>2</sub>O; N5:K5 = 160% N + 120% K<sub>2</sub>O). Considering all the factors, there were 25 treatments repeated in three blocks, each plot consisting of one usable plant, in a total of 75 experimental units. The commercial cultivar of okra used was 'Santa Cruz 47', with characteristics of vigor, short internodes, and fruits of light green color, cylindrical, with slightly curved tip and lower fiber content compared to those of other cultivars.

The lysimeters with 25-L capacity were connected to a 1.0-mm-diameter hose at the base, in order to allow drainage and estimation of water consumption per plant. The lysimeters were filled with a 1.0-kg layer of coarse sand plus crushed stone so that the base was covered to facilitate drainage. After this layer, the lysimeters received 22 kg of non-saline, non-sodic soil collected in the rural area of the municipality of São Domingos - PB, with collection of samples at a depth of 0-0.3 m (Table 1).

Phosphate fertilization was top-dressed with 11.37 g of MAP as recommended by Novais et al. (1991). In each lysimeter, five okra seeds were sown equidistantly and, after stabilization of emergence, thinning was performed at 15 DAS, keeping the most vigorous plant. Nitrogen and potassium doses were applied based on the recommendation of Novais et al. (1991), using 100 mg of N kg<sup>-1</sup> of soil and 150 mg of K<sub>2</sub>O kg<sup>-1</sup> of soil. Nitrogen source was urea - CH<sub>4</sub>N<sub>2</sub>O (45% N) and potassium source was potassium chloride - KCl (60% K), starting the applications at 40 DAS, performed as top-dressing and in circles, split into three portions with an interval of 15 days during the crop cycle.

Weeds were controlled by manual weeding in the pots and weeding in the experimental area, while the control of pests and diseases was established according to the recommendation of the crop. The waters with different salinity levels were prepared using water from the local supply system (0.3 dS m<sup>-1</sup>) and considering the relationship between ECw and salt concentration (10\*meq = 1 dS m<sup>-1</sup> of ECw), extracted from Rhoades et al. (1992), valid for ECw from 0.1 to 5.0 dS m<sup>-1</sup>. To obtain the waters with electrical conductivity from 1.0

**Table 1.** Chemical and physical characteristics of the soil used in the experiment

Chemical characteristics								
pH (H <sub>2</sub> O) (1:2.5)	OM g kg <sup>-1</sup>	P (mg kg <sup>-1</sup> )	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup>
.....Chemical characteristics.....			.....Physical characteristics.....					
ECse (dS m <sup>-1</sup> )	CEC cmol <sub>c</sub> kg <sup>-1</sup>	SAR (mmol L <sup>-1</sup> ) <sup>0.5</sup>	ESP %	Particle-size fraction(gkg <sup>-1</sup> )			Moisture (dag kg <sup>-1</sup> )	
				Sand	Silt	Clay	33.42 KPa <sup>1</sup>	1519.5 KPa <sup>2</sup>
5.58	2.93	39.2	0.23	1.64	9.07	2.78	0.0	8.61
2.15	22.33	0.67	7.34	572.7	100.7	326.6	25.91	12.96

pH - Hydrogen Potential, OM - Organic Matter; Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc at pH 7.0; Al<sup>3+</sup>+H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; ECse - Electrical conductivity of saturation extract; CEC - Cation exchange capacity; SAR - Sodium adsorption ratio of saturation extract; ESP - Exchangeable sodium percentage; <sup>1,2</sup> referring to the limits of field capacity and permanent wilting point.

dS m<sup>-1</sup>, NaCl was diluted according to treatment until reaching the desired EC level, by checking the values with a conductivity meter with temperature adjusted to 25 °C. The waters were stored in plastic containers of 500 L, properly protected to avoid evaporation, entry of rainwater and contamination with materials that could compromise their quality.

Salt stress application started at 25 DAS when irrigation events were performed at one-day intervals. The applied depth was determined based on the water balance in order to replace the average daily consumption of the plants, plus an additional fraction, dividing the value of the volume to be applied (mL) by 0.9 to obtain a leaching fraction corresponding to 10%. The leaching fraction was applied every seven days, to promote the leaching of excess salts from the root zone, originated from irrigation water, according to equation:  $VI = (Va - Vd) / (1 - LF)$ ; where: VI = volume to be irrigated in the next irrigation event (mL); Va = volume applied in the previous irrigation event (mL); Vd = volume drained (mL), and LF = coefficient used to obtain a leaching fraction of approximately 10%.

Photosynthetic pigments were evaluated at 77 days after sowing (DAS). Carotenoid (470 nM), chlorophyll a (663 nM) and chlorophyll b (646 nM) and total chlorophyll contents were analyzed using a spectrophotometer. Eight leaf discs collected from the middle third of the branch in the middle region of the canopy of the plants, corresponding to a total disc area of 9.04 cm<sup>2</sup> per plant, were used. Subsequently, the material was chopped and immersed in 6 mL of 80% acetone, contained in glass containers with a capacity of 10 mL, where the samples remained completely in the dark for 48 hours in a refrigerator at a temperature of 8 °C to extract the pigments from the supernatant. The contents of chlorophyll a =  $((12.21 * A_{663}) - (2.81 * A_{646}))$ ; chlorophyll b =  $((20.13 * A_{646}) - (5.03 * A_{663}))$ ; total chlorophyll =  $((17.3 * A_{646}) + (7.18 * A_{663}))$  and carotenoids  $[(1000 * A_{470} - 1.82 * Cl_a - 85.02 * Cl_b) / 198]$  were analyzed according to the Lichtenthäler (1987).

Gas exchange evaluation was performed with an infrared gas analyzer - IRGA (LCpro+ - ©ADC BioScientific) with constant light of 1200 μmol m<sup>-2</sup> s<sup>-1</sup>. Stomatal conductance (gs - mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration (E - mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and net photosynthesis (A - μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) were evaluated.

The number of fruits, stem diameter, leaf area and fruit fresh mass were evaluated. The leaf area were measured with a graduated ruler, and the sum of the leaf area of the plant was measured according to the

methodology established by Fideles Filho et al. (2010). The equation used was:  $Y = \sum 0.7254 (X)^{2.08922}$ ; where Y = leaf area of the plant and X = midrib length.

The fruits were harvested at complete physiological maturity, the ideal harvesting point, with growth and development where the fruits reached the ideal level of maturation, tender for consumption in natura. The extract was obtained from the ratio 1:1 (m:v) with the aid of a blender (MONDIAL maxis filter 800 W). The extract was transferred to plastic containers identified with lids.

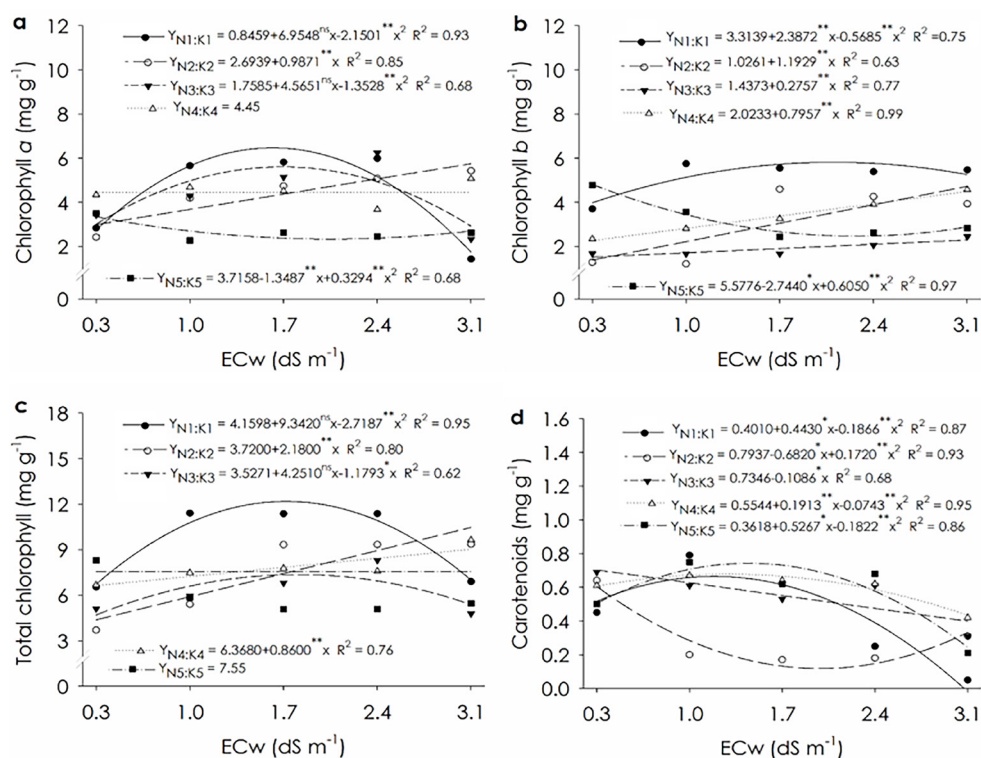
The soluble solids content was determined in a digital refractometer (ITREFD65) with automatic temperature compensation and resolution of 0.2 (IAL, 2008). The titratable acidity was determined according to the methodology of Instituto Adolfo Lutz (IAL, 2008), with the results expressed in percentage of citric acid, equivalent to the amount of NaOH 0.1 N spent in the titration. The ascorbic acid content was estimated from 1.0 g of the extract added to 49 mL of 0.5% oxalic acid and titrated with Tillmans' solution until reaching a pink color according to the method (365/IV) (IAL, 2008).

The data were subjected to analysis of variance and, when significant, a regression analysis was performed. R software (R CORE TEAM, 2021) was used to perform the analyses.

## Results And Discussion

Chlorophylls and carotenoids contents had interaction between the factors. The highest contents of chlorophyll a (6.47 and 5.61 mg g<sup>-1</sup>) were observed with the application of 40% N + 40% K<sub>2</sub>O (N1:K1) and 100% N + 80% K<sub>2</sub>O (N3:K3) at ECw of 1.62 and 1.69 dS m<sup>-1</sup>, respectively. The application of 130% N + 100% K<sub>2</sub>O (N4:K4) and an increase in ECw had a linear increase in this variable. The lowest chlorophyll a content (2.34 mg g<sup>-1</sup>) was observed with the application of 160% N + 120% K<sub>2</sub>O (N5:K5) and ECw of 2.34 dS m<sup>-1</sup>, in addition, the application of 70% N + 60% K<sub>2</sub>O (N2:K2) showed no difference (**Figure 1a**).

The highest chlorophyll b content (5.85 mg g<sup>-1</sup>) and total chlorophyll (12.19 mg g<sup>-1</sup>) were observed with the application of N1:K1 and ECw of 2.10 and 1.72 dS m<sup>-1</sup>, respectively (Figure 1b and 1c). Application of N2:K2 and N3:K3 increased 1.19 (116.16%) and 0.28 (19.18%) mg g<sup>-1</sup> of chlorophyll b per unit increase in ECw, while application of N2:K2 increased 2.18 (58.60%) mg g<sup>-1</sup> of total chlorophyll per unit increase in ECw. The highest (0.74 mg g<sup>-1</sup>) and lowest (0.12 mg g<sup>-1</sup>) carotenoid content was observed with the application of N4:K4 and N2:K2 and ECw of 1.45 and 1.92 dS m<sup>-1</sup>, respectively (Figure 1d).



**Figure 1.** Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and carotenoids (d) of okra (*Abelmoschus esculentus*) grown under combined nitrogen (N) and potassium (K) fertilization and irrigated with saline water (ECw).

Application of nitrogen (N) and potassium (K) mitigated the damaging effects of salt stress in okra plants on chlorophyll and carotenoid contents (Figures 1a-d) due to the nitrogen supply decreasing the toxicity caused by salt stress by modifying the concentration of K<sup>+</sup>, Na<sup>+</sup> and relieving ROS pressure (Yao et al., 2021). Since the chlorophyll content is approximately proportional to the nitrogen content of the leaf (Yu-Kui et al., 2012; Farhangi-AbRiz & Torabian, 2018). In addition, K plays a primary role in chlorophyll and especially in ensuring photosynthetic activity in plants, since K<sup>+</sup> prevents the breakdown of formed chlorophyll and plays a significant role in the formation of the photosynthetic pigment (Naciri et al., 2021).

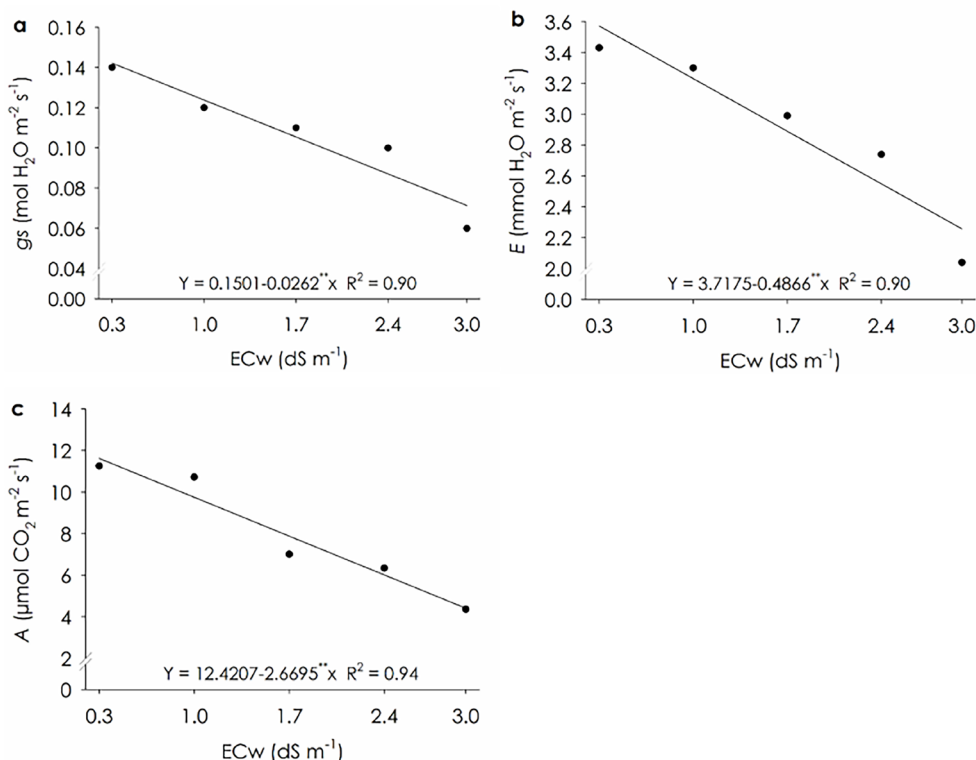
Gas exchange had no interaction between the factors, however, there was an effect of salinity on them. ECw decreased stomatal conductance (g<sub>s</sub>), with a decrease of 0.0262 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (17.46%) per unit increase in ECw (Figure 2a). Increasing ECw also decreased transpiration (E) and net photosynthesis (A), with linear decreases of 0.4866 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (13.09%) and 2.6695 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (21.49%) per unit increase in ECw, respectively (Figure 2b and 2c).

The decrease in gas exchange (Figures 2a-c) may be due to the high concentration of salts in the irrigation water, which increases the osmotic potential of

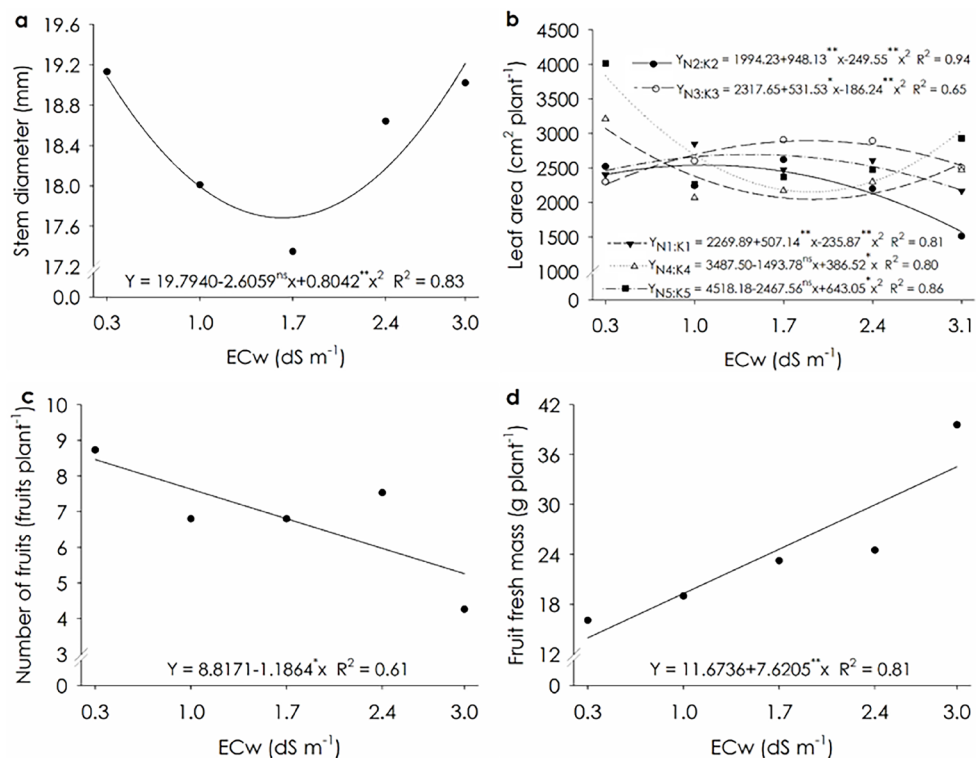
the soil, and decreases water uptake by the plants. Water deficiency caused by salt stress decreased the water potential of the plant leading to stomatal closure and, consequently, transpiration and CO<sub>2</sub> assimilation (Agbna et al., 2017; Azeem et al., 2017). The lack of attenuation from N application may be related to the fact that salt ions, particularly Na<sup>+</sup> and Cl<sup>-</sup>, interact with N, reduce its uptake via ion antagonism, transporter inactivation, restricted water uptake, and/or reduced internal N demand (Ashraf et al., 2018).

Salt stress decreased stem diameter, with the lowest value observed (17.68 mm) at ECw of 1.62 dS m<sup>-1</sup>, with an increase thereafter (Figure 3a). There was interaction among the factors evaluated for the leaf area of okra plants. The highest (2894.80 cm<sup>2</sup> plant<sup>-1</sup>) and lowest (2054.25 cm<sup>2</sup> plant<sup>-1</sup>) leaf area was observed with the application of N2:K2 and N4:K4 at ECws of 1.90 and 1.93 dS m<sup>-1</sup>, respectively (Figure 3b). The number of fruits reduced by 1.19 fruits per plant (13.46%) per unit of increase in ECw (Figure 3c). Fruit fresh mass, on the other hand, increased with ECw, with 7.62 g (65.28%) observed per unit increase in ECw (Figure 3d).

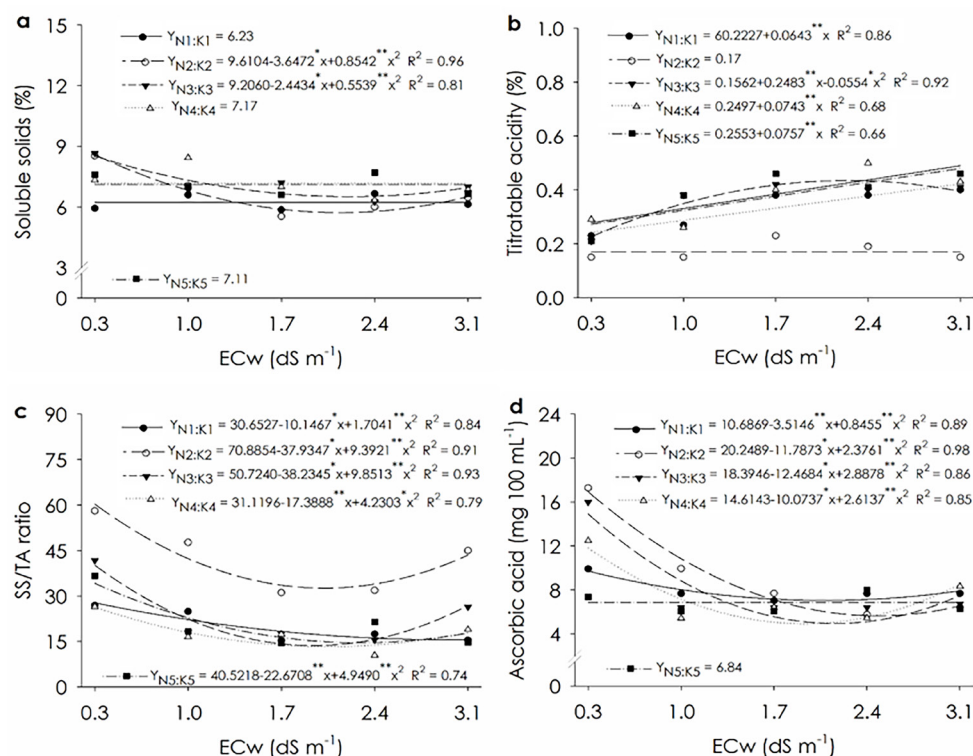
The results showed that increasing salinity decreased stem diameter and fruit number of okra and this was due to excess salts reducing osmosis and increasing ion toxicity, as observed in work by Elshaiikh et al. (2018).



**Figure 2.** Stomatal conductance ( $g_s$  – a), transpiration ( $E$  – b) and net photosynthesis ( $A$  – c) of okra (*Abelmoschus esculentus*) grown under combined nitrogen (N) and potassium (K) fertilization and irrigated with saline water (ECw).



**Figure 3.** Stem diameter (a), leaf area (b), number of fruits (c) and fruit fresh mass (d) of okra (*Abelmoschus esculentus*) grown under combined nitrogen (N) and potassium (K) fertilization and irrigated with saline water (ECw).



**Figure 4.** Soluble solids (a), titratable acidity (b), soluble solids/titratable acidity ratio (SS/TA ratio - c) and ascorbic acid (d) of okra (*Abelmoschus esculentus*) grown under combined nitrogen (N) and potassium (K) fertilization and irrigated with saline water (ECw).

In addition, the reduction in growth and yield may have occurred due to the decrease in gas exchange (Figures 2a-c), which are physiological variables that have a strong relationship with plant growth (Saleem et al., 2011). The accumulation of salts in the leaf and increased water deficiency leads to decreased stomatal conductance that reduces transpiration rate and net photosynthesis, causing decreased growth and yield of okra plants (Wani et al., 2013; Azeem et al., 2017). Salt stress increased the fresh fruit mass (Figure 3d) and this may be related to the partitioning of photo-assimilates, since the number of fruits was reduced with stress, causing the plant to distribute its assimilates to fewer sink, which leads to its increase in size.

N:K fertilization attenuated the damaging effects of salt stress on the leaf area of okra plants (Figure 3b). This may be related to membrane maintenance by increasing the  $K^+$  content in the cell, leading to regulation of  $Na^+/K^+$  pumps, given that toxic  $Na^+$  entering the cell causes membrane depolarization and results in  $K^+$  efflux (Falhof et al., 2016), thus, the leaves could have greater expansion. Under salt stress,  $K^+$ -dependent vital processes are inhibited by  $Na^+$  interference (Munns & Tester, 2008). It is also worth noting that supplying sufficient nitrogen improves salt tolerance, in part by increasing the amino compound against osmotic pressure (Ehltling et al., 2007).

The soluble solids content had a quadratic behavior, with the minimum point (5.72%) observed in

the application of N2:K2 and ECw of 2.13  $dS\ m^{-1}$ . The application of N1:K1, N4:K4 and N5:K5 had no difference (Figure 4a). Titratable acidity had a linear increase with the application of N1:K1, N4:K4 and N5:K5 (28.87, 29.76 and 26.75%, respectively) (Figure 4b). The SS/TA ratio had a decreasing quadratic behavior, with the minimum point (13.25) observed at the application of N4:K4 and ECw of 2.06  $dS\ m^{-1}$  (Figure 4c). The ascorbic acid content had similar behavior as the SS/TA ratio, with the minimum point (4.91  $mg\ 100\ mL^{-1}$ ) at the application of N4:K4 and ECw of 1.93  $dS\ m^{-1}$  (Figure 4d).

N:K fertilization influenced soluble solids, titratable acidity, soluble solids/titratable acidity ratio (SS/TA), and ascorbic acid content. Soluble solids content represents the balance of sugars and acids present in a matrix, which has a major impact on fruit flavor, while titratable acidity is related to the total concentration of free protons that can be neutralized by a strong base (Fundo et al., 2018). The action of K on soluble solids, titratable acidity and SS/TA ratio may be related to the fact that this nutrient promotes the transport of photoassimilates to storage organs and participates in the formation and translocation of sugars (Fernandes et al., 2018). Potassium is responsible not only for increasing production, but also for improving crop quality (Hasanuzzaman et al., 2018). Since the metabolism of sugars is affected by tissue K availability, optimal K fertilization is likely to have a beneficial effect on ascorbic

acid concentration (Magwaza et al. 2017). Furthermore, increased ascorbic acid content can be attributed to the increase in fruit size resulting from the increase in leaf area caused by N application.

### Conclusions

The combined fertilization with nitrogen and potassium (N:K) attenuated the damage caused by saline stress on photosynthetic pigments (chlorophylls and carotenoids), leaf area and fruit quality (soluble solids, titratable acidity, SS/TA ratio and ascorbic acid). However, N:K fertilization did not attenuate the damage caused by salt stress on gas exchange, number of fruit and fruit fresh mass of okra plants. Thus, N:K fertilization can be seen as an important agronomic strategy to improve the performance of okra quality subjected to saline conditions.

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