

MORFOFISIOLOGIA DO CRESCIMENTO INICIAL DE CAFEZEIROS SOB SALINIDADE DA ÁGUA DE IRRIGAÇÃO

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1 RESUMO

A intensidade do estresse salino na planta depende da tolerância, que é variável entre espécies e entre genótipos da mesma espécie. Objetivou-se neste trabalho, avaliar os efeitos da salinidade da água de irrigação sobre o crescimento vegetativo inicial, trocas gasosas, extravasamento de eletrólitos e estado hídrico de clones de café canéfora. O experimento foi conduzido em ambiente protegido usando o delineamento experimental em blocos ao acaso, em esquema fatorial 5x2, com quatro repetições, sendo cinco condutividades elétricas da água de irrigação (CEa): 0,08; 0,88; 1,68; 2,48 e 3,28 dS m⁻¹ e dois clones: C08 e C25. Houve efeito da interação entre salinidade e clones apenas para área foliar aos 60 dias após o transplante (DAT). A CEa acima de 0,08 dS m⁻¹ provocou redução na condutância estomática, concentração interna de CO₂, transpiração, taxa de assimilação de CO₂, número de folhas, altura de planta, diâmetro de caule, área foliar, fitomassa fresca e seca da parte aérea, seca da raiz e relação raiz/parte aérea aos 60 DAT, e no teor relativo de água nos tecidos foliares e dano na membrana celular aos 30 e 60 DAT. Houve efeito significativo dos clones apenas para altura de plantas aos 30 e 60 DAT.

Palavras-chaves: *Coffea canephora* Pierre ex A. Froehner, estresse salino, trocas gasosas, desenvolvimento de plântulas.

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MORPHOPHYSIOLOGY OF THE INITIAL GROWTH OF COFFEE UNDER IRRIGATION WATER SALINITY

2 ABSTRACT

The intensity of salt stress on the plant depends on tolerance, which varies between species and between genotypes of the same species. This work aimed to evaluate the effects of irrigation water salinity on the initial vegetative growth, gas exchange, electrolyte leakage, and water status of coffee *canephora* clones. The experiment was carried out in a greenhouse using a randomized block experimental design, in a 5x2 factorial scheme, with four replications, with five electrical conductivities of the irrigation water (ECw): 0.08; 0.88; 1.68; 2.48, and 3.28 dS m⁻¹ and two clones: C08 and C25. There was an interaction effect between salinity and clones only for leaf area at 60 days after transplanting (DAT). The ECw above 0.08 dS m⁻¹ reduced stomatal conductance, internal CO₂ concentration, transpiration, CO₂ assimilation rate, number of leaves, plant height, stem diameter, leaf area, fresh and dry biomass aerial part, root dryness, and root/shoot ratio at 60 DAT, and in relative water content in leaf tissues and cell membrane damage at 30 and 60 DAT. There was a significant effect of clones only for plant height at 30 and 60 DAT.

Keywords: *Coffea canephora* Pierre ex A. Froehner, salt stress, gas exchange, seedling development

3 INTRODUCTION

In recent years, the state of Rondônia has established itself as the second largest producer of *Coffea canephora*, whose production in 2019 was 2.39 million processed bags, with an average productivity of 36.85 bags ha⁻¹ (CONAB, 2019). This increase in productivity is the result of the use of clones instead of seed crops; better technological packages, such as pruning, irrigation, and fertilization; and the increasing use of fertigation (DUBBERSTEIN *et al.*, 2017).

Among the various physiological processes affected by salinity, water absorption by plants and gas exchange stand out, as saline stress causes morphological changes such as nutritional imbalance, reduced stomatal conductance, a reduced photosynthetic rate, and reduced transpiration. These changes likely occur in response to partial stomatal closure, which is mediated by hormones, photochemical changes, and carbon metabolism (CHAVES; FLEXAS; PINHEIRO, 2009). This stress causes a reduction in osmotic

potential. It is assumed that this behavior may reduce plant growth due to reduced atmospheric CO₂ absorption and, consequently, reduced photosynthesis (PRAXEDES *et al.*, 2010).

Given the problem of the presence of salts in irrigation water, which leads to an increase in the concentration of salts in the soil, alternatives to cultivation must be sought, such as the use of genotypes that are tolerant to salinity, according to Fernandes *et al.* (2011), tolerance to salinity varies between species, between genotypes and between phases of plant development.

When cultivated in saline soil or irrigation water, plant growth is hampered by reduced water and nutrient absorption due to the reduction in osmotic and water potential caused by the increase in the concentration of dissolved salts in the soil solution (NOBRE *et al.*, 2013). This increase also promotes a specific effect of ions (Na, B and Cl) that cause functional disorders and injuries, mainly in the leaves, thus affecting plant metabolism.

With respect to the salinity tolerance of *canephora* coffee plants, there is little information in the literature concerning

Amazonian robustas. Studies indicate that in the Arabica variety, initial plant growth is impaired by water salinity above 1.2 dS m⁻¹ and that plants of this species do not survive under salinity conditions between 1.5 and 6.0 dS m⁻¹ (FIGUEIRÊDO; FARIA; SILVA, 2006; KARASAWA *et al.*, 2003). Therefore, the objective of this study was to evaluate the effects of irrigation water salinity on the initial growth, gas exchange, electrolyte leakage, and water status of canephora coffee clones.

4 MATERIALS AND METHODS

The experiment was developed with canephora coffee plants (*Coffea canephora* Pierre ex A. Froehner) in a greenhouse at the Experimental Field of the Federal University of Rondônia Foundation (UNIR), Rolim de Moura Campus, RO, located at 11° 43' S latitude and 61° 46' W longitude, at an average altitude of 277 m. The climate, according to the Köppen classification, is characterized as Aw, Tropical Rainy. The average annual temperature is 26 °C, and the average annual precipitation is between 1,400 and 2,500 mm.

Randomized blocks, with treatments arranged in a 5x2 factorial scheme, with four replicates and one plant per replicate. The treatments involved combinations of five irrigation water salinity levels (ECw): 0.08, 0.88, 1.68, 2.48 and 3.28 dS m⁻¹ and two coffee clones: 08 and 25. Both clones were medium-sized and had intermediate maturity and high vegetative vigor. In the Zona da Mata region of Rondônia, clone 08 is known as "caroçudo" (caroçudo) because of the size of its fruits, and clone 25 is known as "folhudo" (leafy) because of the size of its leaves.

The soil collected in the experimental area of UNIR to fill the plastic pots with 15 dm³ of soil was classified as Dystrophic Red Yellow Latosol

(EMBRAPA, 2013), whose chemical characteristics were obtained via the methodology proposed by Teixeira *et al.* (2017), namely, pH = 5.4, electrical conductivity of the saturation extract (CEes) = 0.65 dS m⁻¹, P and Na = 5.1 and 0.0 mg kg⁻¹, respectively, organic matter = 43.0 g kg⁻¹, and K, Ca and Mg = 80, 3.0 and 0.8 cmol c kg⁻¹, respectively.

The CEa levels were obtained by dissolving NaCl in water from the local supply system (CEa = 0.08 dS m⁻¹), and the quantity (Q) was determined via Equation (1), which is contained in Rhoades; Kandiah; Mashali (2000):

$$Q \text{ (mg L}^{-1}\text{)} = 640 \times \text{CEa (dS m}^{-1}\text{)} \quad (1)$$

where CEa represents the desired value of the electrical conductivity of water.

Phosphate fertilization in the form of simple superphosphate corresponded to 300 mg of P₂O₅ dm⁻³ of soil, which was applied to the foundation on the day of transplanting. Nitrogen and potassium fertilizers were provided with urea (100 mg dm⁻³ of soil) and potassium chloride (150 mg of K₂O dm⁻³ of soil), respectively; they were divided into two equal applications, one at transplanting and 30 days after transplanting (DAT), following the recommendation of Novais; Neves; Barros (1991) for pots in protected cultivation.

The seedlings were 90 days old and had six pairs of leaves, with an average height of 20 cm and a stem diameter of 4.5 mm. They were obtained from rooting cuttings of orthotropic branches. After transplanting, the seedlings were maintained in soil with a moisture content close to field capacity for 28 days, and supply water with an electrical conductivity (EC) of 0.08 dS m⁻¹ (control treatment) was used.

Treatment application began after this period, with irrigation with salinized

water being carried out according to the respective treatments and on the basis of the plant's water needs, determined by the difference between the volume applied and the volume drained in the previous irrigation, estimated by drainage lysimetry. The soil moisture content was maintained close to field capacity. Irrigation was carried out daily in the late afternoon. The volume of water applied for irrigation after 40 DAT was adjusted to provide a 15% leaching fraction in the soil as a management measure to prevent salt accumulation.

At 30 and 60 DAT, leaf gas exchange measurements were taken (stomatal conductance – g_s , internal CO₂ concentration – C_i , transpiration – E and CO₂ assimilation rate – A) via a portable infrared gas analyzer (IRGA), model LCPro +, from the ADC. All measurements were performed on fully expanded leaves (third leaf from the apex). Readings were taken between 08:00 and 10:00 h using an artificial radiation source with an intensity of $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ under ambient temperature and CO₂ concentration conditions.

The relative leaf water content (RLWC) measured at 30 and 60 DAT was obtained via Equation 2, according to Maia *et al.* (2007):

$$\text{TRAF} = \frac{\text{MMF} - \text{MMS}}{\text{MT} - \text{MMS}} * 100 \quad (2)$$

where MMF represents the mass of fresh matter (g), MT represents the turgid mass (g), and MMS represents the dry matter mass (g).

The turgid weight of the leaf discs ($5.93 \text{ cm}^2_{\text{area}}$) was obtained by hydration for 24 h, followed by weighing, after which excess water was removed from the surface of the tissues; the dry weight of the discs was obtained after drying the material in a forced-air oven at 65°C until a constant weight was reached.

Electrolyte leakage (EE) across the cell membrane was assessed in the leaves at

30 and 60 DAT. Ten leaf discs measuring 5.93 cm^2 were removed from each leaf. These discs were then washed with distilled water to remove any electrolytes adhering to the leaflets. These discs were then placed in beakers containing 30 mL of distilled water and hermetically sealed with aluminum foil. The beakers were kept at 25°C for 6 h, after which the initial electrical conductivity (ECi) was measured. The beakers were subsequently placed in a forced-air oven and heated to 85°C for 2 h. After this period, the readings were taken again. of the final electrical conductivity (CEf). Thus, the extravasation of electrolytes in the cell membrane was obtained according to Equation 3, which was proposed by Scotti-Campos *et al.* (2013) :

$$\text{EE} = \frac{\text{CEi}}{\text{CEf}} * 100 \quad (3)$$

where CEi and CEf represent the initial and final electrical conductivities (dS m^{-1}), respectively.

Vegetative growth analyses of the seedlings at 30 and 60 DAT were performed by determining the plant height (AP (cm)) via a ruler graduated by the distance between the soil and the plant apex; the stem diameter (DC (mm)) was measured with a precision digital caliper two centimeters from the soil surface; and the number of leaves (NF) was counted, considering only those with fully expanded leaf blades. The leaf area (AF (cm^2)) was determined via a nondestructive method for coffee plants according to Equation (4), which was proposed by Barros *et al.* (1973):

$$y (\text{cm}^2) = 0,667.X \quad (4)$$

where y is the AF and X is the area of the rectangle circumscribed around the sheet, corresponding to the product of the longest length and the longest width of the

sheet, obtained on one sheet of each pair of sheets via a graduated ruler.

At 60 DAT, the plants were collected by separating the aerial part from the roots and stored in an air circulation oven at 65 °C until a constant mass was reached. The fresh phytomass – FFPA (g), dry mass of the aerial part – FSPA (g), and of the roots – FSR (g) were subsequently determined on a precision scale; total dry mass – FST (g) corresponded to the sum of the FSPA and FSR, respectively.

The data obtained were subjected to analysis of variance via the F test at the 0.05 and 0.01 probability levels, and in cases of significance, linear and quadratic polynomial regression analyses were

performed for the water salinity levels. The clones were analyzed via the Tukey test of means ($p < 0.05$) via Sisvar statistical software (FERREIRA, 2019).

5 RESULTS AND DISCUSSION

The results of the analysis of variance (Table 1) revealed that salinity (S) significantly affected stomatal conductance (g_s), the internal CO_2 concentration (C_i), transpiration (E) and the CO_2 assimilation rate (A) only at 60 DAT. There was no significant effect for clones or for the interaction between water salinity level and clone on any of the variables studied at 30 and 60 DAT.

Table 1. Summary of analysis of variance (ANOVA) for stomatal conductance (g_s), internal CO_2 concentration (C_i), transpiration rate (E) and CO_2 assimilation rate (A) in canephora coffee clones under irrigation with water of different salinities at 30 and 60 days after transplanting (DAT). Rolim de Moura, RO.

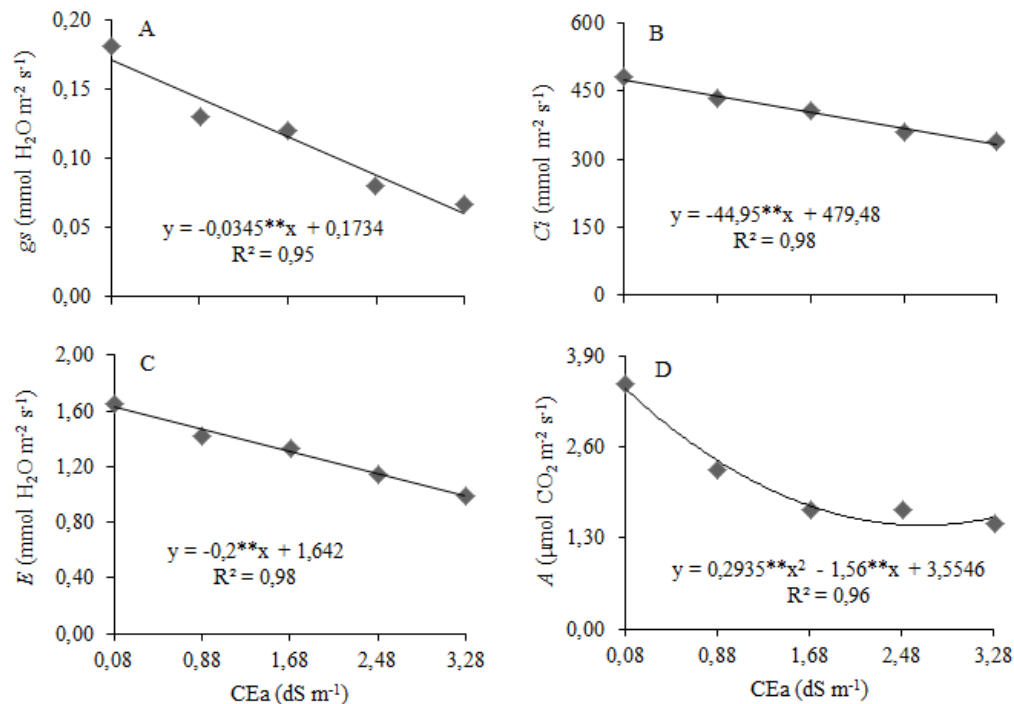
FV	Mean square							
	<i>gs</i>		<i>Ci</i>		<i>AND</i>		<i>THE</i>	
	Days after transplant – DAT							
	30 ¹	60 ¹	30	60	30	60 ¹	30 ²	60 ²
S	0,02 ^{ns}	0,01 ^{**}	940,90 ^{ns}	26214,00 ^{**}	19,11 ^{ns}	0,52 [*]	0,29 ^{ns}	5,33 ^{**}
R.L	0,10 ^{ns}	0,06 ^{**}	3699,20 ^{ns}	103392,20 ^{**}	70,87 ^{ns}	2,06 ^{**}	1,16 ^{ns}	16,72 ^{**}
R.Q	0,00 ^{ns}	0,00 ^{ns}	57,14 ^{ns}	464,10 ^{ns}	5,53 ^{ns}	0,00 ^{ns}	0,00 ^{ns}	3,96 ^{**}
C	0,00 ^{ns}	0,00 ^{ns}	129,60 ^{ns}	48,40 ^{ns}	50,17 ^{ns}	0,02 ^{ns}	0,11 ^{ns}	0,02 ^{ns}
S x C	0,01 ^{ns}	0,00 ^{ns}	1162,85 ^{ns}	5668,60 ^{ns}	28,71 ^{ns}	0,13 ^{ns}	1,22 ^{ns}	0,88 ^{ns}
Resíduo	0,01	0,00	590,90	2858,70	22,68	0,16	0,57	0,62
CV(%)	28.76	27:30	5.08	13.23	16:05	15.97	20.44	16.89

^{**} and ^{*} significant at probabilities of 0.01 and 0.05, respectively, according to the F test; ^{ns} not significant according to the F test; FV – source of variation; S – salinity; RL – linear regression; RQ – quadratic regression; C – clone; CV – coefficient of variation; ^{1,2} statistical analysis performed after the data were transformed into \sqrt{x} and $\sqrt{x+0.5}$, respectively.

Stomatal conductance (g_s) decreased linearly with increasing irrigation water salinity, with a 19.89% reduction per unit increase in ECa and a 64.69% reduction ($0.1104 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) in plants irrigated with 3.28 dS m^{-1} water compared with those in plants irrigated with 0.08 dS m^{-1} water (Figure 1A). This

reduction in g_s may be due to the osmotic effect associated with salt accumulation in the soil and the reduction in root system hydraulic conductivity due to increased suberization and lignification of vascular tissues in the roots of plants under salinity stress (NEVES *et al.*, 2009).

Figure 1. Stomatal conductance – g_s (A), internal CO_2 concentration – C_i (B), transpiration – E (C) and CO_2 assimilation rate – A (D) at 60 days after transplanting (DAT) in canephora coffee clones grown under different electrical conductivities of irrigation water (CEa).



Salinity makes it more difficult for plants to absorb water from the soil, which consequently tends to reduce water loss, resulting in a decrease in stomatal conductance (OLIVEIRA *et al.*, 2017). In this study, the use of saline water, regardless of the clone, reduced g_s due to the reduction in the osmotic potential of the soil solution and, consequently, the increase in the water potential, in addition to the increase in the concentration of salts in the soil sufficient to cause toxicity by specific ions (Na^+ and Cl^-), which resulted in a decrease in stomatal conductance (LEITE *et al.*, 2017).

As previously observed for g_s , the salinity of the irrigation water negatively interfered with the internal CO_2 concentration (C_i) of the canephora coffee clones (Figure 1B), with a linear effect, with a decrease of 9.37% per unit increase in the ECa in C_i . The C_i of the plants irrigated with water with a relatively high salinity level (3.28 dS m⁻¹) was 30.22% lower (143.84 mmol m⁻²

s⁻¹) than that of the plants irrigated with water with an ECa of 0.08 dS m⁻¹. This relative decrease in C_i can be attributed to lower stomatal conductance, a common response of plants to saline stress (PRAXEDES *et al.*, 2010; SILVA *et al.*, 2011).

Regression studies of the transpiration (E) of coffee plants (Figure 1C) revealed that an increase in irrigation water salinity led to a decrease in linear behavior, with a decrease of approximately 12.18% per unit increase in ECa, resulting in a reduction of 39.36% (0.640 mmol H₂O m⁻² s⁻¹) in the E of plants irrigated with water, with an ECa of 3.28 dS m⁻¹, in relation to those irrigated with an ECa of 0.08 dS m⁻¹. The reduction in E is a strategy that the plant adopts in response to water loss, since absorption is impaired by the accumulation of salts in the soil (MUNNS, 2002; NEVES *et al.*, 2009). Furthermore, the decrease in transpiration must have been caused, in part, by the toxic

effects of the salts absorbed by the plants, by the low osmotic adjustment capacity of the crop and by the reduction in the total water potential caused by the increase in salinity concentration (LACERDA *et al.*, 2006; SILVA *et al.*, 2011).

A_2 assimilation rate (A) in canephora coffee plants decreased (Figure 1D) with increasing irrigation water salinity, with the maximum value found in plants when they were irrigated with water, with a CEa of 0.08 dS m^{-1} ($3.43 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and the minimum ($1.59 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was found in those irrigated with water, with a CEa of 3.28 dS m^{-1} . Silva *et al.* (2013) reported that plants close their stomata to reduce water loss through transpiration, resulting in a lower rate of CO_2 assimilation, constituting one of the main causes of reduced growth in species

subjected to salinity stress. However, Kurban *et al.* (1999) reported that reductions in photosynthetic rates in plants caused by salinity stress may be related to damage to the photosynthetic apparatus and/or the CO_2 fixation enzyme system, which is caused more specifically by ionic toxicity to metabolism than by stomatal limitations.

The results of the analysis of variance (Table 2) revealed that the saline level of the irrigation water (S) significantly affected the relative water content of the leaves (TRAF) and the electrolyte leakage (EE) at the 0.01 probability level and the TRAF (60 DAT) at the 0.05 probability level. For the clones (C) and the interaction between salinity and clones (SxC), there was no significant effect on any of the studied variables.

Table 2. Summary of analysis of variance (ANOVA) for relative leaf water content (RLWC) and electrolyte leakage (EE) in canephora coffee clones irrigated with saltwater at 30 and 60 days after transplanting (DAT). Rolim de Moura, RO.

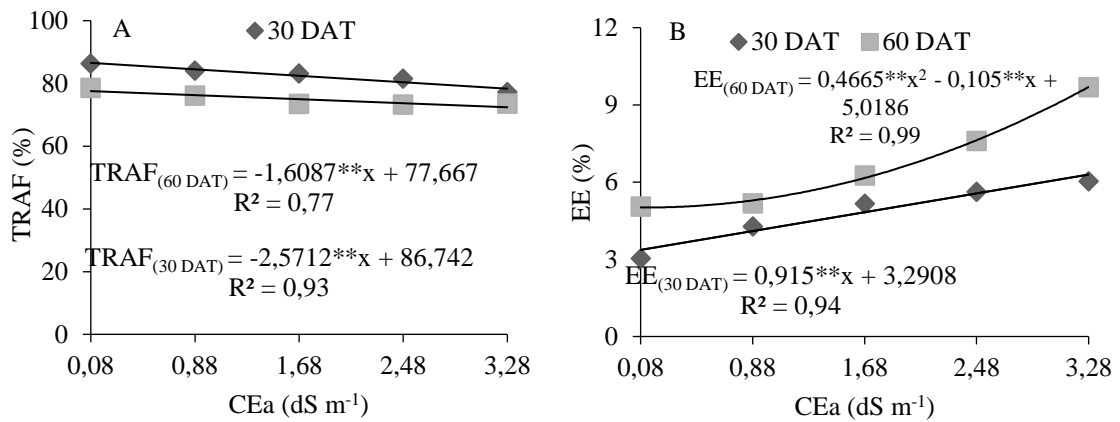
Source of variation	Mean square			
	TRAF – DAT		EE – DAT	
	30	60	30 ¹	60
S	90,90 ^{**}	42,52 [*]	11,39 ^{**}	29,87 ^{**}
Reg. Lin.	338,49 ^{**}	132,38 ^{**}	37,46 ^{**}	109,41 ^{**}
Reg. Quad.	12,58 ^{ns}	34,99 ^{ns}	3,86 ^{ns}	9,90 ^{**}
Clones (C)	0,07 ^{ns}	0,23 ^{ns}	3,86 ^{ns}	0,33 ^{ns}
S x C	16,16 ^{ns}	20,86 ^{ns}	1,13 ^{ns}	0,15 ^{ns}
Bloco	26,52 ^{ns}	25,40 ^{ns}	3,05 ^{ns}	3,18 [*]
Resíduo	10,99	18,03	2,18	0,76
CV(%)	4,02	5,66	15,28	12,93

^{**} and ^{*} indicate significance at probabilities of 0.01 and 0.05, respectively, according to the F test; ^{ns}, not significant according to the F test; CV, coefficient of variation; S, salinity; ¹, statistical analysis performed after the data were transformed into \sqrt{x} .

The relative water content of the leaves (TRAF) decreased with increasing salinity of the irrigation water, with relative decreases of 2.96% (30 DAT) and 2.07% (60 DAT) per unit increase in ECa; that is, plants irrigated with water with an ECa of 3.28 dS m^{-1} suffered a reduction in TRAF of 9.50 and 6.64%, respectively, in relation

to those irrigated with water with 0.08 dS m^{-1} (Figure 2A). These results may be associated with the reduction in water content in plants subjected to high levels of salt in the soil and can be explained by the low water consumption of such plants, as the increase in salinity reduces plant water consumption (SILVA *et al.*, 2011).

Figure 2. Relative leaf water content - TRAF (A) and electrolyte leakage - EE (B) in canephora coffee clones as a function of irrigation water salinity - CEa at 30 and 60 days after transplanting (DAT).



The increase in irrigation water salinity promoted a linear increase in electrolyte leakage, with an EE (Figure 2B) of 27.80% per unit increase in ECw at 30 DAT. According to the regression equation, plants subjected to irrigation with an ECw of 3.28 dS m⁻¹ presented an increase in EE of 87.04% compared with those subjected to an ECw of 0.08 dS m⁻¹. At 60 DAT, a quadratic response was observed (Figure 2B), with a maximum estimated value of 9.69%, which was obtained when a salinity level of 3.28 dS m⁻¹ was applied. This increase may be related to ionic effects, since the increase in the concentration of salts in the water can alter the nutritional balance, including the availability of Ca (FERRAZ *et al.*, 2015; SALAZAR *et al.*, 2017), an

element that is essential for the formation of the cell wall, thus increasing the percentage of electrolyte leakage with increasing salinity.

The salinity factor of irrigation water negatively affected ($p < 0.01$) the growth of plants in terms of the number of leaves (NF), plant height (AP), stem diameter (DC) and leaf area (AF) at 30 and 60 DAT (Table 3). There was a significant effect of the interaction between the salinity of the irrigation water and canephora coffee clone on AF only at 60 DAT. At 30 and 60 DAT, C25 plants presented greater heights (38.59 and 42.77 cm, respectively) than C08 plants did (36.10 and 39.70 cm, respectively), differing statistically from each other ($p < 0.05$).

Table 3. Mean squares for the number of leaves (NF), plant height (AP), stem diameter (DC) and leaf area (AF) of canephora coffee cultivar plants under irrigation with water of different salinities at 30 and 60 days after transplanting (DAT). Rolim de Moura, RO.

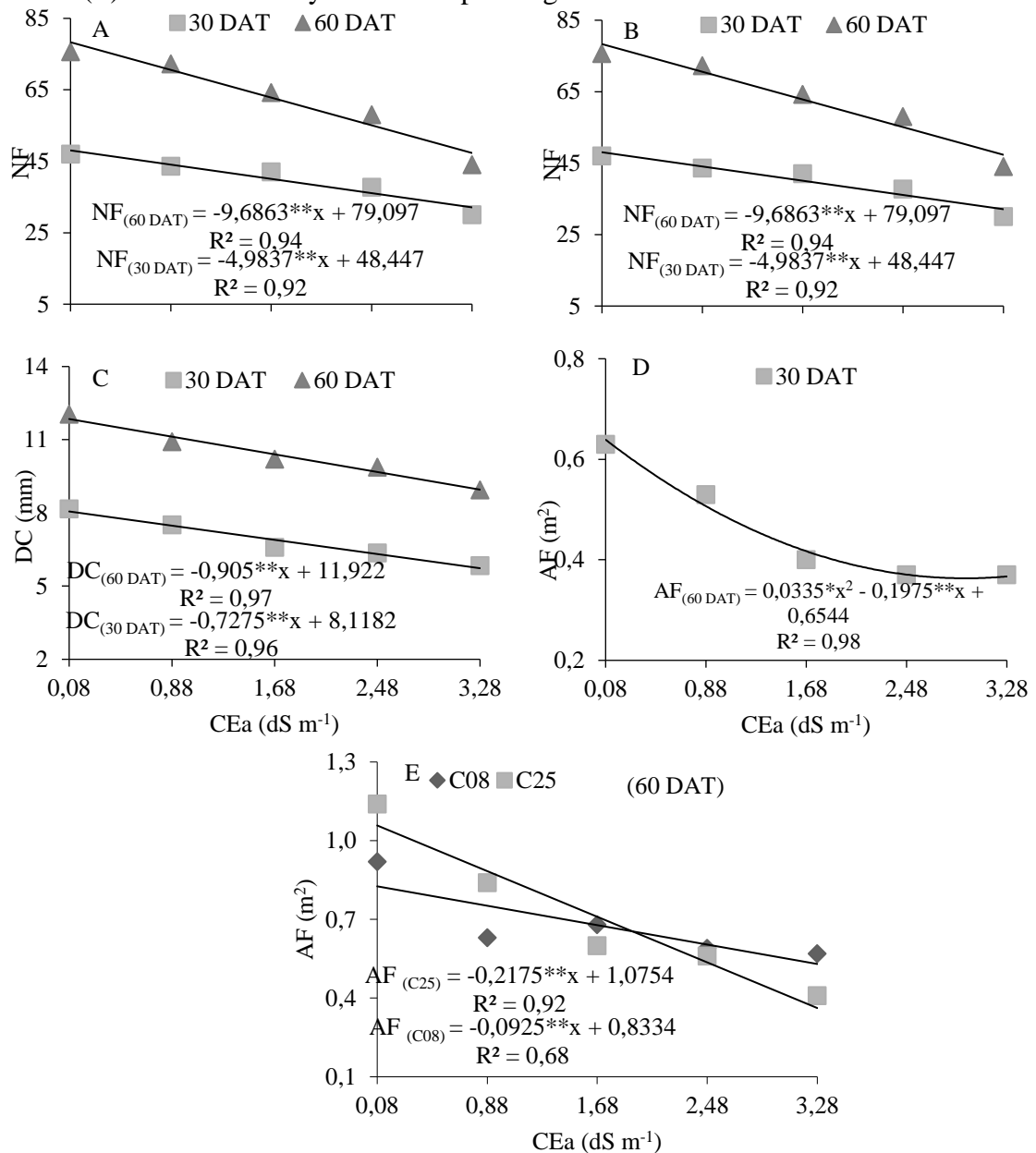
FV	Mean squares							
	NF		AP		A.D		AF	
	30	60	30 ¹	60	30	60	30	60
S	342.35 **	1264.72 **	101.52 **	153.45 **	7.01 **	10.80 **	0.10 **	0.34 **
RL	127.01 **	4805.00 **	366.80 **	512.57 **	27.06 **	41.96 **	0.37 **	1.22 **
RQ	73.93 ^{ns}	217.20 ^{ns}	40.56 ^{ns}	73.12 [*]	0.55 ^{ns}	0.33 ^{ns}	0.04 [*]	0.11 ^{**}
C	70.22 ^{ns}	133.22 ^{ns}	62.25 [*]	94.15 [*]	0.12 ^{ns}	0.08 ^{ns}	0.00 ^{ns}	0.01 ^{ns}
SxC	26.10 ^{ns}	364.72 ^{ns}	12.72 ^{ns}	20.05 ^{ns}	0.10 ^{ns}	1.10 ^{ns}	0.00 ^{ns}	0.06 ^{**}
R	25.06	146.67	13.91	15.70	0.24	1.13	0.01	0.016
CV	12.49	19.28	9.99	9.61	7.10	10.22	11.64	18.45
Media								
C	----- n° -----		----- cm -----		----- mm -----		----- m ² -----	
C ₁	41.40 a	61.00 a	36.10 a	39.70 a	6.95 a	10.35 a	0.46 a	0.6 a
C ₂	38.75 a	64.65 a	38.59 b	42.77 b	6.84 a	10.45	0.46 a	0.7 a
	a.m.							
dms	3.24	7.85	2.42	2.57	0.31	0.69	0.06	0.08

** and * significant at probabilities of 0.01 and 0.05, respectively, according to the F test; ^{ns} not significant according to the F test; FV – source of variation; S – salinity; RL – linear regression; RQ – quadratic regression; C – clone; R – residue; CV(%) – coefficient of variation; C₁ – clone 08; C₂ – clone 25; ¹ statistical analysis performed after transformation of the data into \sqrt{x} ; means followed by the same letter do not differ statistically from each other according to the Tukey test at 5% probability.

Figure 3 shows that at 30 and 60 DAT, as the CEa level increased, the values for NF, AP and DC decreased, with relative decreases of 10.28 and 12.24%; 6.37 and 6.79%; and 8.96 and 7.59%, respectively, per unit increase in ECa, that is, reductions in NF of 33.19% (15.95 leaves) and 39.57% (30.99 leaves), AP of 20.50% (8.53 cm) and 21.86% (10.12 cm) and DC of 28.88% (2.33 mm) and 24.43% (2.90 mm),

respectively, in plants irrigated with water of 3.28 dS m⁻¹ in relation to those irrigated with water of 0.08 dS m⁻¹. When plants are subjected to saline stress conditions, morphological and anatomical changes commonly occur, which are reflected in the reduction in transpiration as an alternative to maintain a low saltwater absorption rate. Another adaptation strategy is to reduce the number of leaves (OLIVEIRA *et al.*, 2013).

Figure 3. Number of leaves – NF (A), plant height – AP (B), stem diameter – DC (C) and leaf area – AF (D) of canephora coffee plants, clones 08 and 25, as a function of irrigation water salinity – CEa and the interaction between salinity \times clones – S \times C (E) at 30 and 60 days after transplanting – DAT.



This considerable reduction in AP can be explained by the way plants adapt to saline conditions, in which they reduce energy expenditure and, consequently, negatively affect their growth (LIU; JIANG, 2015). In accordance with Andrade Júnior *et al.* (2011), salinity affects water absorption and plant growth because of the reduction in the water potential of the

external solution through the osmotic effect of the introduced Na⁺ and Cl⁻ salts. The osmotic and specific effects of these ions slow cell expansion and division, promoting negative effects on the photosynthetic rate and impairing the physiological and biochemical processes of plants (GOMES *et al.*, 2011; NUNES *et al.*,

2012), which consequently also causes a reduction in DC.

The AF also decreased with increasing ECw at 30 DAS, and the results satisfactorily fit the quadratic equation (Figure 3D). The model obtained allows us to state that the highest AF value (0.63 m^2) would be reached with an ECw of 0.08 dS m^{-1} . According to Travassos *et al.* (2012), this decrease in AF is related to the accumulation of salts in the soil, with high NaCl concentrations, which negatively influences water absorption by plants, which is a determining factor for their photosynthetic and metabolic processes, consequently causing a reduction in AF.

The increase in water salinity at 60 DAT linearly inhibited the AF of the plants in each clone (Figure 3E), indicating that the data better fit the linear and decreasing equations. However, despite presenting the same trend, the reduction in AF due to the increase in irrigation water salinity was greater in clone C25. The decreases in AF

per unit increase in the ECw of the clones subjected to salinity stress were 11.09% and 20.22% for C08 and C25, respectively. This result may be due to the mechanisms of adaptation to saline stress in plants, in which, for example, they reduce their transpiring surface. Thus, the reduction in leaf area under these conditions is relevant for maintaining a high water potential in the plant (NOBRE *et al.* 2014).

The results of the analysis of variance presented in Table 4 show that the salinity levels of the irrigation water (S) significantly affected the fresh mass (FFPA) and dry mass (FSPA), root mass (FSR), total mass (FST) and the root/aerial part ratio (RPA) at a 0.01 probability level. However, the clone factor (C) and the interaction between the factors ($S \times C$) did not significantly influence any of the variables analyzed, indicating that the clones behaved similarly at the different water salinity levels tested.

Table 4. Mean squares for fresh (FFPA) and dry weight of aerial parts (FSPA), dry weight of roots (FSR), total dry weight (FST) and the root/shoot ratio (RPA) in canephora coffee plants irrigated with saltwater at 60 days after transplanting (DAT). Rolim de Moura, RO.

Source of variation	Mean squares				
	FFPA	FSPA	FSR ¹	FST	RPA ¹
Salinity (S)	12662.01 **	2704.40 **	16.65 **	7829.35 **	0.03 **
Linear Reg.	49192.28 **	10178.16 **	53.65 **	27647.79 **	0.08 **
Reg. Quadr.	988.77 ^{ns}	554.28 ^{ns}	12.46 **	3465.66 **	0.03 **
Clones (C)	29.61 ^{ns}	1.17 ^{ns}	0.66 ^{ns}	60.63 ^{ns}	0.00 ^{ns}
NS x C	903.97 ^{ns}	128.23 ^{ns}	0.06 ^{ns}	124.78 ^{ns}	0.00 ^{ns}
Resíduo	1016.94	192.75	0.49	282.29	0.00
CV(%)	23.96	22.34	17.99	21.31	6.18

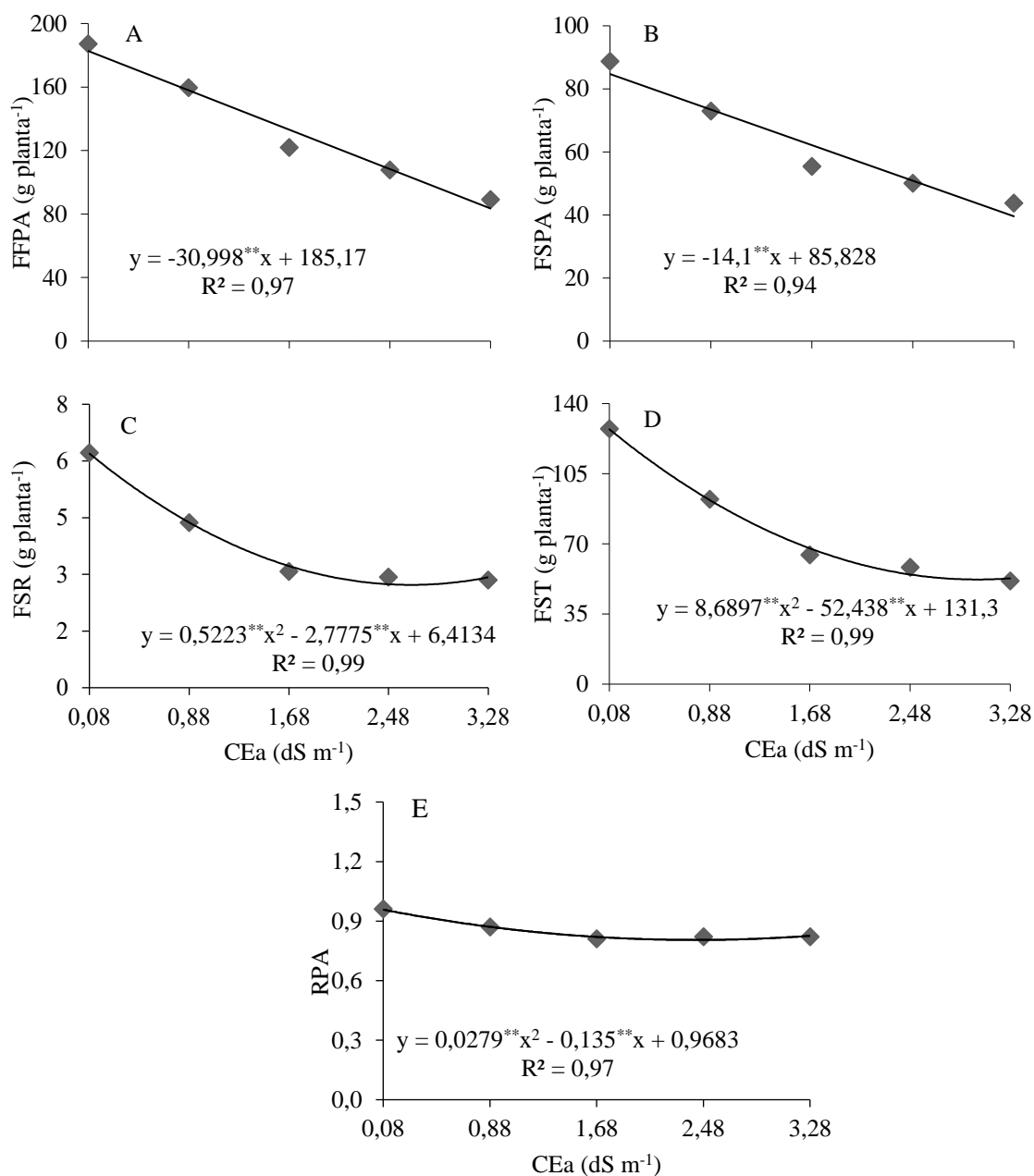
** significant at a probability of 0.01 according to the F test; ^{ns} not significant according to the F test; CV – coefficient of variation; ¹ statistical analysis performed after the data were transformed into $\sqrt{x+0.5}$.

Increasing irrigation water salinity linearly decreased FFPA and FSPA (Figure 4A and B) by 16.74 and 16.42%, respectively, per unit increase in irrigation water electrical conductivity; this result indicates a decrease of 99.19 g (54.29%) in FFPA and 45.12 g (53.27%) in FSPA

between plants irrigated with water of 0.08 and 3.28 dS m^{-1} . To adapt osmotically, plants under saline stress conditions release a certain amount of energy for the accumulation of sugars, organic acids, and ions in the vacuole, and the energy produced under normal conditions can be

converted into phytomass. (SANTOS *et al.*, 2012).

Figure 4. Fresh shoot phytomass – FFPA (A), dry shoot phytomass – FSPA (B), root phytomass – FSR (C), total phytomass – FST (D) and root/shoot ratio – RPA (E) of canephora coffee plants as a function of the electrical conductivity of irrigation water – CEa at 60 DAT.



The reduction in phytomass from ECa levels above 0.08 dS m^{-1} is closely linked to the effects of the concentration of soluble salts, which is a limiting factor for the development of most crops. This behavior can be understood as a possible mechanism of plant adjustment to reduce the effects of salinity because plants can undergo morphological or physiological changes such as a reduction in biomass when subjected to salinity stress (CENTENO *et al.*, 2014).

When the root dry matter (RDF) and total dry matter (TDF) data were analyzed, a quadratic behavior was observed (Figure 4C and D), and when the plants were irrigated with an ECw of 0.08 dS m^{-1} , there was a greater accumulation of RDF (6.19 g), with the lowest value (2.92 g) obtained for the plants irrigated with water, which presented 3.28 dS m^{-1} of electrical conductivity. Evaluating the behavior of the RDF variable as a function of the increase in salinity in the water, a reduction of 52.82% (3.27 g) was also noted between the highest (ECw = 3.28 dS m^{-1}) and the lowest (ECw = 0.08 dS m^{-1}) salinity level of the irrigation water. It can be inferred that the increase in the salinity of the irrigation water directly affects the accumulation of dry matter in canephora coffee plants. The highest value of FST was 127.16 g, which was obtained for plants irrigated with a CEa equal to 0.08 dS m^{-1} . According to Oliveira *et al.* (2015) and Sá *et al.* (2018), excess salts in the soil cause a reduction in water availability for plants, which makes them tend to spend more energy for absorption, resulting in a lower capacity to accumulate reserves. This phenomenon can be observed in this research through the results obtained for FST, which decreased in coffee plants under irrigation with CEa levels higher than 0.08 dS m^{-1} .

The root/shoot ratio (RPA) indicates the level of contribution of reserves stored in the root system in favor of shoot growth.

The results obtained in the present study, according to the regression equation (Figure 4E), revealed that the RPA of the coffee plant decreased to the highest level of ECa studied (3.28 dS m^{-1}), for which the minimum value for this variable was obtained (0.825 g g^{-1}). The estimates obtained via the regression equation also verified that the maximum value for the RPA was found in plants irrigated with an ECa of 0.08 dS m^{-1} (0.957 g g^{-1}). According to Sá *et al.* (2013), this response is related to the greater reduction in root growth in relation to the aerial part, aiming to reduce the absorption of salts from the environment, especially in environments with relatively high salinity levels, a fact confirmed in this work, given the marked reductions in the accumulation of root dry matter (Figure 4C).

6 CONCLUSIONS

When the salinity of irrigation water is greater than 0.08 dS m^{-1} , it reduces stomatal conductance, the internal CO_2 concentration, transpiration and the CO_2 assimilation rate in canephora coffee plants 60 days after transplanting and the relative water content in leaf tissues and electrolyte extravasation at 30 and 60 days after transplanting.

Irrigation with water with a salinity higher than 0.08 dS m^{-1} reduces the initial growth in terms of the number of leaves, plant height, stem diameter, and leaf area and the accumulation of fresh and dry phytomass from clones 08 and 25 of canephora coffee plants.

Considering the analysis of plant height at 30 and 60 days after transplanting, clone 25 was more tolerant to irrigation water salinity than clone 08 was. Notably, at 60 days after transplanting, the leaf area of clone 08 was less affected than that of clone 25.

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