

Photochemical efficiency of cashew rootstocks under salt stress and foliar application of potassium silicate

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Abstract

Cashew is a fruit species of great economic importance in Brazil, especially in its Northeast region. However, abiotic factors such as water and soil salinity stand out as limiting parameters for this crop, affecting all phases of development as well as physiological and biochemical processes. In this scenario, the use of products to mitigate such damage becomes a necessary practice, with silicon and potassium being viable alternatives. From this perspective, the present study aimed to evaluate the photochemical efficiency of cashew rootstocks subjected to water salinity and foliar application of potassium silicate. A randomized block design arranged in a 5x5 factorial with four replications and two plants per plot was used in the experiment. Treatments consisted of different levels of irrigation water electrical conductivity - EC_w (0.3, 1, 1.7, 2.4, and 3.1 dS m⁻¹) and potassium silicate concentrations (0, 250, 500, 750, and 1000 mg L⁻¹) supplied via foliar application. Irrigation water salinity levels from 2.1 dS m⁻¹ reduce the photochemical efficiency of photosystem II by at least 10%, consequently affecting the photosynthetic apparatus of cashew plants 70 days after sowing. The potassium silicate concentration of 250 mg L⁻¹ increases the quantum yield of cashew plants under salt stress conditions.

Keywords: *Anacardium occidentale*, chlorophyll a fluorescence, salinity

Introduction

Cashew (*Anacardium occidentale* L.) is a tropical fruit species highly adapted to the semi-arid climatic conditions of northeastern Brazil, highlighting the importance of its cultivation for the socio-economic development of small and medium producers in the region through an activity that generates more than 50 thousand jobs every year (Almeida et al., 2017). This relevance can be perceived in the cashew growing areas in Brazil, corresponding to 428,937 ha in the national territory, with 99.7% of this total in the Northeast region (IBGE, 2021).

However, discussions have developed with regard to the quality of the water available for irrigation in arid and semi-arid regions, as the low rainfall and high evapotranspiration of such areas can significantly increase salt accumulation in the soil (Dias et al., 2019). These conditions can result in damage to the different

phases of plant development, especially during seedling formation, as the increase in salt concentration in the water may reduce growth due to disturbances in the photosynthetic apparatus, e.g., stomatal closure and, consequently, reductions in the transpiration and net CO₂ assimilation rates, in addition to ionic imbalance (Wang et al., 2017).

Among physiological processes, assessing chlorophyll a fluorescence can contribute to evaluating the effects of environmental changes on plants (Kalaji et al., 2016). This occurs because the changes caused by salinity tend to modify the transport of electrons and the activity of photosystem II, increasing the loss of energy resulting from the oxidation of water molecules and contributing to the production of reactive oxygen species (EROs), which affect the photosynthetic process, metabolic activity and, in severe cases, may result in plant death (Najar et al., 2019).

As an alternative, silicon (Si) minimizes the harmful effects of salt excess on plants (Souza Junior et al., 2020), acting indirectly on several physiological and biochemical aspects, e.g., chlorophyll index, dissipation of light energy, and transpiration flow, in addition to being related to the activity of antioxidant enzymes (Tatagiba., 2017; Santos et al., 2020). Another element that has stood out in this regard is potassium, which acts on enzyme activation, photosynthesis, water-use efficiency, and the synthesis of starch and proteins (Khanghahi et al., 2019). Moreover, it acts on the antioxidant activity of plants under salt stress conditions, resulting in the accumulation of osmolytes, e.g., proline, sugars, and amino acids (Ahanger & Agarwal, 2017).

However, studies that demonstrate the effects of the joint action of these elements on the mitigation of salt stress are still scarce in the literature, including those conducted by Mozafari et al. (2018) with grapevine (*Vitis vinifera*), Moussa and Shama (2019) with potato (*Solanum tuberosum* L.), and Yaghubi et al. (2019) with strawberry (*Fragaria × ananassa* Duch.).

From this perspective, the present study aimed to evaluate the photochemical efficiency of cashew rootstocks subjected to water salinity and foliar application of potassium silicate.

Material and methods

The study was conducted from August to November 2017 in a protected environment (plant nursery) at the Federal University of Campina Grande, Center of Sciences and Agrifood Technology of the Pombal Campus – PB, 06° 46' S and 37° 49' W, at an elevation of 178 meters above sea level. The experimental area was located in the Sertão microregion of Paraíba (Pereira et al., 2015).

The experiment was arranged in a randomized block design using a 5 x 5 factorial with four replications and two plants per plot. Treatments consisted of different levels of irrigation water electrical conductivity - ECw (0.3, 1, 1.7, 2.4, and 3.1 dS m⁻¹) and concentrations of potassium silicate (0, 250, 500, 750, and 1000 mg of K₂SiO₃ L⁻¹) supplied via foliar application.

The salinity levels were established based on a study developed by Sousa et al. (2011), in which the authors observed the effects of salt stress on the growth of cashew seedlings subjected to increasing salinity levels from 1.58 dS m⁻¹. The different water conductivity levels were prepared according to the ratio commonly found in water sources across the Northeast region of Brazil and summarized in the study conducted by Medeiros (1992). The irrigation water was prepared by adding sodium chloride, Ca, and Mg to local water at a ratio of 7:2:1 (0.3 dS m⁻¹), according to the equation proposed by Richards (1954), shown below.

$$Q(\text{mmolc L}) = 10 \times ECw(\text{dS m}^{-1}) \tag{1}$$

Where: Q = Salt content to be applied (mmol c L⁻¹) according to the percentage of sodium chloride, Cl, and Mg; ECw = Water electrical conductivity (dS m⁻¹).

Quimifol Silício®, composed of 10% silicon and 10% water-soluble potassium, was used as the source of K₂SiO₃. The potassium silicate concentrations were determined despite the inexistence of studies on the production of cashew seedlings that considered this parameter. However, the study considered the potassium level of 150 mg K dm⁻³ recommended for pot cultivation proposed by Novais et al. (1991).

The plant material used was the cashew rootstock “Crioulo”, highly adapted to semi-arid conditions and widely used to produce rootstocks in plant nurseries of the region. The seeds came from a commercial area located in the municipality of Severiano Melo – RN and were selected according to size and quality.

The rootstock was grown in 1,150-mL polyethylene bags perforated at the base to allow water drainage. The bags were filled with a substrate containing 85% soil, 10% fine sand, and 5% bovine manure and placed on metallic benches at 0.8 m from the ground.

The physical and chemical characteristics of the substrate used in the experiment (Table 1) were analyzed according to the methodology proposed by Texeira (2017) at the Laboratory of Soil and Plant Nutrition of CCTA/UFCG.

Table 1. Physical and chemical characteristics of the substrate used in the experiment.

pH CaCl ₂ 1:2,5	Chemical attributes								
	CEa	P	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	H + Al ³⁺	
7.41	dS m ⁻¹	mg/dm ³	cmol _c /dm ³					0.00	0.00
	1.21	778	0.43	1.17	7.8	1.7	0.00		
Physical attributes									
Sand	Silt	Clay	ds	dp	Porosity		Textural class		
g kg ⁻¹		g cm ⁻³		%					
778	136	76	1.48	2.86	48		Sandy-loam		

pHes = pH of the saturation extract of the substrate; ECse = Electrical conductivity in the substrate saturation extract at 25°C. P= phosphorus; K⁺ = potassium; Na⁺ = sodium; Ca²⁺ = calcium; Mg²⁺ = magnesium; Al³⁺ = aluminum; ds = soil density; dp = particle density.

Sowing was performed with one nut per bag, whose substrate was at field capacity with low-conductivity water (ECw of 0.3 dS m⁻¹). The nut was inserted vertically with the base (point of insertion to the peduncle) facing up, at an approximate depth of 1 cm. The soil was maintained close to the maximum water retention capacity during seedling emergence, and irrigation was performed daily in the late afternoon (5:00 p.m.) using local water (0.3 dS m⁻¹).

After germination stabilized, 30 days after sowing (DAS), the saline irrigation water began to be manually applied daily using a graduated cylinder according to each treatment. The volume applied at each irrigation was determined using drainage lysimeters, as recommended by Bernardo et al. (2019) in order to achieve water balance, according to equation 2:

$$VI = \frac{Vp - Vd}{1 - FL} \quad (3)$$

Where: VI = Water volume to be applied via irrigation (mL); Vp = water volume applied in the previous irrigation (mL); Vd = drained water volume (mL); and LF = leaching fraction of 0.1.

Foliar fertilization with potassium silicate began 31 days after sowing and was split into five weekly applications in the late afternoon using sprayers. The application of K₂SiO₃ during this period used eight liters of solution, and a total volume of 200 ml was applied per plant, thus resulting in the accumulation of 0, 50, 100, 150, and 200 mg of potassium silicate for each increase of 250 mg L⁻¹ K₂SiO₃ in the studied treatments, respectively.

Preventive control was performed by applying the fungicide Kumulus® DF at sowing, which is recommended for the crop and characterized by rapid initial action and short persistence. In addition, hoeing around the area was performed by removing weeds and storing them in bags to control the incidence of harmful plants.

Chlorophyll *a* fluorescence was evaluated 70 DAS using a pulse-modulated chlorophyll fluorometer, model OS5p, developed by Opti Science®. The F_v/F_m protocol was used to determine the following fluorescence induction variables: initial fluorescence (F₀), maximum fluorescence (F_m), variable fluorescence (F_v = F_m - F₀), and maximum quantum efficiency of photosystem II (F_v/F_m) (Genty et al., 1989). This protocol was performed after leaf adaptation to the dark for 30 min, occurring between 7:00 a.m. and 9:00 a.m. using a leaf clip to ensure that all primary acceptors were totally oxidized, i.e., the reaction centers were opened.

The pulse-modulated chlorophyll fluorometer was also used under light conditions. The 'Yield' protocol was used by applying an actinic light source with a saturating

multi-flash pulse coupled to a photosynthetically active radiation clip (PAR-Clip) to determine the following variables: initial fluorescence before the saturation pulse (F'), maximum fluorescence after adaptation to saturating light (F_m'), electron transport rate (ETR), and quantum efficiency of photosystem II (YII). These results were then used to determine the following variables: minimum fluorescence of the illuminated plant tissue (F₀'), determined by the methodology developed by Oxborough and Baker (1997), using Equation 4; the photochemical quenching coefficient, determined by the lake model (qL), the quantum yield of photochemical quenching (Y_{NPQ}), and the quantum yield of non-photochemical quenching (Y_{NO}), present in equations 5, 6, and 7 and based on the methodology proposed by Kramer et al., 2004, described as follows:

$$Fo' = \left(\frac{Fo}{\left(\frac{Fm - Fo}{Fm}\right) + \left(\frac{Fo}{Fm'}\right)} \right) \quad (4)$$

$$qL = \frac{(Fm' - F')}{(Fm' - Fo)} * \left(\frac{Fo'}{F'}\right) \quad (5)$$

The variables were evaluated by analysis of variance, by the F-test (1 and 5% probability), and, in cases of significant effects, linear and quadratic polynomial regression were employed using the statistical software SISVAR (Ferreira, 2014). Regression was chosen based on the best adjustment using the coefficient of determination (R²) and considering a probable biological explanation.

Results and discussion

There was a significant interaction between irrigation water salinity (ECw) and potassium silicate concentrations (D) under light conditions, except for the variables of quantum efficiency of photosystem II and leaf temperature in cashew seedlings (Table 2), with TF showing an isolated effect on the potassium silicate concentrations.

For the PAR values (Figure 1A), the application of 250 mg L⁻¹ resulted in a quadratic behavior, with a positive effect of potassium silicate up to the estimated ECw of 1.64 dS m⁻¹. However, there was an increasing linear effect when using 1,000 mg L⁻¹, achieving the highest PAR value at the ECw of 3.1 dS m⁻¹, (365.1 μmol m⁻² s⁻¹).

It should be noted that the PAR values showed no statistical difference at the potassium silicate concentrations of 500 and 750 mg L⁻¹, with mean values of 193.0 and 191.4 μmol m⁻² s⁻¹, respectively (Figure 1A). According to Petter et al. (2016), the efficient use of

solar radiation depends on some factors, including the studied species, which may show different morphological characteristics. From this perspective, the use of

photosynthetically active radiation by plants is strongly influenced by biotic and abiotic factors that can affect their growth and development (Zivcak et al., 2014).

Table 2. Summary of the analysis of variance for the initial fluorescence before the saturation pulse (F_i'), maximum fluorescence after adaptation to saturating light (F_m'), quantum efficiency of photosystem II (YII), electron transport rate (ETR), photosynthetically active radiation (PAR), minimum fluorescence of the illuminated plant tissue (F_0'), photochemical quenching coefficient by the lake model (QL), quantum yield of photochemical quenching (Y_{NPQ}), quantum yield of non-photochemical quenching (Y_{NO}), and leaf temperature (TF) of cashew plants subjected to potassium silicate fertilization and irrigation with saline water 70 days after sowing.

FV	DF	Mean square									
		F_i'	F_m'	YII	ETR	PAR	F_0'	QL	Y_{npq}	Y_{no}	TF
ECw (EC)	4	5357**	26080**	0.010 ^{ns}	3148**	44838**	261*	0.020*	0.0018**	0.008**	0.017 ^{ns}
K silicate (D)	4	10604**	6690**	0.074**	3451**	53674**	609**	0.103**	0.0012**	0.027**	0.114*
Interaction (EC x D)	16	1655 ^{ns}	7603**	0.012**	2355**	53394**	306**	0.048**	0.0008**	0.007**	0.074*
Blocks	3	896 ^{ns}	2105 ^{ns}	0.004 ^{ns}	635 ^{ns}	10823 ^{ns}	627**	0.014 ^{ns}	0.0005*	0.016**	1.133**
Residue	72	648	1403	0.004	261	4797	86	0.007	0.0001	0.002	0.03
Means		226.94	577.97	0.562	60.57	237.53	134.13	0.467	0.043	0.391	34.49
CV (%)		11.22	6.48	12.17	20.70	20.16	6.95	18.31	21.97	11.67	0.57

^{ns}, **, * respectively non-significant and significant at $p \leq 0.01$ and $p \leq 0.05$.

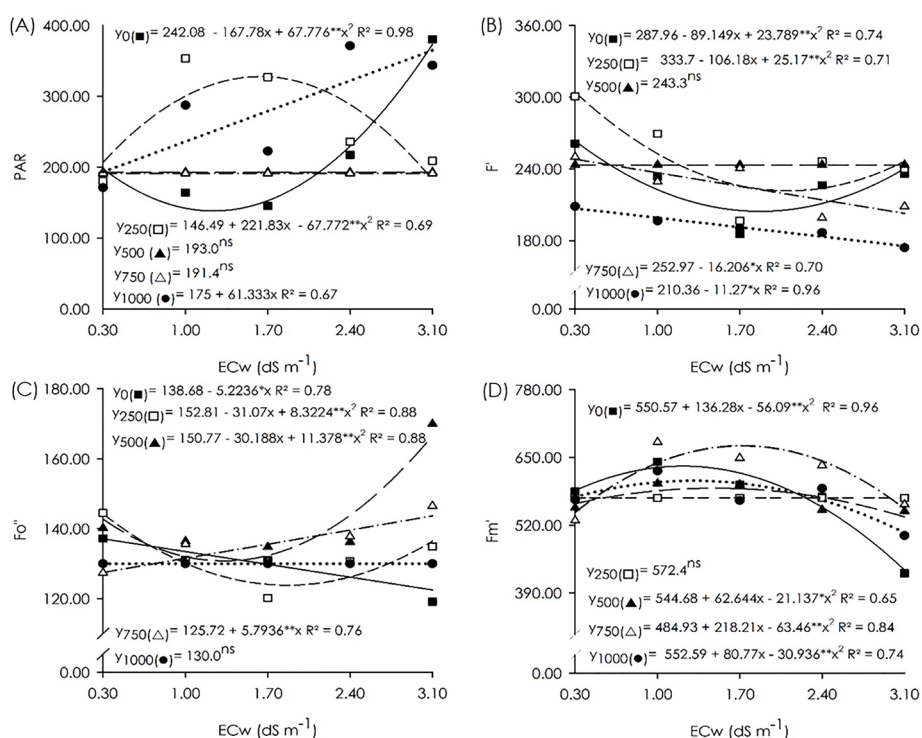


Figure 1. Photosynthetically active radiation – PAR (A), initial fluorescence before the saturation pulse F_i' (B), minimum fluorescence of the illuminated plant tissue – F_0' (C), and maximum fluorescence after adaptation to saturating light F_m' (D) of cashew plants as a function of the interaction between water electrical conductivity– ECw and fertilization with potassium silicate 70 days after sowing.

The initial fluorescence was not significantly influenced by the tested factors at the concentration of 500 mg L⁻¹, showing a mean value of 243.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1B). At the potassium silicate concentrations of 0 and 250 mg L⁻¹, this variable increased from the salinity levels of 1.87 and 2.11 dS m⁻¹, respectively. However, at the highest concentrations (750 and 1000 mg L⁻¹), there were linear reductions of 18.3 and 15.2%, respectively. These results highlight that, under ambient light, the

combination of Si and K in cashew plants under salt stress results in a more efficient use of light energy.

For minimum fluorescence, the absence of potassium silicate resulted in a linear decrease as irrigation water salinity increased, suggesting that the salinity levels tested did not cause photoinhibition in cashew plants (Figure 1C). At the potassium silicate concentrations of 250 and 500 mg L⁻¹, F_0' decreased by 18.98 and 13.28% until the estimated ECw of 1.87 and 1.33 dS m⁻¹, respectively.

However, the concentration of 750 mg L⁻¹ promoted a linear increase as a function of the salinity levels, with a 14.28% increase at the EC_w of 3.1 dS m⁻¹; in contrast, no significant difference was observed at 1,000 mg L⁻¹, with a mean of 130 electrons quantum⁻¹. These values highlight that salinity levels above 1.87 dS m⁻¹ combined with high potassium silicate concentrations induce lower efficiency in the transference of light energy to reaction centers, resulting in PSII inactivation (Najar et al., 2018).

In cashew plants without potassium silicate fertilization, the maximum fluorescence after adaptation to saturating light decreased with estimated EC_w levels from 1.21 dS m⁻¹, expressing the maximum value of 633.35 electrons quantum⁻¹. Under potassium silicate application, the best performance was achieved at 750 mg L⁻¹, with 672.51 electrons quantum⁻¹ at the EC_w of 1.71 dS m⁻¹ (Figure 1D). The increase in F_m' indicates that more electrons are passing through the photosystems and highlights their

importance in protecting the photosynthetic apparatus (Kalaji et al., 2016; Wang et al., 2017).

Moreover, in plants without potassium silicate application, there was a linear increase in ETR as the salinity levels increased. However, potassium silicate application also increased this variable, more markedly at the concentration of 1,000 mg L⁻¹ in plants irrigated with up to 2.11 dS m⁻¹ (Figure 2A). It can be deduced that water salinity did not cause photoinhibition and, consequently, did not limit carbon fixation in cashew plants. Moreover, the application of potassium silicate induced the electron transport rate, which may be related to the maintenance of the photosynthetic apparatus provided by the double silicon layer in the leaves and the metabolic activity of potassium, thus maintaining the high efficiency of the photochemical activity in the plant (Santos et al., 2020; Souza Junior et al., 2020).

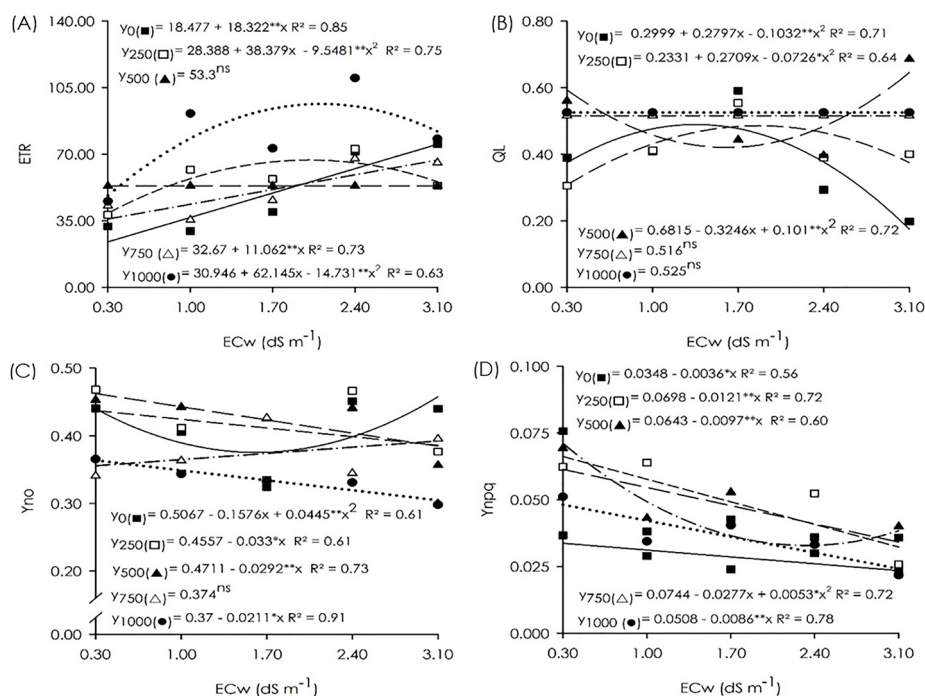


Figure 2. Electron transport rate – ETR (A), photochemical quenching coefficient by the lake model – QL (B), quantum yield of non-photochemical quenching – Y_{no} (C), and quantum yield of photochemical quenching – Y_{npq} (D) in cashew plants as a function of the interaction between water electrical conductivity– EC_w and fertilization with potassium silicate 70 days after sowing.

The plants cultivated under the potassium silicate concentrations of 0 and 250 mg L⁻¹ showed an increase in QL up to the estimated EC_w levels of 1.36 and 1.87 dS m⁻¹, respectively (Figure 2B). However, the concentration of 500 mg L⁻¹ caused the opposite effect, reducing the QL until the salinity of 1.61 dS m⁻¹. It should be noted that the concentrations of 750 and 1,000 mg L⁻¹ did not significantly influence this variable, with mean values of 0.516 and 0.525, respectively (Figure 2B). QL indicates

the degree of openness of the PS II reaction centers, representing the joint occurrence of photosynthesis and respiration (Wang et al., 2017). This increase in QL suggests the greater participation of an alternative electron drain, e.g., photorespiration. On the other hand, the highest concentrations did not cause a significant difference, probably because the cashew plants could not channel the flow of electrons to NADPH synthesis (Azevedo Neto et al., 2011).

The increase in Y_{NO} is a strong indicator of photoinhibition (Wang et al., 2017). From this perspective, the application of 1,000 mg L⁻¹ potassium silicate evidenced the lowest inhibition rates by reducing this variable, with a linear decreasing behavior with the increase in salinity (Figure 2C). Corroborating the previous statement, Khanghahi et al. (2019) reported that the values of Y_{NO} are negatively correlated with the quantum efficiency of PSII, making it an excellent photodamage indicator.

The use of potassium silicate decreased the Y_{NPQ} by 30.3, 51.5, 44.3, 40.9, and 50% at the concentrations of 0, 250, 500, 750, and 1000 mg L⁻¹, respectively (Figure 2D). The reduction in Y_{NPQ} indicates that less excitation energy was dissipated as heat, supposedly favoring energy dissipation to the photochemical stage, resulting in significant photosynthetic rate gains (Tatagiba et al.,

2017).

The increase in water salinity resulted in a linear decrease in the quantum efficiency of the photosystem, with an 8.9% reduction in plants cultivated under the ECw of 3.1 dS m⁻¹ compared to the treatment with the lowest water salinity (Figure 3A). However, the application of potassium silicate levels up to 666.7 mg L⁻¹ increased the YII by 23.3% when considered in isolation (Figure 3B). This reduction as a function of salinity is an indicator that the solar light absorbed was not converted into photochemical energy and was possibly dissipated as heat (Najar et al., 2018). On the other hand, the positive effect with the increase in the concentrations occurred because these nutrients contribute to energy storage, enzyme activation, and the increase of photosynthetic capacity (Souza Junior et al., 2020).

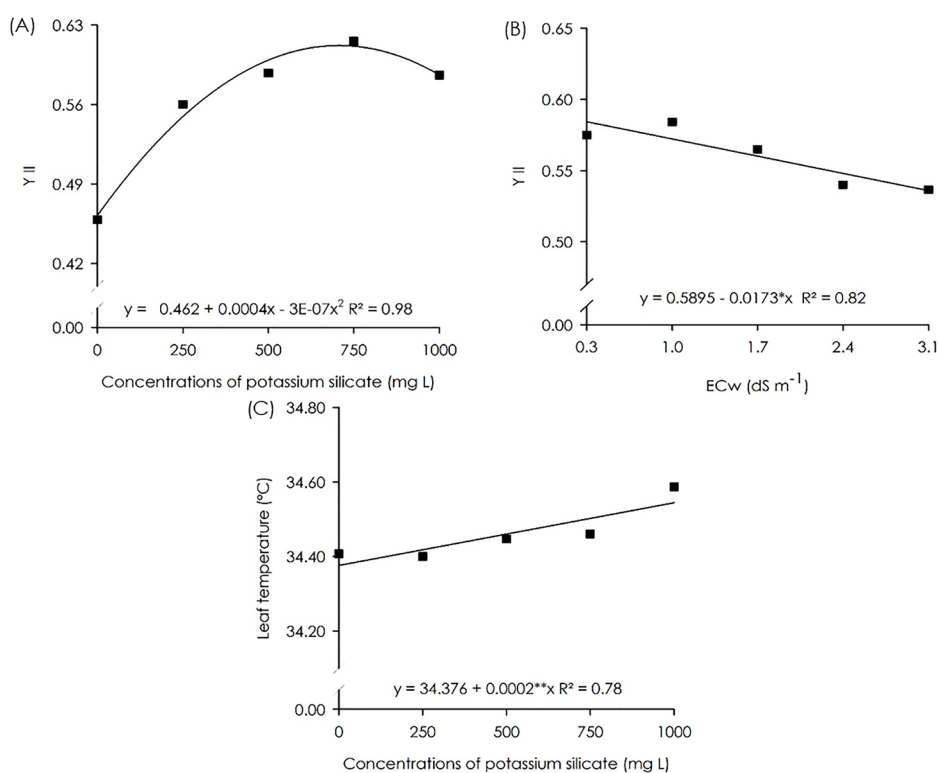


Figure 3. Quantum efficiency of photosystem II – YII (A and B) and leaf temperature – TF (C) of cashew plants as a function of water electrical conductivity – ECw (A) and fertilization with potassium silicate 70 days after sowing.

Leaf temperature increased linearly with the increase in potassium silicate concentrations (Figure 3C). The increase in leaf temperature may be related to the fact that Si modifies the leaf structure, improving the opening angle and allowing greater contact with light, resulting in increased heating (Santos et al., 2020).

Under dark conditions, there was a significant interaction between irrigation water salinity (ECw) and potassium silicate levels (D) for all variables analyzed (Table 3).

For initial fluorescence, the potassium silicate concentrations of 0 and 250 mg L⁻¹ did not influence this variable significantly. However, the concentrations of 500, 750, and 1000 mg L⁻¹ resulted in F_0 increases of 27, 15, and 10%, respectively, as a function of the increase in salinity (Figure 4A). The increase in salinity can damage the photosynthetic apparatus due to low water availability, decreasing the transfer of excitation energy to PSII (Ghassemi-Golezani & Lofli, 2015).

Table 3. Summary of the analysis of variance, by mean square values, for initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), maximum quantum yield of PSII (F_v/F_m), and F_v/F_0 ratio of cashew plants subjected to potassium silicate fertilization and irrigation with saline water 70 days after sowing.

FV	GL	Mean square				
		F_0	F_m	F_v	F_v / F_m	F_v / F_0
ECw (EC)	4	727.39**	1028.08*	5155.59**	0.00217**	0.631**
K silicate (D)	4	321.84**	3077.52**	2629.16**	0.00159**	0.245**
Interaction (EC x D)	16	298.35**	2211.20**	2592.66**	0.00126**	0.258**
Blocks	3	914.10**	11002.90**	7601.59**	0.00033 ^{ns}	0.089 ^{ns}
Residue	72	71.43	307.06	542.07	0.00026	0.057
Means		133.10	568.81	430.25	0.7619	3.258
CV (%)		6.35	3.08	5.41	2.14	7.38

^{ns}, **, * respectively non-significant and significant at $p \leq 0.01$ and $p \leq 0.05$.

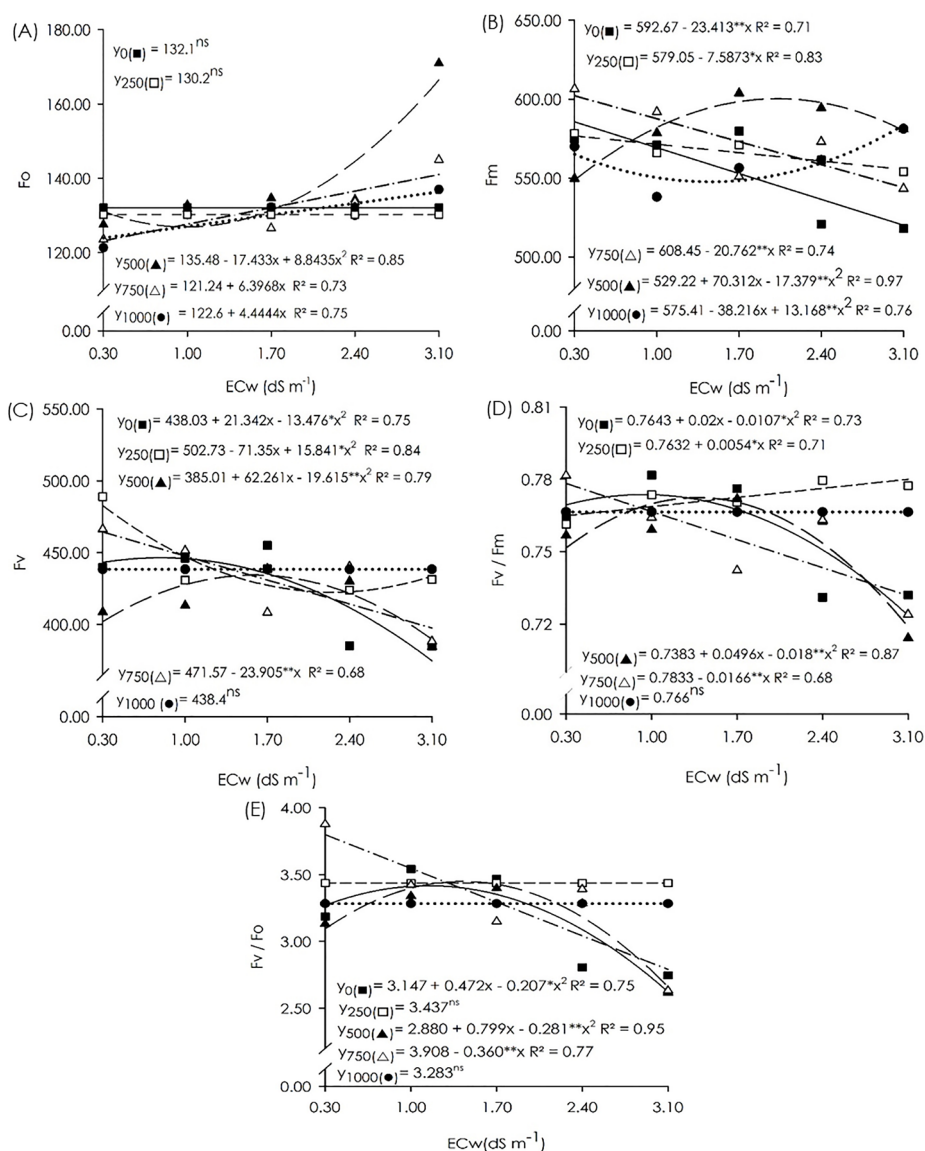


Figure 4. Initial fluorescence - F_0 (A), maximum fluorescence - F_m (B), variable fluorescence - F_v (C), maximum quantum yield of PSII - F_v/F_m (D), and F_v/F_0 (E) ratio of cashew plants as a function of the interaction between water electrical conductivity – ECw and potassium silicate fertilization 70 days after sowing.

Maximum fluorescence (Figure 4B) followed the same trend as F_0 (Figure 4A), decreasing with the increase in irrigation water salinity. However, the potassium silicate concentration of 500 mg L⁻¹ provided positive effects up

to the estimated CEa of 2.02 dS m⁻¹ (Figure 4B). F_m is an indicator of when the reaction centers of PSII are closed and reach their maximum capacity, signaling the failure of PSII in reducing plastoquinone A (Zivcak et al., 2014;

Souza Junior et al., 2020).

Similar to F_0 and F_m , the highest salinity levels caused reductions in F_v , both in the presence and absence of potassium silicate, except at the concentration of 1,000 mg L⁻¹, which showed no significant difference (Figure 4C). This behavior can be associated with the F_m values obtained, highlighting a strong correlation between these variables, with F_v referring to the potential energy active in PSII (Sá et al., 2018).

The application of potassium silicate increased the maximum quantum yield of PSII (Figure 4D), with the concentration of 250 mg L⁻¹ showing linear gains as the salinity levels increased (Figure 4D). These results suggest that, under salt stress conditions, this concentration of Si and K resulted in a higher quantum yield of PSII in cashew plants. However, the decrease verified at 0 mg L⁻¹, for example, could be associated with chlorophyll degradation by chlorophyllase, especially chlorophyll *a* (Ahanger & Agarwal, 2017).

For the F_v/F_0 ratio, the potassium silicate levels of 0, 500, and 750 mg L⁻¹ provided the best results at the respective EC_w of 1.14 (3.41), 1.42 (3.45), and 0.3 (3.91) dS m⁻¹, subsequently decreasing (Figure 4E). For Zivcak et al. (2014), the F_v/F_0 ratio refers to the efficiency of the photochemical reactions of PSII. Therefore, it could be inferred that the increase in water salinity caused photochemical damage to cashew plants (Ghassemi-Golezani & Lofti, 2015).

Conclusions

Irrigation water salinity levels from 2.1 dS m⁻¹ reduce the photochemical efficiency of photosystem II by at least 10%, consequently affecting the photosynthetic apparatus of cashew plants 70 days after sowing.

The potassium silicate concentration of 250 mg L⁻¹ increases the quantum yield of cashew plants under salt stress conditions.

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