ISSN 1807-1929



# Revista Brasileira de Engenharia Agrícola e Ambiental

Brazilian Journal of Agricultural and Environmental Engineering

v.25, n.12, p.853-861, 2021

Campina Grande, PB - http://www.agriambi.com.br - http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v25n12p853-861

# Growth and physiology of ornamental sunflower under salinity in function of paclobutrazol application methods<sup>1</sup>

Crescimento e fisiologia do girassol ornamental sob salinidade em função de métodos de aplicação de paclobutrazol

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#### HIGHLIGHTS:

Application of paclobutrazol (PBZ) maintains the external diameter of chapter in sunflower greater than without PBZ under salt stress. The number of sunflower petals decreases with salt stress without PBZ application.

The PBZ application via soil is more effective than foliar application in sunflower plants.

**ABSTRACT:** The ornamental sunflower has great importance in the floriculture sector due to the color and vitality of its flowers. However, the production and quality of flowers decrease under salt stress, which can be mitigated with paclobutrazol application. The objective of the present study was to evaluate the effects of different application methods of paclobutrazol in ornamental sunflower 'Sol Noturno' irrigated with brackish waters. The experimental design was randomized blocks arranged in a  $5 \times 3$  factorial scheme, corresponding to five electrical conductivities of irrigation water - ECw (0.4; 1.9; 3.4; 4.9, and 6.4 dS m<sup>-1</sup>) and three paclobutrazol application methods (foliar application, via soil and a control treatment - without paclobutrazol), with four replicates. The increase in salinity of irrigation water reduced gas exchange, photosystem II photochemical efficiency, SPAD index, plant height, and chapter diameter. The paclobutrazol application via soil or foliar increased stomatal conductance and transpiration by 21.09 and 17.80%, respectively, in comparison to plants without application, whereas photosynthesis and instantaneous carboxylation efficiency increased by 28.33 and 31.18% via soil and 14.40 and 16.12% via foliar, respectively. The paclobutrazol application, mainly via soil, favored 'Sol Noturno' sunflower plants under salt stress, increasing chlorophyll SPAD index and external chapter diameter, and keeping the number of the petals.

Key words: Helianthus annuus L., gas exchange, growth regulator

**RESUMO:** O girassol ornamental tem grande importância no setor de floricultura devido a cor e exuberância de suas flores. Porém, sua produção e qualidade das flores diminuem sob estresse salino, o qual pode ser atenuado com a aplicação de paclobutrazol. O objetivo foi avaliar os efeitos de diferentes métodos de aplicação de paclobutrazol em girassol ornamental 'Sol Noturno' irrigado com águas salobras. O delineamento experimental consistiu em blocos casualizados em esquema fatorial  $5\times3$ , correspondendo a cinco condutividades elétricas da água de irrigação - CEa (0,4;1,9;3,4;4,9 e 6,4 dS m<sup>-1</sup>) e três métodos de aplicação de paclobutrazol (aplicação foliar, via solo e uma testemunha - sem paclobutrazol), com quatro repetições. O aumento da salinidade da água de irrigação reduziu as trocas gasosas, eficiência efetiva do fotossistema II, índice SPAD, altura de plantas e diâmetros do capítulo. A aplicação de paclobutrazol na folha e via solo aumentou a condutância estomática e a transpiração por 21,09 e 17,80%, respectivamente, em relação às plantas sem aplicação, enquanto a fotossíntese e eficiência instantânea de carboxilação aumentaram 28,33 e 31,18% via solo, e 14,40 e 16,12% via foliar, respectivamente. A aplicação de paclobutrazol, principalmente via solo, favoreceu as plantas de girassol 'Sol Noturno' sob estresse salino, aumentando o índice de clorofila SPAD e o diâmetro externo do capítulo, e mantendo o número de pétalas.

Palavras-chave: Helianthus annuus L., trocas gasosas, regulador de crescimento

• Accepted 31 May, 2021 • Published 01 Jul, 2021

Edited by: Hans Raj Gheyi

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<sup>•</sup> Ref. 247758 – Received 19 Jan, 2021

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## Introduction

The ornamental sunflower is a highly valued plant as a flower for cultivation in vases, mainly due to the different colors and exuberance of its inflorescence (Brito et al., 2016). However, the plant has a large size, difficult for the production and commercialization of this species for ornamental purposes, considering that small architectural plants are sought for cultivation in vases with a mean height of 35 cm (Wanderley et al., 2014). Besides, the production and quality of flowers can be affected when only brackish water is available for irrigation, reducing the external and internal diameter of the chapter, besides the number of petals (Nobre et al., 2014; Santos Júnior et al., 2016).

The plant responses to salt occur in two main phases. The first is characterized by reduced growth independent of ions, which occurs within minutes or days, cause stomatal closure and inhibition of cell expansion, mainly the aerial part. The second phase occurs over days or even weeks and relates to increased levels of cytotoxic ions, which delay metabolic processes, cause premature senescence, and, finally, cell death (Isayenkov & Maathuis, 2019; Lima et al., 2019; Alves et al., 2020). However, cultivation alternatives have been used to minimize the deleterious effects of salinity, such as the use of paclobutrazol (Hu et al., 2017; Waqas et al., 2017; Xia et al., 2018).

Paclobutrazol - PBZ [(2 RS, 3 RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1 H-1,2,4-trizol-1-yl)-pentan-3-ol] is a triazole compound with plant growth regulating properties. The PBZ is used, mainly in the foliar application or via soil, being reported in literature important performance in plant growth and biomass (Hu et al., 2017), promoting increase in the photosynthetic pigments (Kishor et al., 2009) and stomata functioning, and maintaining photochemical efficiency under salt stress conditions (Waqas et al., 2017).

PBZ also operate in the absence of stress as in *Catharanthus roseus*, which increased the photosynthetic rate (Jaleel et al., 2007), in *Solanum lycopersicum* increased stomatal conductance (Silva et al., 2020) and sunflower decreased plant height without affecting biomass production (Barbosa et al., 2009; Brito et al., 2016). Despite these reports of PBZ on sunflower, the effects of different application methods are not known under salt stress conditions. Therefore, the study aimed to evaluate the effects of PBZ application methods on ornamental sunflower 'Sol Noturno' irrigated with brackish waters.

# MATERIAL AND METHODS

The study was carried out from June to September 2019, under greenhouse conditions at the Centro de Tecnologia e Recursos Naturais of the Universidade Federal de Campina Grande, Campina Grande, Paraiba, Brazil, in the 'Agreste Paraibano' mesoregion, situated by the geographic coordinates 7° 15' 18" S, 35° 52' 28" W, and altitude of 550 m.

In the greenhouse, the maximum values of temperature and relative air humidity were 31.4 °C and 96%, and the minimum values were 20.4 °C and 33%, respectively. During

the experiment, the mean daily temperature and relative air humidity were 27.9 °C and 64.5%, respectively.

The experimental design was in randomized blocks and factorial arrangement 5 x 3, with four replicates, being the treatments obtained from the combination of five electrical conductivities of irrigation water - ECw (0.4; 1.9; 3.4; 4.9 and 6.4 dS  $\rm m^{-1}$ ) and three methods of PBZ application (foliar application, application via soil and a control treatment - without PBZ application).

Seeds of the ornamental sunflower, 'Sol Noturno', which has a red chapter, were purchased from Isla\*, previously disinfected. The seeds were placed to germinate in tubes of 280 mL, filled with commercial substrate, and kept in a greenhouse with daily irrigation until the time of transplanting, which occurred 20 days after sowing.

The transplanting was performed in plastic pots with a capacity of 20 L, filled with 18 kg of soil, with a drain of 4 mm on the lower base to allow drainage of the leachate. The outer end of the drain was coupled to a plastic bottle to collect the drained water, aiming to estimate water consumption by the plant. Therefore, based the water volume applied was calculated by water balance, using Eq. 1:

$$VI = \frac{Va - Vd}{1 - LF} \tag{1}$$

where:

VI - volume of water to be applied in the irrigation event (mL);

 $Va \quad \text{-volume applied in the previous irrigation event (mL);} \\$ 

Vd - volume drained (mL); and,

 $\,$  LF  $\,$  - leaching fraction of 0.10, aimed at reducing the accumulation of salts in the soil.

Previously, the field capacity was determined by the difference between the applied volume and the volume drained 48 hours after the irrigation event.

The physical and chemical characteristics of soil are shown in Table 1.

Table 1. Physical and chemical characteristics of soil used

Physical	Values				
Sand (g kg <sup>-1</sup> )	688.00				
Silt (g kg <sup>-1</sup> )	96.00				
Clay (g kg <sup>-1</sup> )	216.00				
Soil bulk density (kg dm <sup>-3</sup> )	1.16				
Particle density (kg dm <sup>-3</sup> )	2.65				
Total porosity (m <sup>-3</sup> m <sup>-3</sup> )	0.56				
Textural classification	Sandy clay loam				
Chemical					
pH (Water - 1:2.5)	5.3				
P (mg dm <sup>-3</sup> )	3.88				
K <sup>+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.20				
Ca <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	1.67				
Mg <sup>2+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	1.56				
Na <sup>+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.04				
SB (cmol <sub>c</sub> dm <sup>-3</sup> )	3.47				
Al <sup>3+</sup> (cmol <sub>c</sub> dm <sup>-3</sup> )	0.40				
$H^+ + Al^{3+}$ (cmol <sub>c</sub> dm <sup>-3</sup> )	7.21				
CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	10.68				
OM (g kg <sup>-1</sup> )	28.63				

OM - Organic matter; SB - Sum of bases; CEC - Cation exchange capacity

The water with different electrical conductivities was prepared by addition of NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O, and MgCl<sub>2</sub>·6H<sub>2</sub>O, in equivalent proportions 7:2:1, dissolved in public-supply water (ECw = 0.4 dS m<sup>-1</sup>), considering the relationship between ECw and the concentration of salts (mmol<sub>c</sub> L<sup>-1</sup> = 10 x ECw, in dS m<sup>-1</sup>), according to Richards (1954).

Irrigation with different saline treatments began ten days after acclimatization of seedlings.

The PBZ application was performed twice during the cycle, one day after transplanting and 20 days thereafter, because it refers to the vegetative phase of the crop, before the flower bud emission (Castiglioni et al., 1997). The concentration used was 300 mg  $\rm L^{-1}$ , and 100 mL was applied through both methods. In the soil, the paclobutrazol solution was applied directly on the substrate of each pot, near the base of plants (Brito et al., 2016). The foliar application was performed using a small hand-operated sprayer until the excess solution started to drop. The sprays were performed on the adaxial and abaxial sides of the leaves.

Fertilization was carried out with nitrogen, phosphorus, and potassium, based on the recommendation of Novais et al. (1991) and considering the concentrations presented in the soil analysis, with the equivalent of 100 mg N, 300 mg  $P_2O_5$ , and 150 mg  $K_2O$  kg<sup>-1</sup>, using urea, monoammonium phosphate, and potassium chloride respectively. As a source of micronutrients, the equivalent of 2 kg ha<sup>-1</sup> of boron was used (Jardini et al., 2014), through the mix of micronutrients (Dripsol'), containing [N (68.89%); P (61%); K (60%); Mg (9%); Ca (18.5%); S (28.9%); B (0.85%); Cu (0.5%); Fe (3.4%); Mn (3.2%); Zn (4.2%), and Mo (0.06%)].

At 25 days after transplanting, corresponding to 15 days after the beginning of irrigation with saline treatments, physiological and biometric traits were evaluated.

The gas exchange was measured through stomatal conductance - gs (mol  $\rm H_2O~m^{-2}~s^{-1}$ ), transpiration - E (mmol  $\rm H_2O~m^{-2}~s^{-1}$ ), photosynthesis rate - A (µmol  $\rm m^{-2}~s^{-1}$ ) internal  $\rm CO_2$  concentration (µmol  $\rm m^{-2}~s^{-1}$ ) (Ci); intrinsic water-use efficiency - iWUE (µmol  $\rm m^{-1}~s^{-1}$ ), and instantaneous carboxylation efficiency - iCE (µmol  $\rm m^{-2}~s^{-1}$ ), obtained based on the ratio between photosynthesis rate with stomatal conductance and internal  $\rm CO_2$  concentration, respectively. These measurements were made on the third leaf, counted from the apex, using the portable photosynthesis meter "LCPro+" from ADC BioScientific Ltda., operating with control of temperature at 25 °C, irradiation of 1200 µmol photons  $\rm m^{-2}~s^{-1}$ , airflow of 200 mL min<sup>-1</sup>, and at the atmospheric level of  $\rm CO_2$ , between 9:00 and 11:00 a.m.

The SPAD index was determined from five readings in the third expanded leaf from the apex, using SPAD-502. The results were expressed by the average of the readings per plant, estimated as chlorophyll index.

The maximum photochemical efficiency of PSII (Fv/Fm) was measured in leaves pre-adapted to the dark using leaf clips for 30 min, between 10:00 and 11:00 a.m., the same leaves used for gas exchange evaluations, using a modulated pulse fluorometer model OS5p (Opti Science), according to Baker & Rosenqvist (2004). The evaluations were performed under light conditions through Yield protocol. An actinic lighting source with a saturating multi-flash pulse was applied to obtain readings, attached to a clip for determining photosynthetically active radiation (PAR-Clip), aiming to measure the photosystem II photochemical efficiency (YII).

The growth of sunflower plants was evaluated based on plant height, measured from the base to insertion point of the apical meristem. Stem diameter was measured at 2 cm from the soil surface, and leaf number counting those fully expanded with a minimum length of 2 cm in each plant. In the R5 stage, the external diameter (EDC) and the internal diameter of the chapter (IDC) in a completely open inflorescence were measured in the horizontal direction; at the time, the number of the petals (PN) was also counted.

After verifying the homogeneity of variances, the obtained data were subjected to analysis of variance. In cases of significance, polynomial regression analyses were carried out for the electrical conductivities of irrigation water. The Tukey test was used to compare the means for the paclobutrazol application methods at  $p \leq 0.05$ , using the statistical program SISVAR version 5.6 (Ferreira, 2014).

## RESULTS AND DISCUSSION

The stomatal conductance (gs), transpiration (E), photosynthesis rate (A), and instantaneous carboxylation efficiency (iCE) were significantly affected by PBZ application methods and salinity levels. There was no effect of the interaction between factors for any of these characteristics (Table 2). Meanwhile, the internal CO<sub>2</sub> concentration was affected only by salinity, and intrinsic water use efficiency (iWUE) was not influenced by any factor.

**Table 2.** Summary of analysis of variance of paclobutrazol application method (M), electrical conductivities of irrigation water (S), and their interaction (M  $\times$  S) for stomatal conductance (gs), transpiration (E), photosynthesis rate (A), internal CO<sub>2</sub> concentration (Ci), intrinsic water use efficiency (iWUE), instantaneous carboxylation efficiency (iCE), maximum photochemical efficiency (Fv/Fm), photosystem II photochemical efficiency (YII), SPAD index, plant height (PH), stem diameter (SD), number of leaves (NL) and petals (NP), external chapter diameter (ECD) in sunflower, and internal chapter diameter (ICD)

SV	DF	P-value					
		gs	Е	Α	Ci	iWUE	
Block	3	0.351	0.059	0.432	0.324	0.147	
M	2	0.001**	$0.000^{**}$	$0.000^{**}$	$0.347^{ns}$	$0.778^{ns}$	
S	4	0.011*	$0.003^{**}$	$0.000^{**}$	$0.008^{**}$	0.485 <sup>ns</sup>	
$M \times S$	8	0.713 <sup>ns</sup>	$0.537^{ns}$	0.687 <sup>ns</sup>	$0.343^{ns}$	0.147 <sup>ns</sup>	
Error	42						
CV (%)		22.70	14.94	12.95	12.78	16.73	
		iCE	Fv/Fm	Y(II)	SPAD	PH	
Block	3	0.089	0.348	0.647	0.358	0.341	
M	2	0.000**	$0.306^{\text{ns}}$	$0.002^{**}$	0.000**	$0.007^{**}$	
S	4	0.000**	0.102 <sup>ns</sup>	0.000**	0.001**	0.000**	
$M \times S$	8	0.628 <sup>ns</sup>	0.342 <sup>ns</sup>	$0.875^{ns}$	$0.010^*$	0.000**	
Error	42						
CV (%)		20.87	1.19	5.79	4.63	12.74	
		SD	NL <sup>1</sup>	NP <sup>1</sup>	ECD	ICD	
Block	3	0.234	0.400	0.683	0.197	0.031	
M	2	0.008**	$0.062^{ns}$	$0.020^{*}$	0.001**	$0.072^{ns}$	
S	4	0.389 <sup>ns</sup>	$0.490^{ns}$	$0.340^{ns}$	0.000**	$0.005^{**}$	
$M \times S$	8	$0.035^{*}$	0.146 <sup>ns</sup>	$0.005^{**}$	$0.003^{**}$	0.094 <sup>ns</sup>	
Error	42						
CV (%)		7.42	5.02	8.67	6.84	11.29	

ns, \*\*, \* - Not significant, significant at  $p \le 0.01$  and  $p \le 0.05$  by F test, respectively;  $^1$  data transformed in square root; SV - Source of variation; DF - Degrees of freedom; CV - Coefficient of variation

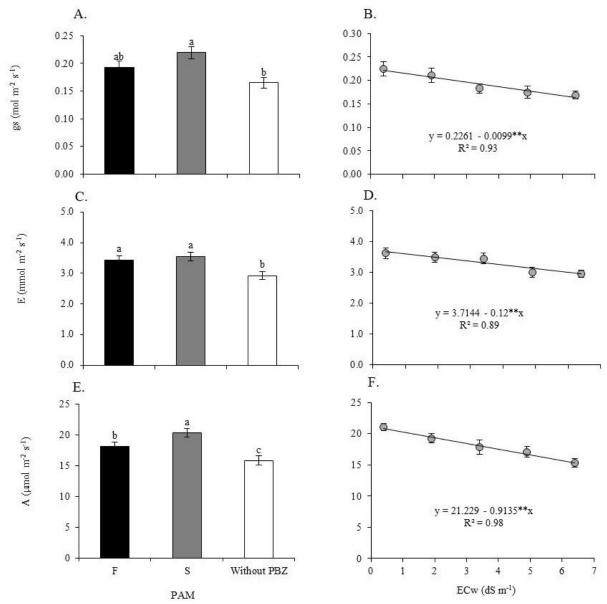
Considering PBZ application methods, both foliar and soil application increased gs and E of ornamental sunflower plants by 21.09 and 17.80% compared to those without PBZ application (Figures 1A and C). While for photosynthesis, application via soil promoted higher A followed by the foliar application, corresponding to 28.33 and 14.40%, respectively higher than without PBZ (Figure 1E). A positive linear correlation between the gas exchange was found when plants received PBZ application via leaf and soil, but not in plants without PBZ, indicating that salinity may have affected more intensely plants not treated with PBZ (Table 3).

These effects on the gas exchange may be associated with the improvement promoted by the application of PBZ over stomatal density on both the adaxial and abaxial surfaces of leaves of plants under salt stress (Waqas et al., 2017), which favors stomatal conductance, transpiration, and photosynthesis, as observed in the sunflower plants. Also, triazole compounds, such as PBZ, reduce the biosynthesis of gibberellin and increase the content of abscisic acid and cytokinin, which helps plants to

maintain a better water balance under stress (Hu et al., 2017).

The higher net photosynthesis promoted by the application of PBZ via soil may be related to the more efficient action of this growth regulator on the xylem and phloem vessels (Jaleel et al., 2007), favoring greater availability of water and nutrients to the roots for the aerial part, which contributed to photosynthesis rate, since PBZ interferes in the source-sink relationship (Silva et al., 2020). PBZ induced improvement in gas exchange was also observed in plants of *Catharanthus roseus* (Jaleel et al., 2007), *Paeonia lactiflora* (Xia et al., 2018), *Solanum lycopersicum* (Silva et al., 2020), and forest species (Cregg & Ellison-Smith, 2020) in the absence of stress, besides *Chenopodium quinoa* under salt stress (Waqas et al., 2017).

The increased salinity of irrigation water promoted a reduction in gas exchange characteristics, except for internal CO<sub>2</sub> concentration. At the higher salt level (6.4 dS m<sup>-1</sup>) gs, E and A decreased 26.73, 19.63, and 26.27%, respectively, compared to those irrigated with ECw 0.4 dS m<sup>-1</sup> (Figures 1B, D, and F), while Ci increased by 14.26% in the same range (Figure 2A).

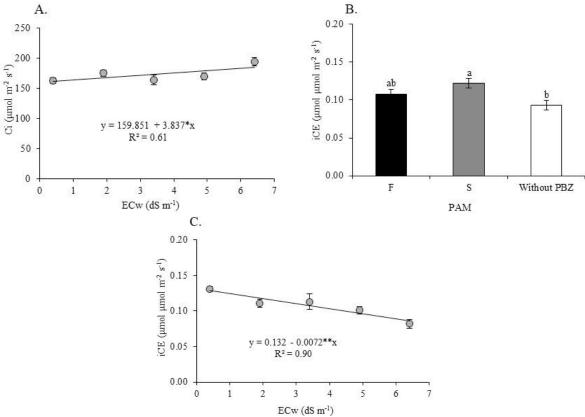


Bars with the same letters indicate that the treatments do not differ by Tukey test at  $p \le 0.05$ ; \*\* - Significant at  $p \le 0.01$  by the F-test; F - Foliar application of PBZ; S - PBZ application via soil Figure 1. Stomatal conductance - gs (A and B), transpiration - E (C and D), and net photosynthesis - A (E and F) of sunflower as a function of irrigation water electrical conductivity (ECw) and paclobutrazol application method (PAM)

**Table 3.** Pearson correlation coefficients between physiological and morphological characteristics in sunflower plants under different paclobutrazol (PBZ) application methods

	E	A	Y(II)	SPAD	PH	SD	NP	EDC	IDC
				PBZ foliar	application				
gs	0.79**	0.83**	0.03	-0.03	-0.23	-0.47*	-0.39	0.02	0.23
E		0.80**	-0.06	-0.12	-0.47*	-0.73**	-0.28	0.17	0.34
4			0.16	-0.21	-0.15	-0.59*	0.34	0.00	0.24
/(II)				-0.06	0.07	0.30	-0.07	0.32	0.31
SPAD					0.01	-0.08	0.37	-0.29	0.00
PH						0.50*	0.05	-0.42	-0.15
SD							0.15	-0.18	-0.35
NΡ								-0.30	0.07
EDC									0.64**
				PBZ soil a	application				
gs	0.83**	0.71**	0.15	0.46*	-0.27	0.20	0.01	0.55*	0.18
js E		0.69**	0.15	0.65**	-0.12	0.12	0.04	0.52*	0.09
4			0.08	0.44*	-0.22	0.04	-0.11	0.63**	0.28
/(II)				0.31	-0.04	0.00	-0.31	0.38	-0.19
SPÁD					-0.18	-0.08	-0.10	0.17	-0.22
PH						0.00	-0.08	-0.08	-0.12
SD							0.59**	0.15	0.29
NΡ								-0.27	-0.01
EDC									0.55*
				Witho	ut PBZ				
gs	0.29	0.25	-0.10	0.09	0.27	0.51*	0.02	0.15	0.02
		0.20	0.07	0.56**	0.07	0.18	-0.04	0.13	0.03
4			0.75**	0.46*	0.70**	0.13	0.37	0.71**	0.27
/(II)				0.62**	0.75**	0.00	0.45	0.79**	0.46*
SPÁD					0.49*	-0.06	0.01	0.49*	0.43
PH						0.13	0.47	0.64**	0.27
SD							0.00	0.20	0.28
ΝP								0.47*	0.22
EDC									0.65**

gs - Stomatal conductance; E - Transpiration; A - Net photosynthesis; YII - Photosystem II photochemical efficiency, SPAD index; PH - Plant height; SD - Stem diameter; NP - Number of petals; EDC - External diameter of chapter; IDC - Internal diameter of chapter; \* - Significant at  $p \le 0.05$  by t test



Bars with the same letters indicate that the treatments do not differ by the Tukey-test at p < 0.05; \*\* and \* - Significant at  $p \le 0.01$  and  $p \le 0.05$  by the F-test, respectively; F - Foliar application of PBZ; S - PBZ application via soil

**Figure 2.** Internal CO<sub>2</sub> concentration - Ci (A) and instantaneous carboxylation efficiency - iCE (B and C) in sunflower plants as a function of irrigation water electrical conductivity (ECw) and paclobutrazol application method (PAM)

For the instantaneous carboxylation efficiency, the PBZ application via soil promoted greater value, followed by foliar application, which corresponded to 31.18 and 16.12%, respectively higher than without PBZ (Figure 2B). Among salinity levels, iCE decreased 33.45% between 0.4 and 6.4 dS m<sup>-1</sup> (Figure 2C).

The reduction in gas exchange, initially, can occur due to osmotic tolerance that starts relatively fast, including a decrease in stomatal conductance to preserve water with an increasing solute concentration in soil solution and reduction of osmotic potential. This involves fast signaling mechanisms for a long distance between root and shoot (Isayenkov & Maathuis, 2019). With the decrease in the stomatal opening, besides reducing water loss, there is also a limitation in the assimilation of  $\mathrm{CO}_2$  and, as a result, a reduction in the production of photoassimilates by the plant.

The increase in the internal concentration of  $\mathrm{CO}_2$  due to the increase in salinity indicates that there was also a non-stomatal limitation in sunflower plants, a fact that may indicate low activity of the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), as well as the lack of ATP and NADPH from the electron transport chain of photosystem II (Silva et al., 2014). This was verified due to the low instantaneous efficiency of carboxylation with increased salinity in sunflower plants.

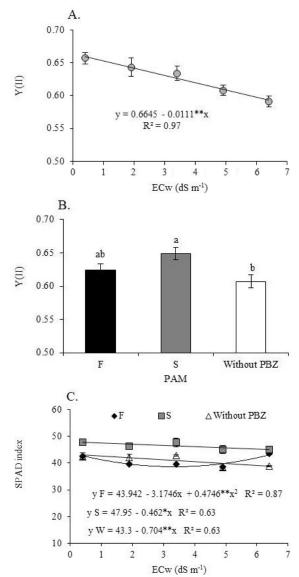
The effective photochemical efficiency of PSII (YII) was significantly affected by application methods of PBZ and by salinity levels. The SPAD index was affected by the interaction between factors (Table 2).

The photosystem II photochemical efficiency reduced 1.66% by a unit increase in water salinity, which corresponds to a decrease of 10.09% between irrigated plants with higher and lower electrical conductivity (Figure 3A). This reduction in the photosystem II photochemical efficiency is due to the damage to the photosynthetic apparatus caused by salinity, limiting the electron transport rate in chloroplasts, with a consequent reduction in the photosystem II photochemical efficiency (Monteiro et al., 2018; Alves et al., 2020).

The PBZ application via soil provided larger YII, followed by the foliar application that corresponded to 6.91 and 2.80%, respectively greater than without PBZ (Figure 3B). These positive effects of PBZ in improving the photosystem II photochemical efficiency, mainly in the application via soil, are probably due to the improvement in chlorophyll content, verified by the SPAD index. According to Xia et al. (2018), the photosynthetic process in plants is directly related to the chlorophyll content, which absorbs light energy and favors carbon fixation.

The SPAD index decreased with increasing salinity in all PBZ application methods. However, when applied via soil, it had higher SPAD in all saline levels, except in the highest level (6.4 dS m<sup>-1</sup>), in which leaf application was equal, staying higher 12.27% than the treatment without PBZ (Figure 3C). In treated plants with PBZ via soil the SPAD index correlated with gs ( $r = 0.46^*$ ), E ( $r = 0.65^{**}$ ), and A ( $r = 0.44^*$ ), and in plants without PBZ, it correlated with E ( $r = 0.56^{**}$ ), A ( $r = 0.46^*$ ), and YII ( $r = 0.62^{**}$ ) (Table 3).

Barbosa et al. (2009) evaluated the application of PBZ doses in the Golden sunflower cultivar in the absence of stress. They



Bars with the same letters indicate that the treatments do not differ by Tukey test, at p<0.05; \*\* and \* - Significant at  $p\leq0.01$  and  $p\leq0.05$ , respectively by the F-test; F - Foliar application of PBZ; S - PBZ application via soil

**Figure 3.** Photosystem II photochemical efficiency - Y(II) (A and B) and SPAD index (C) in sunflower plants as a function of irrigation water electrical conductivity (ECw) and paclobutrazol application method (PAM)

observed an increase in the SPAD index until the dose of 5.08 mg per pot. These authors report that the rise in this index by PBZ reflects the increase in the intensity of the green color of the leaves, enabling greater contrast with the yellow of the inflorescence, which increases visual attractiveness and, after that, the commercialization, indicating that PBZ application via soil is a viable alternative for sunflower cultivation. This change in leaf color, promoted by PBZ, was also found in *Paeonia lactiflora* (Xia et al., 2018) and forest species (Cregg & Ellison-Smith, 2020).

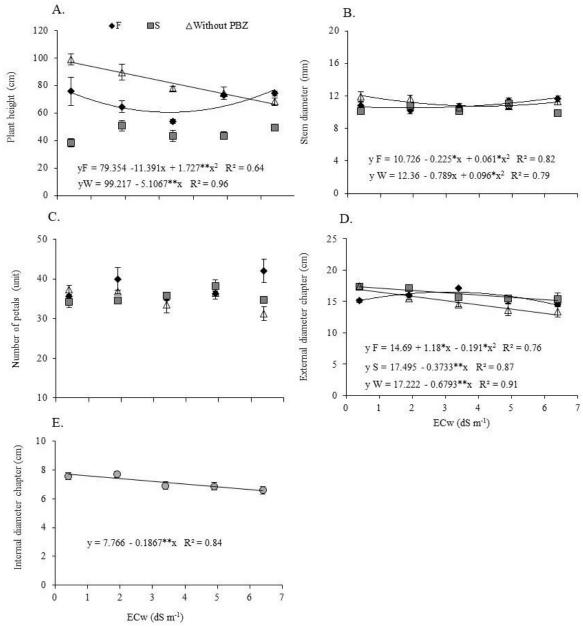
The plant height (PH), stem diameter (SD), number of petals (NP), and external chapter diameter (ECD) were significantly affected by the interaction between the PBZ application methods and salinity levels. In contrast, internal chapter diameter (ICD) was influenced only by the saline levels (Table 2).

The plant height was drastically reduced in plants where PBZ was applied via soil at all saline levels but did not adjust to any mathematical model that expressed an acceptable regression coefficient (y = 41.718 + 1.010x and  $R^2$  = 0.22) (Figure 4A). In the absence of PBZ, plant height decreased 5.25% per unit increase of water salinity, a corresponding decrease of 31.55% between irrigated plants with lower and higher saline water. In contrast, in the plants with the foliar application, the plant height decreased until the estimated value of 60.57 cm at the salinity level of 3.29 dS m $^{-1}$ , increasing from that to the highest saline level.

The plant height was inversely correlated with E (r = -0.47\*) in the treatment with foliar PBZ application and positively with A (r = 0.70\*\*), YII (r = 0.75\*\*) and SPAD (r = 0.49\*) in plants without PBZ (Table 3). In the plants with PBZ application via soil, there was no correlation of PH with no other variable, possibly due to the effect of this application method on plant height, which did not range among saline levels.

The performance of PBZ in the growth of sunflower plants, mainly when applied via soil, was also observed in cultivar Multissol at 30 and 45 days after PBZ application, in which height growth inhibition was 49.40 e 39.50%, respectively, concerning control (Brito et al., 2016). According to the authors, the effect of regulator in plant growth is linked to inhibition of the conversion of ent-kaurene to ent-kaurenoic acid, resulting in a reduction in gibberellin biosynthesis, with the consequent decrease in elongation and cell division rates.

The stem diameter ranged among the application methods only in the lowest and highest saline level, respectively, being the application via soil method not varied in any of the saline levels, and therefore, not adjusted to any mathematical model that expressed acceptable regression coefficient (y = 10.459 - 0.0180x and  $R^2 = 0.006$ ) (Figure 4B). When the plants were irrigated with water ECw 0.4 dS m<sup>-1</sup>, the stem diameter was



\*\* and \* - Significant at  $p \le 0.01$  and  $p \le 0.05$ , respectively by the F-test; F - Foliar application of PBZ; S - PBZ application via soil; W - Without application of PBZ Figure 4. Plant height (A), stem diameter (B), number of petals (C), external chapter diameter (D), and internal chapter diameter (E) of sunflower plants as a function of irrigation water electrical conductivity (ECw) and paclobutrazol application method (PAM)

higher at 11.97% in plants without PBZ than plants treated with PBZ. At the same time, in ECw of 6.4 dS m<sup>-1</sup>, SD was higher in treated plants with foliar PBZ application and without PBZ, both 14.35% higher than plants with the application via soil.

With these results, PBZ application via soil favored the plants of sunflower 'Sol Noturno' under conditions of the high salinity of irrigation water since the plants had lower height, regardless of salinity level, but had higher gas exchange values, photosynthetic efficiency, and chlorophyll SPAD index. Otherwise, in plants without PBZ, correlations suggest that plant height was influenced by photosynthesis, Photosystem II photochemical efficiency, and SPAD index, indicating that the reductions caused by salt stress in the photosynthetic apparatus of these plants reflected in its growth. The use of water for irrigation with higher ECw means high salt content becomes a limiting factor for agricultural production since salinity inhibits plant growth due to the osmotic and toxic effects of ions (Monteiro et al., 2018; Lima et al., 2019). This is because salinity negatively affects water absorption by the roots, consequently limiting primary and secondary plant growth, as observed in this study, mainly with ornamental sunflower plants and without application PBZ.

The number of petals did not range among salt levels when the plants were treated with PBZ, and therefore, not adjusted to any mathematical model (Figure 4C). In the absence of PBZ application, although there was a decreasing linear behavior for the number of petals with the increase in water salinity from 0.4 to 6.4 dS m<sup>-1</sup> (y = 37.827 - 0.8167 x), the coefficient of determination was less than 0.60 (R<sup>2</sup> = 0.57). The number of petals positively correlated with SD (r = 0.59\*\*) in plants with PBZ application via soil (Table 3), indicating that the stability in the stem diameter of these plants under saline conditions contributed to the chapter structure. Thus, it was verified that PBZ positively influenced the visual appearance of inflorescences, maintaining the number of petals in plants grown under salt stress.

These effects of PBZ in sunflower plants under salinity are probably due to lower degradation of cell membranes and chlorophylls, as observed in *Cucumis sativus* under thermal stress (Baninasab & Ghobadi, 2011) and *Myrica rubra* under salinity (Hu et al., 2017). This is because PBZ may be related to the endogenous cytokinin content, where plants treated with PBZ synthesize more cytokinin, increasing chlorophyll biosynthesis and preventing degradation of these (Baninasab & Ghobadi, 2011).

The external chapter diameter was lower with the foliar application of PBZ under low salinity conditions (ECw  $0.4 \, dS \, m^{-1}$ ). However, from 1.9 dS  $m^{-1}$ , plants not treated with PBZ had lower ECD (Figure 4D). In the two highest levels of salinity (4.9 and  $6.4 \, dS \, m^{-1}$ ) without PBZ, there was lower ECD 11.30 and 14.70%, respectively, than plants treated with PBZ, irrespective of the method used. The ECD not correlated to other variables in plants with foliar PBZ application but have correlated with the net photosynthesis, for example, in treated plants with PBZ via soil ( $r = 0.63^{**}$ ) and without PBZ ( $r = 0.71^{**}$ ) (Table 3), indicating that the external chapter diameter in these plants is associated with the net photosynthesis.

The internal chapter diameter ranged only among salinity levels, with a reduction in ICD of 2.41% per unit increase of water salinity, which corresponds to a decrease of 14.54% between irrigated plants with lower and higher salinity water (Figure 4E). The reduction in ICD was also reported by Nobre et al. (2014), a decrease of 33.33% in plants irrigated with water of 3.0 dS  $m^{-1}$  in relation ECw 0.3 dS  $m^{-1}$ .

The external chapter diameter increased in the plants treated with PBZ under high levels of salinity, indicating that the inflorescences of these plants had greater petal length, besides maintained among saline levels, with PBZ application. Thus, PBZ increased inflorescences in the 'Sol Noturno' sunflower grown under saline conditions, acting, therefore, as a mitigator of salt stress. These results corroborate with several studies involving the effect of PBZ and salinity in different plant species, as in Triticum aestivum in which the treatment with PBZ increased the length and root dry mass (Hajihashemi et al., 2007), in Mangifera indica increased chlorophyll content and reduced damage to the membrane (Kishor et al., 2009), in Chenopodium quinoa increased chlorophyll content and stomatal density (Waqas et al., 2017), and in Myrica rubra increased root dry mass and chlorophyll content (Hu et al., 2017). This was probably due to the reduction in by accumulation of toxic ions Na+ and Cl-, in plant tissues promoted by PBZ (Hajihashemi et al., 2007; Kishor et al., 2009).

### **Conclusions**

- 1. The increased electrical conductivity of irrigation water decreased gas exchange and photosystem II photochemical efficiency in sunflower 'Sol Noturno' plants.
- 2. Paclobutrazol application, mainly when applied via soil, attenuates the effects of electrical conductivity of irrigation water, increasing chlorophyll SPAD index, which improves the quality of flowers due to increase in external chapter diameter and maintenance of the number of petals.

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