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# Sugar Metabolism and Photosynthesis of Tomatoes Irrigated with Water Treated with Low-Frequency Electromagnetic Resonance Fields in Different Fertigation Doses

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**Abstract:** Management of irrigation and fertilization in greenhouses, if not done correctly, can cause soil salinization. The use of water treated with very low-frequency electromagnetic resonance fields (WVLF) can reduce salinization effects on the photosynthetic and biometric systems. Thus, the purpose of the research to evaluate the metabolism of photosynthesis and the impact of WVLF on the cultivation of tomato crops subjected to different levels of fertigation. For this, the gas exchange parameters were evaluated, as well as chlorophyll a fluorescence, sugar contents, sucrose, chlorophylls, and phaeophytins and fruit production. The gas exchange parameters had greater activity when subjected to irrigation with electromagnetic water, consequently the production of sugar and sucrose increased. Photosynthetic System II showed less salinity effect, being favored by very low-frequency electromagnetic resonance fields. The production increased by 20% for the dose of 2.5 d·Sm<sup>-1</sup> of WVLF reducing the effects caused by higher doses. Hence, the induction of water by electromagnetic fields can provide less damage to the photosynthetic system and to the cultivation of the tomato crop when subjected to saline stress and, consequently, favor the production of fruits by this crop under such conditions.

Keywords: fertilizers; Lycopersicon esculentum L.; photosynthesis II; salinization; sucrose

## 1. Introduction

Tomato (*Solanum lycopersicum*) is one of the main vegetable crops grown all over the world [1,2]. Some characteristics inherent to the tomato crop make it susceptible to pests, diseases, and sudden changes in weather conditions. Thus, some techniques used for tomato production, such as cultivation in a protected environment, fertigation, foliar fertilization, and topsoil coverage, favor the development of the tomato plant and, consequently, its nutrient absorption process [3,4].

However, the inadequate use of irrigation and fertilizers in overdoses can lead to soil salinization, whose effects are a decrease in quality, nutritional value, and productivity of sensitive crops, such as the tomato [5]. Salinity not only affects agricultural production, but also several aspects of plant physiology and biochemistry [6].

High levels of ions in the soil induce osmotic effects that reduce water uptake by the roots. Along with this, there is an excessive increase in absorption of ions that causes toxicity as well as an imbalance of pH and nutrients in the plant [7,8]. Thus, the exposure



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of plants to saline stress causes physiological changes, the main consequences of which are the degradation in photosynthetic pigments, destruction of chloroplasts, and reduction in chlorophyll fluorescence [9–11]. Furthermore, the accumulation of toxic ions leads to deterioration in the chloroplast membrane, which serves as a sensitive indicator of the cellular metabolic state in a way that its downturn causes a reduction in chlorophyll [12]. Studies also show that there are changes in kinetics of the emissions in chlorophyll fluorescence, photosystem II efficiency,  $CO_2$  assimilation and/or producing compatible osmolytes for water absorption adjustment, transport, and ion accumulation [13,14].

The changes caused in the photosynthetic system lead to the production of carbohydrates, which are a source of energy for plant growth and productivity. Furthermore, sugars (i.e., sucrose, fructose, glucose) are known to act as important osmoregulatory substances to maintain cell turgor under osmotic stress (water or saline) [15].

Furthermore, salinity not only affects agricultural production, but also several aspects of plant physiology and biochemistry, impairing the quality and nutritional value of the tomato fruit. Thus, there is a need to look for complementary approaches to minimize the negative effects of soil and water salinity.

Studies show that when water is induced to a magnetic or electromagnetic field, there are changes in their molecular interactions, in which there may be variations in the crystal structure of calcium carbonate [16,17] and changes in the distribution of salts between the soil layers, reducing its content in the upper layers [18]. In addition, studies have already verified changes in water characteristics such as its viscosity, surface tension, light refraction index, electrical conductivity, and light absorption capacity [19], as well as changes in soil characteristics such as moisture reduction in the applied volume of water in irrigation [20,21]. With the above, the importance of developing studies that seek to explain the behavior of water when exposed to magnetic and electromagnetic treatments is remarkable, as it is noted that studies on these topics are scarce, especially those involving soil salinization [22,23].

The treatment of water with electromagnetic fields has shown results that increase production, cow pea and brinjal [24], cotton [25], opulus  $\times$  euramericana 'Neva' under NaCl stress [26], wheat [27], tomato [28].

Thus, we hypothesize that irrigation of tomato crops using electromagnetically treated water under protected cultivation conditions can reduce the damage caused by salinity. Thus, the objective of the present work was to evaluate the impacts of irrigation with the use of water treated with low-frequency electromagnetic resonance fields in the photosynthetic system and sugar metabolism of tomato crops subjected to different levels of ion concentration in the soil.

## 2. Materials and Methods

## 2.1. Experimental Area

The experiment was carried out under protected cultivation conditions of the tunnel type, between June and September 2018, at the School of Sciences and Engineering, Tupã city Campus, São Paulo State, Brazil, with a geographical location defined by the coordinates 22°51′ S and 48°26′ W and an average altitude of 786 m above the sea level.

#### 2.2. Planting and Plant Management

The tomato cultivar used in the experiment was the HS 1188, an Italian type from the Horticeres brand. Sowing was carried out in plastic trays of 160 pots filled with a mixture based on pine bark and BIOPLANTTM coconut fiber. Three seeds were sown per pot, which, after germinating, were submitted to thinning, leaving only one seedling per pot. The transplant of the seedlings occurred 35 days after sowing (DAS) and, after the development of the crop, the lateral shoots were eliminated, in order to maintain only one main branch, up to approximately two m in height. The main branch was, also, cut off, thus paralyzing the vertical development. In addition, plant tutoring was performed with the aid of plastic tape, supported by a wire installed above and in the direction of the cultivation lines. The other cultural treatments were carried out according to the technical recommendations for tomato cultivation from the tomato cultivars table.

#### 2.3. Treatments

The randomized blocks design was adopted, in a 2  $\times$  5 factorial scheme, composed of two types of water (WVLF-water treated with very low-frequency electromagnetic resonance fields) and UW = untreated water), as well as five fertigation levels (1.5; 2.5; 4.0; 5.5 and 7.0 dS m<sup>-1</sup>) for tomato cultivation. Hence, the experiment consisted of ten treatments, with five replications, totaling 50 plots.

These treatments were arranged in eight dm<sup>3</sup> pots (one plant per pot) filled with coconut fiber substrate. The pots were allocated in five rows, with a spacing of 0.5 m between plants and 1 m between rows. Each row represented a level of salinity and received both types of water.

#### 2.4. Water Treated with Very Low-Frequency Electromagnetic Resonance Fields (WVLF)

The AQUA4D system aims to perform water treatment, in which it is carried out through physical treatment based on the process of quantum mechanism and electromagnetism. The equipment's maximum treatment potential is  $3.6 \text{ m}^3 \cdot \text{h}^{-1}$  [24]. For water treatment, the Aqua-4D device generates an EMF playing the role of an external impulse was used, which will influence the magnetization of water with a speed of 0.2 m s<sup>-1</sup>, T = 26.5 °C and pH = 8.25 [24].

## 2.5. Irrigation and Fertigation Management

Irrigation was performed using a localized system, in which, approximately every 20 min, the system was activated and remained so for two minutes. The system, it is important to mention, did not activate at night.

Fertigation was performed according to the phenological phase of the cultivation. The solution adopted in the initial phase containing concentrations of 1.05 g L<sup>-1</sup> of calcium nitrate, 0.1 g L<sup>-1</sup> of urea, 0.4 g L<sup>-1</sup> of potassium nitrate, 0.3 g L<sup>-1</sup> of potassium chloride, 0.1 g L<sup>-1</sup> of potassium sulfate, 0.2 g L<sup>-1</sup> of magnesium nitrate, 0.1 g L<sup>-1</sup> of magnesium sulfate, 0.28 g L<sup>-1</sup> of MKP, 0.003 g L<sup>-1</sup> of zinc sulphate, 0.008 g L<sup>-1</sup> of boric acid, and 0.02 g L<sup>-1</sup> of iron in the form of 80% EDDHA. For the flowering and production phase, the used solution contained 1.114 g L<sup>-1</sup> of calcium nitrate, 0.11 g L<sup>-1</sup> of urea, 0.43 g L<sup>-1</sup> of potassium nitrate, 0.23 g L<sup>-1</sup> of urea, 0.43 g L<sup>-1</sup> of potassium nitrate, 0.03 g L<sup>-1</sup> of potassium chloride, 0.11 g L<sup>-1</sup> of urea, 0.43 g L<sup>-1</sup> of potassium nitrate, 0.03 g L<sup>-1</sup> of potassium chloride, 0.11 g L<sup>-1</sup> of negative sulphate, 0.20 g L<sup>-1</sup> of magnesium nitrate, 0.11 g L<sup>-1</sup> of potassium sulphate, 0.28 g L<sup>-1</sup> of magnesium nitrate, 0.11 g L<sup>-1</sup> of potassium sulphate, 0.20 g L<sup>-1</sup> of magnesium nitrate, 0.11 g L<sup>-1</sup> of potassium sulphate, 0.20 g L<sup>-1</sup> of magnesium nitrate, 0.11 g L<sup>-1</sup> of potassium sulphate, 0.20 g L<sup>-1</sup> of magnesium nitrate, 0.11 g L<sup>-1</sup> of magnesium sulphate, 0.30 g L<sup>-1</sup> of MKP, 0.0033 g L<sup>-1</sup> of zinc sulphate, 0.009 g L<sup>-1</sup> boric acid, and 0.022 g L<sup>-1</sup> iron in the form of 80% EDDHA.

The concentrations were prepared for each liter of solution, thus obtaining the conductivity of 2.5 dS m<sup>-1</sup>. For the others, the calculations were proportionally performed to achieve the required conductivity. This way, five reservoirs were adopted where the solutions were stored in a concentrated manner. When the irrigation system was activated, fertilizers were injected.

## 2.6. Determination of Total Sugar and Sucrose Contents

Plant tissue samples, of approximately 1 g, were extracted and macerated in 10 mL of the methanol, chloroform, water (MCW) solution (60% methanol, 25% chloroform, and 15% deionized water). After this procedure, the material was centrifuged at 10,000 rpm, for 10 min and at 4 °C, and stored in the refrigerator for an approximate period of 48 h. Then, 4 mL of the supernatant was collected, while 1 mL of chloroform and 1.5 mL of deionized water were added. About 24 h later, there was the separation of the alcoholic and aqueous phases, which was used for the determination of sucrose and total sugars. Analyses were performed after the beginning of flowering.

## 2.6.1. Determination of Total Sugars

To determine the total soluble sugars, the sulfuric phenol method was used [25]. This method consists of pipetting: 500  $\mu$ L of MCW extract supernatant; 500  $\mu$ L of 5% Phenol, and 2 mL of H<sub>2</sub>SO<sub>4</sub>. After homogenization, the reading was performed at absorbance of 490 nm and in quadruplicate. The difference in the blank reading was only the replacement of the sample supernatant by deionized water.

### 2.6.2. Sucrose Determination

For sucrose determination, the method described by Van Handel [26] was used, which consists of pipetting: 500  $\mu$ L of MCW extract supernatant; 500  $\mu$ L of 30% KOH, and 2 mL of H<sub>2</sub>SO<sub>4</sub>. After homogenization, the tubes were placed in a dry bath at 100 °C for 10 min. The tubes were allowed to cool down to room temperature for readings at absorbance of 490 nm and in quadruplicate.

## 2.6.3. Determination of Photosynthetic Pigments

Samples were collected at the beginning of flowering. Photosynthetic pigments (chlorophylls and carotenoids) were determined according to Lichtenthaler [27].

#### 2.7. Gas Exchange

Gas exchange analyses were performed on the same leaf and time, in which chlorophyll fluorescence evaluations were performed, in an open photosynthesis system, with CO<sub>2</sub> analyzer and infrared water vapor radiation (Infra-Red Gas Analyzer-IRGA, model LI-6400, LI-COR). The gas exchange characteristics that were analyzed were: CO<sub>2</sub> assimilation rate (A,  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); transpiration rate (E, mmol water vapor m<sup>-2</sup> s<sup>-1</sup>); stomatal conductance (gs, mol m<sup>-2</sup> s<sup>-1</sup>); internal CO<sub>2</sub> concentration in the leaf (Ci,  $\mu$ mol CO<sub>2</sub> mol<sup>-1</sup>); water use efficiency (WUE,  $\mu$ mol CO<sub>2</sub> (mmol H<sub>2</sub>O)<sup>-1</sup>); and the carboxylation efficiency (A/Ci) [28].

#### 2.8. Chlorophyll Fluorescence

The fluorescence measurements of chlorophyll a were performed by a fluorometer coupled to the LI-6400, according to the saturated pulse method (Maxwell and Johnson, 2000), using the nomenclature recommended by Baker and Rosenqvist [29]. The evaluations were carried out at the beginning of flowering, from 8:00 am to 11:00 am, selecting five plants from each treatment, in which the second leaf was chosen and standardized with the limbus fully expanded. Before fluorescence analyses, aluminum foil was placed on each selected leaf for approximately 20 min, in order to keep the leaf in the dark so that the entire electron photosynthetic transport system oxidized.

In the presence of artificial light, it was measured, according to Schreiber et al. [30]: maximum fluorescence (Fm'), fluorescence in the state of dynamic equilibrium (F'), and minimum fluorescence (F0'); potential quantum efficiency of FSII (Fv/Fm); quantum efficiency of the antenna (Fv'/Fm'); photochemical extinction coefficient (Pq); non-photochemical extinction coefficient (NPq), and apparent electron transport rate (ETR). For the calculation of ETR, the fraction of excitation of energy distributed to the PSII was considered 0.5, and the fraction of photosynthetically active photon flux density (PAPFD) absorbed by the leaf was considered 0.84 [31].

## 2.9. Determination of Production

Commercial production was determined by weighing the fruits that were in commercial conditions and had a red color. It did not take into account fruits with a black background and fruits with defects. The production is determined in g·plant<sup>-1</sup>.

#### 2.10. Statistical Analysis

Analysis of variance (ANOVA) was carried out with subsequent use of the Tukey test. A 5% confidence level was considered in all statistical tests. For the purposes of the described tests, the R software was used.

## 3. Results and Discussion

## 3.1. Sugar Metabolism

Irrigation with WVLF influenced the total sugar levels for tomato culture when submitted to different doses of fertigation. (Figure 1A). The total sugar contents, in general, were higher for the plants irrigated with WVLF, when compared to untreated water (UW), regardless of the applied level of fertigation. Fertigation application with 4 dS m<sup>-1</sup> increased total sugar levels in plants that received UW, while applications with 7 dS m<sup>-1</sup> reduced the total sugar levels in relation to other levels of fertigation. However, with the use of WVLF, total sugar contents were higher for the application of 4 and 5.5 dS m<sup>-1</sup>. Furthermore, with the use of WVLF, there was no reduction in the sugar content even for the highest tested fertigation level (7 dS m<sup>-1</sup>), which was statistically equal for the doses of 1.5 and 2.5 dS m<sup>-1</sup>.



**Figure 1.** Total sugar (**A**) and sucrose (**B**) contents in tomato leaves for different levels of fertigation, and the handling of irrigation with electromagnetically treated (WVLF) and untreated water (UW). Means followed by the same letter (lowercase compares the type of water and uppercase compares the salinity doses) do not differ statistically (p < 0.05) according to the Tukey test. Error bars indicate the standard deviation in the mean of four replicates (n = 4).

On the other hand, sucrose contents presented different responses for the interaction between water types and fertigation levels. There was no statistical difference between the types of water used for fertigation with 2.5 and 5.5 dS m<sup>-1</sup>. The combination of UW with the fertigation of 7 dS m<sup>-1</sup> showed lower sucrose content when compared to the other

salinity levels. However, when WVLF was used, there was a difference in sucrose content only between the doses of 2.5, 4 and 5.5 dS m<sup>-1</sup>, with higher sucrose content for a dose of 4 dS m<sup>-1</sup>. There was no statistical difference for the combination of WVLF plus fertigation at 1.5 and 7 dS m<sup>-1</sup> and the other fertigation levels.

Total sugars contribute to the regulation of reactive oxygen species (ROS) signaling, as well as osmotic adjustments during abiotic stress [32]. Furthermore, they are involved in the metabolism and protection of both ROS production and elimination pathways, such as mitochondrial respiration, photosynthesis, and the oxidant-pentose-phosphate pathway [33,34].

For sucrose, it was observed that the levels of 1.5, 4 and 7 dS m<sup>-1</sup> showed the highest values when irrigated with WVLF. By comparing the types of water, it can be seen that the highest contents were observed for 1.5, 4 and 7 dS m<sup>-1</sup>, differing significantly (Figure 1B).

Some studies show that saline stress promotes an increase in the concentration of sugars, as well as in the amount of organic acids and percentage of dry mass, even though there is a reduction in tomato size and production [35,36].

For Patel [37], sugars in leaves may reflect the metabolic situation of photosynthesis and/or their translocation efficiency to reserve tissues. Thus, the doses and treatments that presented the highest amount of total sugars and sucrose possibly presented greater photosynthetic capacity, which shows their better adaptation to the submitted treatment conditions.

#### 3.2. Photosynthetic Pigments

The levels of chlorophylls and pheophytins A, B, and Total of the tomato crop showed significant effects when subjected to different doses of fertigation and irrigated with UW and WVLF (Figure 2). Pheophytins are products of the degradation in chlorophylls and can interfere with their determination as they capture light and fluoresce in the same region of the spectrum. If this pheopigment is present in the sample, significant errors in the concentration of chlorophylls may occur [38]. It is possible to notice that the behavior of chlorophylls and pheophytins were very similar, which indicates that there was no interference in the reading.

It was found that the use of WVLF increased the levels of chlorophyll and pheophytins A, B, and Total, when compared to the use of UW, for conductivities of 1.5, 4 and 7 dS m<sup>-1</sup>. Other studies have shown that an increase in chlorophyll occurs as a result of irrigation with magnetized water, as in the cultivation of peanuts [39] and grape [40].

The reduction in chlorophyll content under saline stress is a commonly reported phenomenon and is generally attributed to adverse effects on membrane stability [41,42].

Saline stress can stimulate the activity of the chlorophylase enzyme, which is responsible for degrading photosynthetic pigment molecules and, therefore, induces the structural destruction of the chloroplast, resulting in unbalanced and reduced activity of pigmentation proteins [43,44].

The results obtained corroborate those attained by Sadeghipour [45], who observed that irrigation with electromagnetically treated water, for the cowpea crop, presented a significant increase in photosynthetic pigment concentrations. Rawabdeh et al. [46] also observed an increase in contents of chlorophyll a, b and Total for pepper leaves subjected to magnetic treatment. Higher levels of photosynthetic pigments, for the wheat crop, were observed when using electromagnetically treated water, indicating the activation effect of the concentration of ions such as K<sup>+</sup> and GA<sub>3</sub> [47], which causes an increase in the number of chloroplasts per cell. The increase in photosynthetic pigment in the present study can also be explained by the larger leaf area that was observed. Thus, electromagnetic treatment technology can cause an increase in the phospholipids/sterol ratio, leading to an increase in the fluidity of the lipid membrane, while sterols act as a barrier to prevent leakage in the biological membranes.



**Figure 2.** Chlorophyll A contents (**A**); Pheophytin A (**B**); Chlorophyll B (**C**); Pheophytin B (**D**); Chlorophyll A + B (**E**); and Pheophytin A + B (**F**) for the different levels of fertigation, and the handling of irrigation with electromagnetically treated (WVLF) and untreated water (UW). Means followed by the same letter (lowercase compares the type of water and uppercase compares the salinity doses) do not differ statistically (p < 0.05) according to the Tukey test. Error bars indicate the standard deviation of the mean of four replicates (n = 4).

The contents of carotenoids, which are accessory pigments in the absorption and transfer of radiant energy, were significantly affected as a function of the water type and electrical conductivity (Figure 3). In general, the carotenoid contents showed a behavior very similar to chlorophylls and pheophytins in response to treatments.



**Figure 3.** Carotenoid contents for different levels of fertigation, in response to irrigation management with electromagnetically treated (WVLF) and untreated water (UW). Means followed by the same letter (lowercase compares the type of water and uppercase compares the salinity doses) do not differ statistically (p < 0.05) according to the Tukey test. Error bars indicate the standard deviation of the mean of four replicates (n = 4).

It is possible to observe that there was an effect of WVLF on the content of carotenoids in the tomato leaf tissue. Therefore, the highest concentrations were found at the doses of 1.5 and  $7 \text{ dS m}^{-1}$ , while the lowest were at the dose of  $4 \text{ dS m}^{-1}$ .

It can be noted that there was a gradual reduction in the levels of carotenoids up to the dose of 4 dS m<sup>-1</sup>. This behavior can be due, among other reasons, to the fact that the accumulation of salts delays the production of photosynthetic pigments, which induces the degradation of  $\beta$ -carotene, and causes a reduction in the content of carotenoids [48]. Carotenoids are integrated components of thylakoids, and act on the absorption and transfer of light to chlorophyll [49]. However, for the dose of 5.5 dS m<sup>-1</sup> it is possible to observe an increase in this pigment. This may have occurred as carotenoids can also act as antioxidant agents, protecting the lipid membranes from oxidative stress generated in plants exposed to salinity [50].

The results found for treatments with UW corroborate with Melo et al. [51] who studied the culture of *Atriplex nummularia*. The authors observed that plants grown under saline stress present reductions in the levels of a, b, and Total chlorophylls, indicating possible oxidative stress and membrane degradation. Furthermore, the increase in salinity increased the content of carotenoids in the leaves of *Atriplex nummularia*, preventing further damage to the photosynthetic apparatus. In the present study, the deleterious effects caused by increased salinity on photosynthetic pigments were minimized with the use of electromagnetic water. According to Al-Khazan et al. [52], the high content of carotenoids in plants irrigated with magnetized water can be considered a good adaptive factor under stress conditions, since carotenoids protect chlorophyll and allow them to complete their life cycle.

#### 3.3. Gas Exchange

There are several physiological stress markers that can be determined in plants and provide information on how photosynthesis and photosynthetic components behave under stressful conditions. Thus, the gas exchange was analyzed through:  $CO_2$  assimilation rate (A); Stomatal conductance (gs); Internal concentration of  $CO_2$  (Ci); Respiration (E); Water use efficiency (WUE); and Carboxylation Efficiency (A/Ci).

The tomato crop, when irrigated with WVLF, showed higher values for the following parameters: A, gs, E, WUE and A/Ci. Besides, there was an interaction between the factors Fertigation Level and Water Type (Table 1).

Table 1. Gas exchange of tomato crop subjected to different levels of conductivity and water types.

		A <sup>1</sup>	gs <sup>2</sup>	Ci <sup>3</sup>	E <sup>4</sup>	WUE <sup>5</sup>	A/Ci <sup>6</sup>
Water type	UW <sup>7</sup>	5.89 b	0.16 b	238 a	3.21 b	1.98 b	0.02 b
	WVLF <sup>8</sup>	7.84 a	0.21 a	223 b	3.70 a	2.20 a	0.03 a
$1.5 (dS m^{-1})$	UW	5.37 Ab	0.15	227 Aa	2.79 Bb	1.78B Ca	0.023 b
	WVLF	9.50 Aa	0.27	242 Aa	5.34 Aa	1.93 Ca	0.039 a
$2.5 (dS m^{-1})$	UW	6.27 Aa	0.20	232 Aa	2.59 Ba	2.42 ABb	0.027 a
	WVLF	6.73 Ba	0.24	239 Aa	2.33 Ca	2.88 Aa	0.028 a
$4 (dS m^{-1})$	UW	5.60 Ab	0.08	183 Bb	2.15 Bb	2.61 Aa	0.030 a
	WVLF	7.85 ABa	0.16	241 Aa	3.17 Ca	2.47 ABa	0.030 a
$5.5 (dS m^{-1})$	UW	6.15 Ab	0.19	239 Aa	3.87 Aa	1.62 CDa	0.020 b
	WVLF	8.53 Aa	0.21	237 Aa	4.36 BCa	1.95 Ca	0.035 a
$7.0 (dS m^{-1})$	UW	6.07 Aa	0.19	235 Aa	3.30 Ab	1.32 Db	0.028 a
	WVLF	6.57 Ba	0.15	229 Aa	4.63 ABa	2.00 BCa	0.025 a
CV (%)	UW	10.12	24.26	4.59	12.54	9.55	11.64
W.T.	WVLF	3.10 *	4.77 **	5.96 **	9.78 *	10.45 **	25.43 **
L.C.	UW	58.46 **	6.66 **	14.40 **	21.46 **	31.52 **	2.30 <sup>ns</sup>
W.T. $\times$ L.C.	WVLF	725 *	2.62	8.81 **	16.78 **	5.09 *	5.02 *

<sup>1</sup> A: CO<sub>2</sub> assimilation rate; <sup>2</sup> gs: stomatal conductance; <sup>3</sup> Ci: Internal CO<sub>2</sub> concentration; <sup>4</sup> E: transpiration rate; <sup>5</sup> WUE: Water Use Efficiency; <sup>6</sup> A/Ci: Carboxylation Efficiency; <sup>7</sup> UW: Untreated water; <sup>8</sup> WVLF: Water treated with very low-frequency electromagnetic resonance fields. W.T.: Water type; L.C.: levels of conductivity. Means followed by the same letter (lowercase compares the type of water and uppercase compares the fertigation doses) do not differ statistically (p < 0.05) according to the Tukey test. Error bars indicate the standard deviation of the mean of five repetitions (n = 5). \* significant at 1% and \*\* significant at 5%, <sup>ns</sup>: not significant.

The transpiration rate (E) showed similar values for all fertigation doses, and the use of WVLF caused an increase of 5%. WVLF irrigation showed higher CO<sub>2</sub> assimilation rates for doses of 1.5, 4 and 5.5 dS m<sup>-1</sup>. For gs, it was observed that UW values remained similar, with a significant difference (p < 0.05) only for the dose of 4 dS m<sup>-1</sup>.

According to Moles et al. [7] the reduction in gs and A can be considered a biophysical strategy for plants in order to reduce water losses from the stomata and diminish salt uptake according to the transpiration flow. Therefore, the considerable increase in plants irrigated with WVLF, for these parameters, by hypothesis, may mean that the deleterious stomatal effects caused by salinity were minimized.

The different concentrations of salts did not influence the Ci when comparing the UW and WVLF treatments. Only the dose of 4 dS m<sup>-1</sup> showed a significant difference, both in relation to conductivity and water type. However, WVLF stood out in relation to UW, indicating that this treatment provided higher Ci. Usually, the increase in Ci is followed by the increase in gs; hence, stomatal limitation is an extremely relevant factor regarding the limitation of photosynthetic performance, given that the larger the stomatal opening, the greater the diffusion of CO<sub>2</sub> into the substomatal chamber [53].

According to Bunce [54], if the Ci values are too low, the entry of  $CO_2$  into the mesophyll cells is limited. This way, plants use  $CO_2$  from respiration to keep the photosynthetic rate to a minimum, limiting it. In general, the transpiration rate increased as the fertigation dose increased in tomato plants irrigated with UW. The water use efficiency increased up to a dose of 4 dS m<sup>-1</sup> and then decreased with UW. As for the carboxylation efficiency in tomato plants irrigated with UW, the highest value was for the dose of 4 dS m<sup>-1</sup> and the lowest for the dose of  $5.5 \text{ dS m}^{-1}$ .

Closing the stomata is one of the first defense mechanisms of plants under stress. This behavior reduces leaf transpiration, in addition to causing a decrease in the normal flow of  $CO_2$  towards the carboxylation site, leading to reduced photosynthesis [55].

It was possible to verify that, despite the increase in transpiration, the water use efficiency did not decrease, which is due to the increase in  $CO_2$  assimilation. Furthermore, the evaporation force triggers an increase in the stomatal opening, which leads to a greater input of  $CO_2$ . According to [55], this would explain why the WUE was not reduced even with the values found for transpiration.

According to Ramos et al. [56], high values of Ci associated with the elevation of gs indicate an increase in A/Ci, which occurs due to the availability of ATP, NADPH, and the substrate for the Rubisco enzyme. Thus, for photosynthesis, A/Ci depends on the availability of  $CO_2$  in the leaf mesophyll, as well as the amount of light and temperature conditions, and enzymatic activity.

By analyzing gas exchange in cowpea culture, Shoukat et al. [57] reported that the salinity of irrigation water reduced gas exchange, resulting in a decrease in the following parameters: stomatal conductance; photosynthesis; growth; and production.

The results of the present study are in agreement with Ospina-Salazar et al. [55], for the analyzed parameters of gas exchange in the corn crop, in which electromagnetic water had a positive value.

## 3.4. Chlorophyll Fluorescence A

For the tomato crop fluorescence, it was verified that there was no statistical difference in PSII Potential Quantum Efficiency (Fv/Fm), Antenna Quantum Efficiency (Fv'/Fm'), and Photochemical Extinction Coefficient (qP). For the Non-photochemical Extinction Coefficient (qNR), there was a higher concentration when irrigated with UW. For the Apparent Electron Transport Rate (ETR), the highest concentration was given by WVLF (Table 2).

The results found here are in line with Zribi et al. [58] and Ospina-Salazar et al. [59], who also verified the effect of salinity in tomatoes, analyzing chlorophyll fluorescence. In this case, salinity does not seem to affect the primary photochemistry in PSII, which may not be the only target of saline stress. The same trend, also detected in Fv/Fm, was observed in all parameters measured in light-adapted leaves, that is, measurements performed during 'daytime' (qP).

Ramos et al. [60] and Saddiq et al. [61] studied the fluorescence of chlorophyll a in wheat leaves to verify the effects of salinity on PSII. They found that the rapid decline in photosynthesis under saline stress is reversible and specific for osmotic stress, where the slow decline is irreversible and specific to ionic stress. Furthermore, they concluded that saline stress inhibits the rate of electron transport, but found that the damage caused to PSII can be largely reversible.

Saline stress imposes stomatal limitations on photosynthesis, which are accompanied by a decrease in the consumption rate of ATP and NADPH for  $CO_2$  assimilation, which would result in a reduction in AETR and, consequently, harm the PSII. However, the functioning of the water-water cycle in C3 plants, the increase in photorespiration, under stress conditions, can maintain the AETR similar to those observed in leaves of non-stressed plants, despite the reduction in the  $CO_2$  assimilation rate [62–66].

		Fv/Fm <sup>1</sup>	Fv'/Fm' <sup>2</sup>	qP <sup>3</sup>	qNR <sup>4</sup>	AETR <sup>5</sup>
Water type	UW <sup>6</sup>	0.93	0.45	0.50	2.26 a	116.72 b
water type	WVLF <sup>7</sup>	0.93	0.45	0.52	2.15 b	130.52 a
1 = (10 = -1)	UW	0.94	0.48	0.46	1.98	118.53
$1.5 (dS m^{-1})$	WVLF	0.93	0.47	0.47	2.01	118.51
$2 \in (40 \text{ m} - 1)$	UW	0.93	0.45	0.47	2.27	115.44
$2.5 (dS m^{-1})$	WVLF	0.94	0.48	0.44	2.08	126.71
$4 (dCm^{-1})$	UW	0.94	0.40	0.57	2.55	120.51
$4(a S m^{-1})$	WVLF	0.94	0.44	0.55	2.24	137.17
$E = (dE m^{-1})$	UW	0.94	0.45	0.42	2.05	101.88
5.5 (d5 m <sup>-1</sup> )	WVLF	0.92	0.42	0.57	2.14	126.76
$70(46 m^{-1})$	UW	0.92	0.41	0.58	2.44	127.24
7.0 (d5 m )	WVLF	0.94	0.45	0.55	2.28	143.44
CV (%)	-	3.06	6.92	18.64	6.50	10.96
W.T.	-	0.68 <sup>ns</sup>	1.51 <sup>ns</sup>	0.21 <sup>ns</sup>	4.53 *	7.78 *
L.C.	-	0.04 <sup>ns</sup>	3.43 *	1.60 <sup>ns</sup>	8.63 **	2.32 <sup>ns</sup>
$W.T \times L.C.$	-	0.55 <sup>ns</sup>	1.80 <sup>ns</sup>	0.94 <sup>ns</sup>	1.94 <sup>ns</sup>	0.68 <sup>ns</sup>

**Table 2.** Fluorescence of chlorophyll a of tomato crop subjected to different levels of fertigation and water types.

<sup>1</sup> Fv/Fm: PSII potential quantum efficiency; <sup>2</sup> Fv'/Fm': Quantum antenna efficiency; <sup>3</sup> qP: Photochemical extinction coefficient; <sup>4</sup> qNR: Non-photochemical extinction coefficient; <sup>5</sup> AETR: Apparent electron transport rate. <sup>6</sup> UW: Un-treated water; <sup>7</sup> WVLF: Water treated with very low-frequency electromagnetic resonance fields, W.T.: Water type; L.C.: levels of conductivity. Means followed by the same letter (lowercase compares the type of water) do not differ statistically (p < 0.05) according to the Tukey test. Error bars indicate the standard deviation of the mean of 5 repetitions (n = 5). \* significant at 1% and \*\* significant at 5%, <sup>ns:</sup> not significant.

## 3.5. Production

The production of the tomato crop when subjected to different fertigation doses showed a reduction from the dose of 2.5 dS m<sup>-1</sup> and so forth. That dose was proved to be the tolerant threshold for the crop (Figure 4).



**Figure 4.** Tomato production in response to different levels of fertigation and irrigation management with electromagnetically treated (WVLF) and untreated water (UW). Means followed by the same letter (lowercase compares the type of water and uppercase compares the salinity doses) do not differ statistically (p < 0.05) according to the Tukey test. Error bars indicate the standard deviation of the mean of four replicates (n = 4).

When irrigated with electromagnetized water, it was found that there was greater production for doses of 1.5, 2.5, and 7 dS m<sup>-1</sup>. It is worth highlighting that the recommended dose for tomato crops should be around 2.5 dS m<sup>-1</sup>. For this dose, production reached 13% more when irrigated with WVLF.

#### 4. Conclusions

From the analysis of the data in the present study, we can conclude that the electromagnetic treatment of water reduced the impacts caused by fertigation levels above the dose of  $2.5 \text{ dS m}^{-1}$ , which proved to be the threshold for tomato development, that is, for its metabolism photosynthetic. Such facts are evidenced as the gas exchange parameters that should have been reduced with the presence of salinity were not verified. Another parameter that should have been reduced, but was not, was the fluorescence of chlorophyll a. The production, per plant, at the recommended dose presented approximately 20% more in production. This shows that the use of water treated with very low-frequency electromagnetic resonance fields has the potential to minimize impacts on plants subjected to saline stress.

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