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Influence of Mixed Substrate and Arbuscular Mycorrhizal Fungi on Photosynthetic Efficiency, Nutrient and Water Status and Yield in Tomato Plants Irrigated with Saline Reclaimed Waters

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Abstract: The use of reclaimed water (RW) is considered as a means of maintaining agricultural productivity under drought conditions. However, RW may contain high concentrations of salts. The use of some practices, such as biofertilizers and organic substrates, is also becoming increasingly important in agricultural. production. The aim of this study was to evaluate the application of a mixed substrate (with coconut fibre) and arbuscular mycorrhizal fungi (AMF) on water relations, nutrient uptake and productivity in tomato plants irrigated with saline RW in a commercial greenhouse. Saline RW on its own caused a nutrient imbalance and negatively affected several physiological parameters. However, the high water-holding capacity of coconut fibre in the mixed substrate increased water and nutrient availability for the plants. As a consequence, leaf water potential, gas exchange, some fluorescence parameters (PhiPSII, F_v'/F_m', qP and ETR) and fruit size and weight improved, even in control irrigation conditions. The use of AMF improved only some parameters because of the low percentage of colonization, suggesting that AMF effectiveness in commercial field conditions is slower and dependent of several factors.

Keywords: biofertilizer; coconut fibre; fluorescence; gas exchange; leaf water potential; salinity

1. Introduction

Increasing water scarcity is a major challenge for sustainable development. The competition for water will intensify as the world population will increase to 9–10 billion people by around 2050 [1]. Agricultural production is, at the same time one of the main causes and victims of this water shortage, since more than 70% of the world's fresh water supply is used by agriculture [2]. Increasing competition between municipal, industrial, and agricultural sectors for good quality freshwater makes it important for farmers to adopt practices and policies that focus on the use of poor-quality water [3].

The use of reclaimed water (RW) as an additional source of water has been considered as a potential approach to overcome the water scarcity as an environmentally sustainable practice for maintaining agricultural productivity and the security of food supplies [4,5]. In Spain, the use of RW for agricultural purposes in the Community of Valencia and Region of Murcia accounts for 75.4% of the total of regenerated water of the country, with the provinces using 113.5 hm^3 $year^{-1}$ and 83.5 hm^3 $year^{-1}$, respectively [6]. Both areas are characterized as having very limited water resources. However, there are also risks in using RW for agricultural irrigation, including salinization due to the continuous supply of phytotoxic salts. A high concentration of Na, B and Cl also reduces crop productivity and marketability [7]. Therefore, when saline reclaimed water (RW) is used, its success in terms of crop production is dependent on the salinity level in the water, crops tolerance to saline conditions and the strategies used to mitigate the effect of the salts on plant growth and physiology. The tomato is one of the most important crops in Spain, representing 23% of the total value of production in the agricultural sector [8]. The region of Murcia is the third largest tomato producer in the country, with a total of 2459 ha, a yield of 145,504 kg ha^{-1} and production of 260,084 tonnes in 2018 [9]. Tomato plants are considered as moderately sensitive to salinity [10]. The growth and yield of the crop begins to decline when the electrical conductivity (EC) of the nutrient solution used in cultivation exceeds 2.5–4.0 $dS{\cdot}m^{-1}$ [11]. Consequently, under these conditions, an appropriate management of the crop is essential to maintain productivity and fruit quality [12]. In this sense, the use of environmentally sustainable practices, such as biofertilizers and organic substrates, is becoming increasingly important in agriculture [13], since such practices allow greater water preservation in the soil and a sufficient release of mineral nutrients to sustain crop yields. At the same time, they can maximize the stable soil organic carbon recovery, accompanied by improvements in soil fertility [14].

After composting, many organic wastes or byproducts are suitable as commercial organic substrates in soilless culture, where they also act as a renewable resource [15–17]. However, since the nutrient elements and other characteristics of each organic substrate differ from those of others due to their different origins and different type of treatment during composting [18,19], not all organic substrates fulfill the requirements of the plant [20].

Arbuscular mycorrhizal fungi (AMF) have been demonstrated to enhance plant growth and tolerance to both environmental and production stresses, including issues related to water quality. They are soil microorganisms that form mutualistic symbioses with about 80% of land plant species, including several agricultural crops. Many AMF have been found to be able to provide host plants with mineral nutrients (especially nitrogen and phosphorous) and water in exchange for photosynthetic products [21], even in abiotic stress conditions, such as drought, salinity and flooding [22]. The fine hyphae of AMF can explore pores in the soil that are inaccessible to roots, allowing them to reach water and nutrient sources which are otherwise inaccessible to the plant. There is a variety of AMF products and commercial inocula on the market. Nevertheless, although marketable inoculants are always advertised as being suitable for a broad variety of environmental conditions and plants, the actual gains to the host might not always be positive [23].

The aims of this study were to evaluate the application of a mixed substrate (a combination of a traditional agricultural soil with a specific organic substrate) combined with the application of arbuscular mycorrhizal fungus in a commercial greenhouse experiment, studying the photosynthetic efficiency, nutrient and water imbalance and yield of tomato plants irrigated with saline reclaimed water.

2. Materials and Methods

2.1. Plant Material and Experiment Conditions

The experiment was performed using 30 cm-high tomato plants (*Solanum lycopersicum* var HULK F1, provided by CapGen) (n = 352) grown in a commercial greenhouse located in the E.D.A.R. of Balsicas (Murcia, Spain) from April 2018 until September 2018. The microclimatic conditions showed that during the experimental period, the average values of the air temperature, relative humidity and radiation were around 25 °C, 65% and 500 Wm^{-2}, respectively. The experimental plot inside the greenhouse consisted of 8 rows, with a total length of approximately 17 metres and 44 plants per row (0.4 m × 1.2 m planting frame). The first and last row were left untreated. Irrigation and agronomic management were established by the farmer. Plants were irrigated by localized irrigation system (40 to 60 min) twice a day, with one lateral pipe per plant row and one emitter (3 L h^{-1}), calculated according to the evapotranspiration rate of the crop and the retention capacity of the mixed substrate.

2.2. Preparation of Treatments

Before transplanting, two types of soil were prepared in the greenhouse: A traditional agricultural soil (T) (the soil used normally in the greenhouse), and a mixed substrate (M), which consisted of an organic substrate mixed with the traditional agricultural soil. The organic substrate (provided by PELEMIX S.L) consisted of washed coconut fibre (20 L lineal metre of soil^{-1}), containing up to 20% of medium length fibres, 89.3% organic matter, 80 g L^{-1} da, 60–70% humidity, EC < 1 ds cm^{-1} and pH: 5.5–6.7.

Half of the plants were inoculated with fungus *Glomus iranicum* var *tenuipharum* (a mixture of spores, mycorrhizal root fragments and rhizospheric soil), provided by SYMBORG S.L., which was added manually to the soil or substrate (500g m^{-3}) (+). The other half remained without AMF (−). The fungus was obtained under extreme saline soil conditions. Multiplication of the strain was carried out as proposed by Fernández and Juárez [24].

Plants were irrigated to 100% water-holding capacity using two types of water: saline reclaimed water (EC ≈ 3 dS m^{-1}) (S) from the tertiary treatment effluent of a wastewater treatment plant (Roldán-Balsicas, Murcia, Spain), and control water (EC: 1 dS m^{-1}) (C) from an Irrigation Community (Table 1). Therefore, over a 20-week period, plants were exposed to eight treatments: Three factorial combinations of soil type (T, M), AMF application (+, −) and irrigation water (S, C).

Table 1. Physicochemical analysis of the irrigation water. Data are values from samples collected at the beginning of the experiment.

	Control	**Saline RW**
EC (dS m^{-2})	0.994	3.107
pH	7.22	7.16
B (ppm)	0.69	0.75
Ca (ppm)	21.39	93.97
Fe (ppm)	0.03	0.08
K (ppm)	15.38	56.19
Mg (ppm)	4.76	19.33
Na (ppm)	167.60	535.60
P (ppm)	2.12	6.73
Zn (ppm)	0.01	0.05

The treatments followed a randomized design, with three replications per treatment (11 plants per replication, 33 plants per treatment). Three rows were irrigated with control water and the other three with saline RW in the plot.

2.3. Percentage of Root Colonization

Halfway through the experiment (week 13) young root samples with the surrounding rhizosphere soil were collected at a depth of 10–20 cm to assess symbiotic development. Six root samples per treatment (two per replication) were collected. The percentage of mycorrhizal root colonization was estimated following the gridline intersect method [25] under a microscope (100 × magnification) after cleaning washed roots in (i) 10% KOH for 10 min at 90 °C, (ii) HCl_2N for 10 min and (iii) staining with 0.05% (*v/v*) trypan blue dissolved in lactic acid [26].

2.4. Determination of Mineral Content in Leaves, Soil and Water

The inorganic mineral content of dry soil and dry leaves was determined halfway through the experiment in three plants per treatment (one sample per replication) by means of emission spectrophotometry. The leaves and soil were oven dried at 80 °C, ground, and sieved through a 2-mm nylon mesh before analysis. A chemical analysis of water irrigation treatments was performed. Electrical conductivity (EC) was measured with a multirange Cryson-HI8734 electrical conductivity meter (Cryson Instruments, S.A., Barcelona, Spain). The pH was calculated with a Cryson-507 pH-meter (Cryson Instruments, S.A., Barcelona, Spain). The nutrient concentrations were determined in an extract digested with HNO3:HClO4 (2:1, *v/v*) using an Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). Chloride (Cl) ion was analysed by ion chromatography (Metrohm, Herisau, Switzerland).

2.5. Water Relations

Leaf water relations were measured near the beginning (week 5), in the middle (week 13) and at the end (week 17) of the experiment in six plants per treatment (two plants per replication). Leaf water potential (Ψ_{leaf}) was measured at midday, collecting a mature leaf according to Scholander et al. [27] using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA). Leaves were placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s^{-1} [28]. Adjacent leaves were also collected, frozen immediately in liquid nitrogen (−196 °C) and subsequently stored at −30 °C. After thawing, the osmotic potential (Ψ_{os}) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. [29]. The leaf osmotic potential at full turgor (Ψ_{100s}) was estimated as indicated above for Ψ_{os} after placed in distilled water overnight to reach full saturation.

2.6. Gas Exchange and Chlorophyll Fluorescence Parameters

Leaf gas exchange and chlorophyll fluorescence were measured simultaneously at midday using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA), fitted with an infrared gas analyser attached to a leaf chamber fluorometer (LCF) (6400-40B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, NE, USA). The reference CO_2, photosynthetically active radiation (PAR) and speed of the circulating air flow inside the system were set at 400 ppm, at 2000 µmol m^{-2} s^{-1} and at 300 µmol s^{-1}, respectively. The leaf photosynthetic rate (P_n), stomatal conductance (g_s), internal CO_2 concentration (C_i), the excitation capture efficiency of open centres (F_v'/F_m'), the effective quantum efficiency of photosystem II (Phi PSII), photochemical quenching coefficient (qP) and the electron transport rate (ETR) were measured [30]. The intrinsic water use efficiency (WUE_i) was determined as the P_n/g_s ratio. The relative chlorophyll content (RCC) was measured using a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan).

2.7. Yield and Fruit Measurements

The yield (kg fruit $plant^{-1}$) and number of fruits per plant, as well as mean fruit weight, were determined. In addition, fruit size (length and diameter) were also calculated in 30 fruits per treatment (10 fruits per replication).

2.8. Statistics

In the experiment, tomato plants (n = 264) were randomly assigned to each treatment, with three replications for each treatment. The data were analysed by one-way ANOVA and three-way ANOVA using IBM SPSS Statistics 25. The independent variables were soil type, AMF inoculation and irrigation water. Treatment means were separated with Duncan's Multiple Range Test ($p \leq 0.05$).

3. Results

3.1. Root Colonization

Plants inoculated with AMF showed an increase in root mycorrhizal colonization compared with the non-inoculated plants under both irrigation conditions at week 13 (Figure 1). However, root colonization did not exceed 16% in inoculated plants. There were no significant differences in the percentage of root colonization resulting by the soil and irrigation water type.

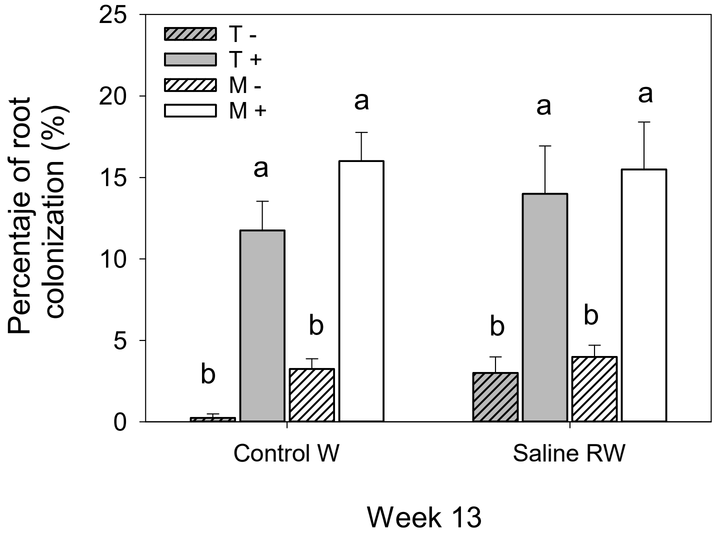

Figure 1. Percentage of root colonization in tomato plants irrigated with control water (Control W) and saline reclaimed wastewater (Saline RW) growing in traditional soil (T) or mixed substrate (M) without (−) and with AMF (+) inoculation. Different lowercase letters indicate significant differences between treatments according to Duncan test at $p \leq 0.05$.

3.2. Ions Content

The concentrations of Na, Ca, B, K, Fe, Mg, Zn and P ions were higher in the saline RW than control water (Table 1).

However, the use of saline RW reduced the P content of the soil (Table 2). The K content was higher with saline RW, while the rest of the elements did not show any changes. An increase in the P content was observed for the application of mixed substrate, regardless of the type of water and the application or not of AMF. There were no significant differences resulting from AMF inoculation (Table 2).

Table 2. Soil mineral content according to soil type (s) (traditional: T and mixed: M), AMF inoculation (A) (with: + or without: −), irrigation water (I) (saline: S, control: C), and interaction among them. Statistical significance according to one-way and three-way ANOVA tests. Values are means of three samples.

Ion (ppm)	Soil Type (s)		AMF (A)		Irrigation Water (I)		P			
	T	M	+	−	S	C	s	A	I	S * A * I
Na	1063.5	1137.3	1242.5	958.3	1374.9	826.0	ns	ns	ns	ns
Ca	157,287.5	151,612.5	153,666.7	155,233.3	157,116.7	151,783.3	ns	ns	ns	ns
P	1162.0	1783.7	1511.2	1432.5	911.5	1034.2	*	ns	***	ns
Mg	11,245.4	10,761.7	11,207.5	10,799.6	11,176.3	10,830.8	ns	ns	ns	ns
B	181.6	188.2	190.2	179.9	183.3	186.8	ns	ns	ns	ns
K	10,665.0	11,009.2	10,907.9	10,766.3	11,290.8	10,383.3	ns	ns	*	ns

* $p < 0.05$ and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

Regardless of the type of soil and AMF application, there was also a greater accumulation of leaf K and Na caused by irrigation with saline RW (Table 3), but the K/Na ratio remained unaltered.

Table 3. Leaf mineral content according to variables including Soil type (s) (traditional: T and mixed: M), AMF inoculation (A) (with: + or without: −), irrigation water (I) (saline: S, control: C), and interaction among them. Statistical significance according to one-way and three-way ANOVA tests. Values are means of three samples.

Ion (ppm)	Soil Type (s)		AMF (A)		Irrigation Water (I)		P			
	T	M	+	−	S	C	s	A	I	S * A * I
Na	5063.9	4122.1	4778	4408.0	5472.0	3714.2	ns	ns	**	ns
Ca	52,890.8	54,111.3	53,546.3	53,455.8	51,070.0	55,932.1	ns	ns	*	ns
P	4238.3	6537.1	5326.0	5449.4	5186.3	5589.2	***	ns	ns	ns
Mg	11,245.4	10,761.7	11,207.5	10,799.6	11,176.3	10,830.8	ns	ns	ns	ns
B	535.2	527.5	537.6	525.1	522.2	540.5	ns	ns	ns	ns
K	8818.3	10,050.4	9062.1	9806.67	10,297.5	8571.3	*	ns	**	ns

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

Irrigation with RW water induced a decrease in the leaf Ca content, which was reflected in the value of the Ca/Na ratio (Figure 2A). The mixed substrate increased the leaf P and K content. The increase in K led to a higher K/Na ratio in this type of substrate. (Table 3 and Figure 2B). The application of AMF did not significantly affect the leaf mineral content (Table 3).

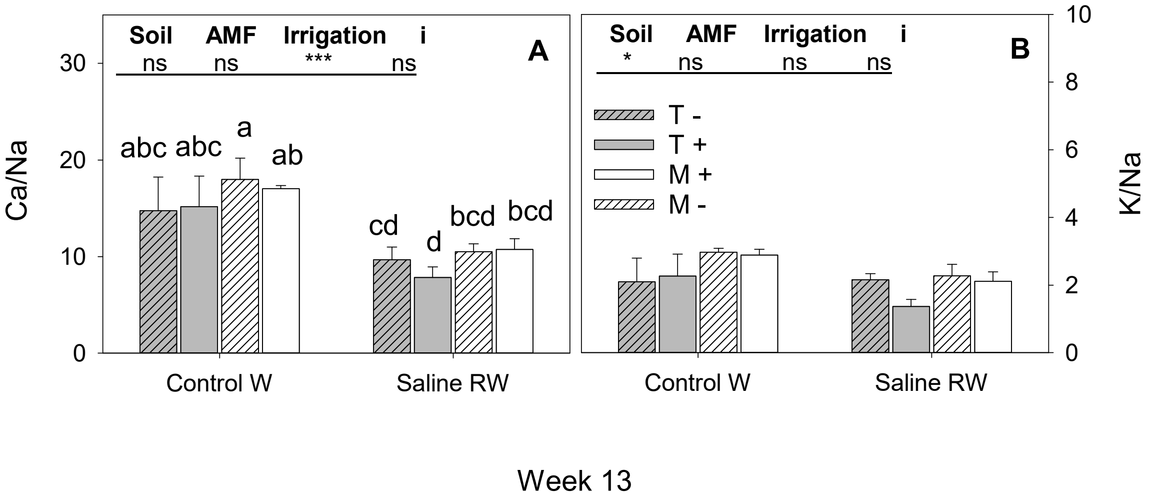

Figure 2. Leaf Ca/Na (**A**) and K/Na (**B**) ratios for irrigation with control water (Control W) and saline reclaimed wastewater (Saline RW) in traditional soil (T) or mixed substrate (M) without (−) and with AMF (+) inoculation. Results of one-way and three-way ANOVA tests on these parameters, for independent variables (soil, AMF and irrigation) and their interaction (i). Different lowercase letters indicate significant differences between treatments according to Duncan test at $p \leq 0.05$. * $p < 0.05$, and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

3.3. Leaf Water Relations

Irrigation with the saline RW induced a reduction of leaf water potential in the second half of the experiment (from week 13), as the non-inoculated plants grown in traditional soil reached the lowest values (Figure 3B,C). The application of mixed substrate had slightly increased these values by week 13, especially with saline RW (Figure 3B), and by week 17, values also increased under control irrigation (Figure 3C). The leaf osmotic potential decreased from week 13, especially toward at the end of the experiment (Figure 3E,F). Saline RW induced a reduction in Ψ_{os}, while mixed substrate increased the value of this parameter under both irrigation conditions. Thus, the lowest values were found for saline RW applied to plants grown with traditional soil, and the highest values were found under control conditions in plants grown with mixed substrate (Figure 3F). The Ψ_{100s} showed a similar trend, although the effect of mixed substrate was not so marked as in the case of Ψ_{os} (Figure 3H,I). The application of AMF did not alter any water relation parameters.

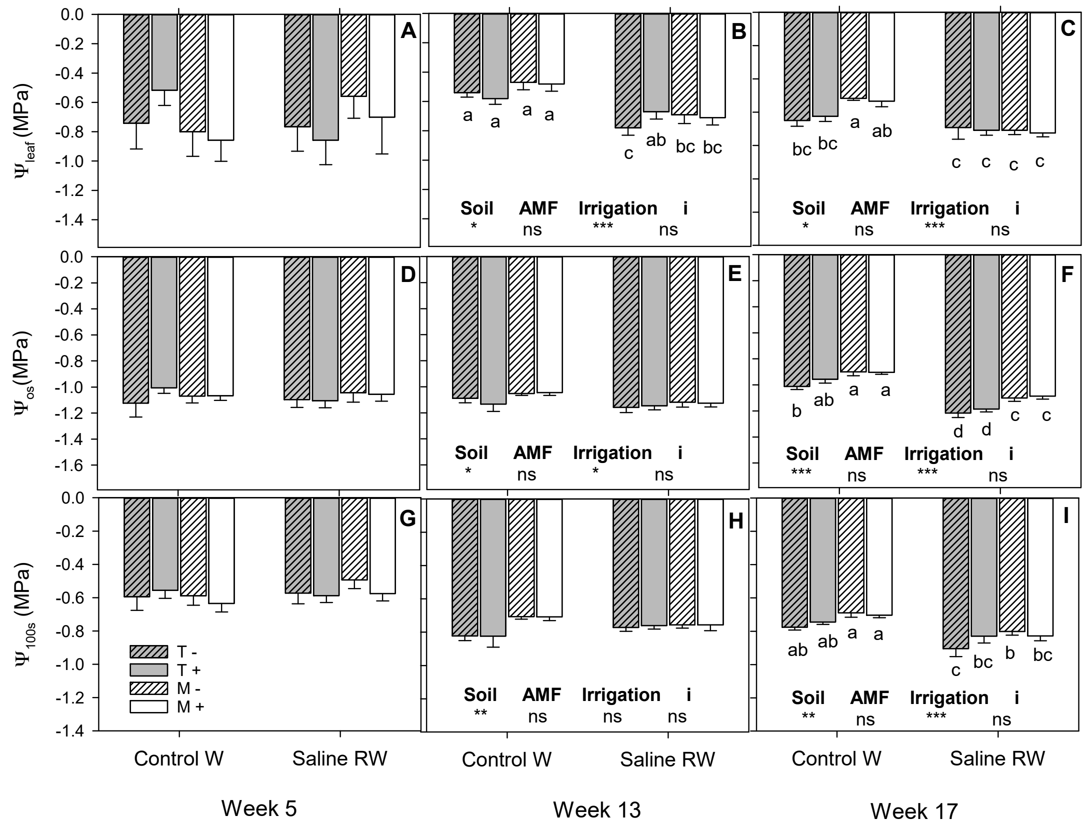

Figure 3. Leaf water potential (Ψ_{leaf}) (**A**, **B**, **C**), osmotic water potential (Ψ_{os}) (**D**, **E**, **F**) and osmotic water potential at full turgor (Ψ_{100s}) (**G**, **H**, **I**) in plants irrigated with control water (Control W) and saline reclaimed wastewater (Saline RW) growing in traditional soil (T) or mixed substrate (M) without (−) and with AMF (+) inoculation, at weeks 5, 13 and 17. Results of one way and three-way ANOVA tests in these parameters, for independent variables (soil, AMF and irrigation) and their interaction (i). Different lowercase letters indicate significant differences between treatments according to Duncan's test at $p \leq 0.05$. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

3.4. Gas Exchange and Photosynthetic Efficiency

Gas exchange was negatively affected by saline RW from week 5, while mixed substrate improved this parameter (Figure 4A–C). At the end of the experiment, the highest values of g_s and P_n were observed in plants growing in mixed substrate (Figure 4B,C). The plants grown with AMF also showed improved stomatal conductance and net photosynthetic rate at week 17 (Figure 4C,F). The WUE_i increased in plants irrigated with saline RW compared with those irrigated with control water since the second half of the experiment, regardless of AMF application or soil type. However, there were no statistical differences between treatments according to one-way ANOVA analyses (Figure 4G–I). On the other hand, the RCC showed lower values at weeks 5 and 17 (Figure 4J,L), but showed higher values at week 13 after irrigation with saline RW (Figure 4K). The application of mixed substrate had a positive effect on RCC from week 13 onward (Figure 4K,L), while AMF application did not lead to any statistically significant changes.

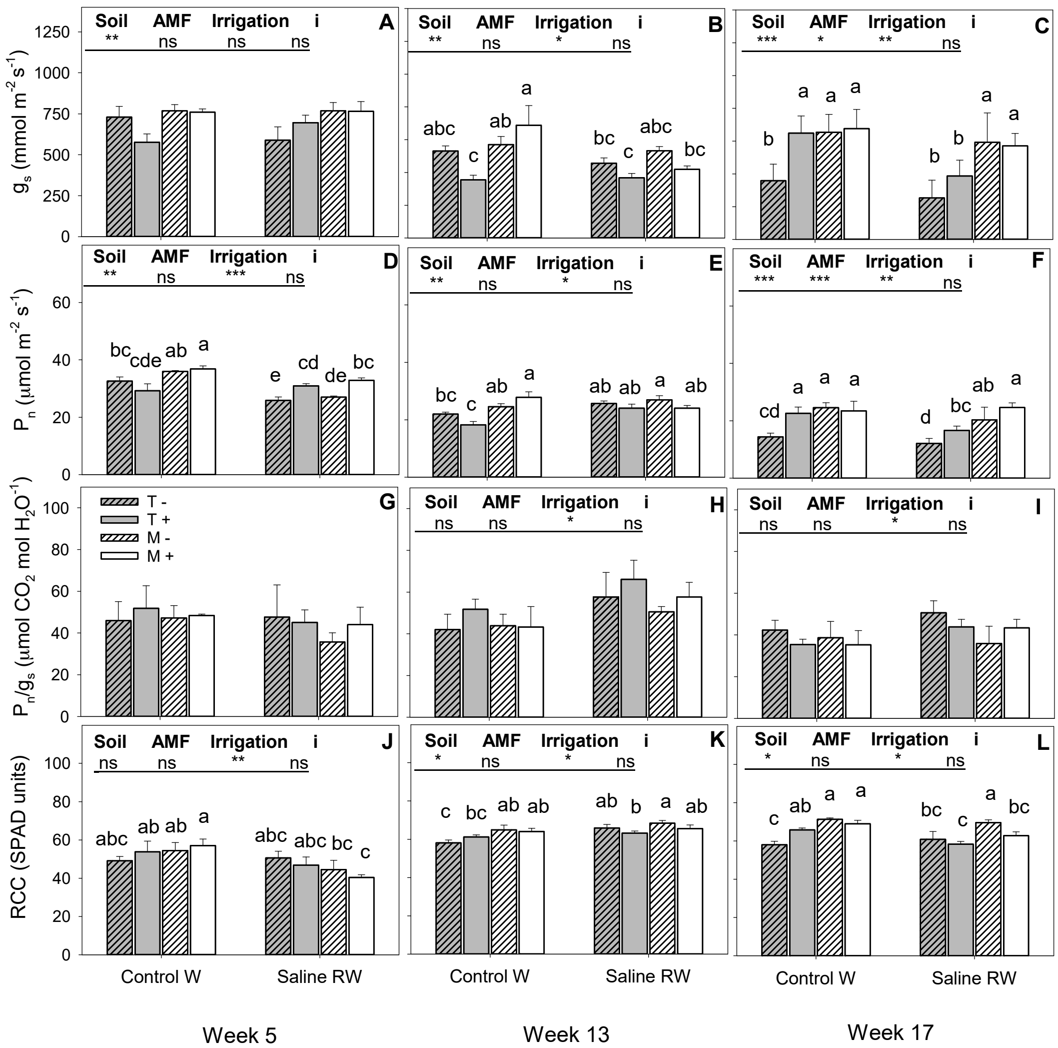

Figure 4. Stomatal conductance (gs) (**A**, **B**, **C**), net photosynthetic rate (Pn) (**D**, **E**, **F**), intrinsic water use efficiency (P_n/g_s) (**G**, **H**, **I**) and relative chlorophyll content (RCC) (**J**, **K**, **L**) in plants irrigated with control water (Control W) and saline reclaimed wastewater (Saline RW) growing in traditional soil (T) or mixed substrate (M) without (−) and with AMF (+) inoculation, at weeks 5, 13 and 17. Results of one way and three-way ANOVA tests in these parameters, for independent variables (soil, AMF and irrigation) and their interaction (i). Different lowercase letters indicate significant differences between treatments according to Duncan test at $p \leq 0.05$. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

The electron transport rate (ETR) and the effective quantum efficiency of photosystem II (Phi PSII) showed similar trends. At weeks 5 and 17, these parameters showed lower values in saline RW but showed higher values at week 13 (Table 4). The mixed substrate enhanced both parameters at week 17. The internal CO_2 (C_i) showed increased values at week 5 and decreased values at week 13 following saline irrigation. The application of mixed substrate had the opposite effect on C_i to that of saline RW at week 13 (Table 4). The AMF application led to reduced values of this parameter at week 13. The excitation capture efficiency of open centres (F_v'/F_m') was lower in saline RW at week 17, but higher in mixed substrate at week 13. The photochemical quenching coefficient (qP) increased in the saline RW plants by week 13 and also in the mixed substrate plants by week 17 (Table 4).

Table 4. Fluorescence parameters (Ci: Internal CO2 concentration; F_v'/F_m': The excitation capture efficiency of open centres; PhiPSII: PSII effective quantum yield; qP: Photochemical quenching coefficient; and ETR: Apparent electron transport rate), depending on soil type (s) (traditional: T and mixed: M), AMF inoculation (A) (with: + or without: −), irrigation water (I) (saline: S, control: C), and interaction among them, at weeks 5, 13 and 17. Statistical significance according to one-way and three-way ANOVA tests. Values are means of six samples.

Parameter		Soil Type (s)		AMF (A)		Irrigation Water (I)		P			
	Week	T	M	+	−	S	C	s	A	I	S * A * I
C_i	5	290.26	297.62	293.38	294.50	300.56	287.32	ns	ns	*	ns
	13	274.64	285.27	275.76	284.15	269.88	290.03	**	*	***	ns
	17	294.99	298.47	299.06	294.40	292.65	300.80	ns	ns	ns	ns
F_v'/F_m'	5	0.539	0.551	0.544	0.546	0.541	0.549	ns	ns	ns	ns
	13	0.574	0.587	0.581	0.580	0.575	0.585	*	ns	ns	ns
	17	0.558	0.567	0.569	0.558	0.550	0.578	ns	ns	*	ns
PhiPSII	5	0.281	0.286	0.276	0.291	0.267	0.299	ns	ns	*	ns
	13	0.201	0.197	0.193	0.206	0.209	0.189	ns	ns	*	ns
	17	0.144	0.193	0.172	0.165	0.160	0.178	***	ns	*	ns
qP	5	0.52	0.518	0.506	0.532	0.494	0.544	ns	ns	ns	ns
	13	0.351	0.337	0.332	0.356	0.364	0.323	ns	ns	**	ns
	17	0.260	0.341	0.304	0.297	0.294	0.307	***	ns	ns	ns
ETR	5	238.43	242.68	234.18	246.94	226.98	254.14	ns	ns	*	ns
	13	170.85	167.75	163.84	174.75	178.04	160.55	ns	ns	*	ns
	17	122.75	164.28	146.36	140.68	135.98	151.06	***	ns	*	ns

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

3.5. Fruit Yield

Neither the yield (kg fruit plant^{-1}) nor the number of fruits per plant was significantly affected by any treatment (Table 5). However, the average fruit weight, as well as the size (length and diameter), were reduced by irrigation with saline RW, but fruit length and weight increased in mixed substrate. No changes were found as a result of AMF application (Table 5).

Table 5. Yield and fruit size depending on soil type (s) (traditional: T and mixed: M), AMF inoculation (A) (with: + or without: −), irrigation water (I) (saline: S, control: C), and interaction among them. Statistical significance according to one-way and three-way ANOVA tests. Values are means of 10 samples.

Parameter	Soil Type (s)		AMF (A)		Irrigation Water (I)		P			
	T	M	+	−	S	C	s	A	I	S * A * I
Kg fruit plant^{-1}	0.678	0.713	0.662	0.73	0.7	0.69	ns	ns	ns	ns
N° fruits plant^{-1}	11.72	12.30	11.58	12.43	12.38	11.63	ns	ns	ns	ns
Mean weight (g)	38.78	43.03	41.5	40.31	35.33	46.48	**	ns	**	ns
Length of fruit (mm)	50.62	52.22	51.78	51.07	49.69	53.15	*	ns	***	ns
Fruit diameter (mm)	36.95	37.42	37.09	37.28	34.82	39.54	ns	ns	***	ns

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.05$. $p > 0.05$ non-significant differences are indicated by "ns".

4. Discussion

The use of low-quality waters for crop irrigation can result in a nutritional imbalance in the plant due to a salt accumulation in the root zone. It is well-known that salinity inhibits the absorption of essential nutrients, such as phosphorus and potassium, through the plant [31]. In spite of the competitive relationship between Na and K, the higher selectivity of tomato plants for K rather than Na [32] led to an increase in the leaf K content in plants irrigated with saline RW. Therefore, the plants were able to maintain the K/Na ratio close to the values of plants irrigated with control water. The K/Na ratio is considered a good indicator of salinity stress of crops [33]. However, the Ca/Na ratio was reduced by salinity. The accumulation of P and K in leaf tissues in mixed substrate has also been observed in other studies with plants growing in soilless cultivation systems [34], probably due to the initial mineral composition and caption exchange capacity of the substrate applied [35,36] or the more efficient utilization of nutrients [37]. Even though AMF are generally linked with increased leaf P uptake [38], no statistical differences were observed under our conditions. In this study, the dependence of *Glomus iranicum* var *tenuipharum* on tomato plants was not clear, since the percentage of root colonization did not reflect the values found in other studies with the same species [39] or genus [40].

Irrigation with saline water causes degradation of the soil structure, altering its physical and chemical properties, resulting in decreased soil hydraulic conductivity [41]. In our experiment, leaf water potential was reduced by irrigation with saline reclaimed water, as a result of plants reducing their ability to acquire water, which is referred to as the osmotic or water-deficit effect of salinity [31,42]. The results for Ψ_{os} and Ψ_{100s} in plants suggested an osmotic adjustment process to maintain the leaf cell turgor, which is a common mechanism in plants with a certain salinity tolerance [43]. The application of mixed substrate improved plant water relations, probably because of the composition and characteristics of coconut fibre. The light weight of the fibres, 20% of which were of medium length, thus ensuring a good aeration, the porosity (95%) and the low bulk density probably helped to increase the water holding capacity and water retention [44–46]. In addition to the lack of any beneficial effect of the AMF on nutrient uptake, water relations were not improved by AMF application in either of the irrigation conditions. These results differ from those described by Ghorbani et al. [47]., who found that, the fungus *Piriformospora indica* inoculated into tomato plants not only reduced Na accumulation and increased K levels in leaves, but also improved the leaf water potential [47]. The authors suggested that accumulation of compatible osmolytes by AMF seems to facilitate the absorption of water in saline conditions.

As a consequence of the reduction of leaf water potential with saline irrigation, stomatal closure occurred, thus reflecting a low CO_2 assimilation rate [42,48,49]. Nevertheless, an improvement of WUE_i was noted under salinity conditions as an adaptation of moderately salinity tolerant plants [50]. The greater amount of leaf K accumulated in these plants than in those irrigated with control water would also help to prevent a greater reduction in water relations by regulating cellular osmotic potentials and thus, stomatal closure [51].

Changes in chlorophyll fluorescence are used as a stress indicator in plants exposed to different stressful conditions [52,53]. Lower C_i would normally be expected to be accompanied by lower stomatal conductance (g_s). However, by the end of the experiment, C_i had not decreased significantly, suggesting that nonstomatal factors were also occurring in the limitation of CO_2 assimilation activity [54–56]. In our case, qP was increased or sometimes not affected by the salinity level of the reclaimed water used, which was an indication that the proportion of reaction centres remaining open was similar under both control and saline conditions [57]. However, at the end of the experiment, the reduction in PhiPS2 caused by the saline RW would have increased the excitation pressure on PSII [58] and led to the photoinhibition of PSII [59]. In addition, the electron transport rate (ETR) was also inhibited. The reduction of F_v'/F_m' might have changed the thylakoid structure and damaged the PSII reaction centres, since chloroplasts are the most sensitive organelle [60,61], as confirmed by the reduction in the relative chlorophyll content in our experiment. Similar results have previously been found in tomato crops irrigated with saline waters [62–64]. The mixed substrate applied was able to increase the gas exchange and some fluorescence parameters under both irrigation conditions as a consequence of an improved plant water status. Carbon absorption processes have also been related with leaf nutrition uptake. The accumulation of K and P in leaves as a result of the use of mixed substrate probably contributed to the effective regulation of stomatal opening, as well as the influence on photosynthetic metabolism [65,66]. In this sense, Paradiso and De Pascale [34] reported that gerbera plants grown in a substrate of coconut fibre showed more intense evapotranspiration and higher leaf P and K content compared with plants grown in other substrates, leading to a better water status and an improvement in gas exchange, thus enhancing plant growth [57]. The inoculation of AMF also induced an increase in CO_2 assimilation, although no positive response in leaf nutrition or water status was observed. Therefore, mechanisms other than those studied must have been in operation. Several studies have demonstrated an improvement in gas exchange and fluorescence parameters following the application of AMF in horticultural crops [67–69], including tomato [70]. In our case, the low relative percentage of root colonization prevented greater changes in plant physiology from being observed. In fact, the combination of AMF and mixed substrate had a positive synergic effect in plants irrigated with saline RW, since, at the end of the experiment, the plants treated with both showed a greater improvement in gas exchange than plants inoculated only with AMF.

Although salt stress may reduce fruit yield by up to 10% for each unit of increased EC [71], not all tomato cultivars are affected equally by salinity. In our case, irrigation with saline RW did not alter tomato yield, although fruit weight and size were reduced. Nevertheless, the application of mixed substrate enhanced fruit weight and length under both irrigation conditions, which could be related to the greater water consumption of these plants and accumulation of P, an element responsible for crop plant growth and yield [72]. The use of AMF did not result in changes in tomato yield. Based on our results, several factors such as variety-fungus incompatibility, field-loading capacity, abundance and priority effects [73,74] were probably responsible for the low AMF activity.

5. Conclusions

Irrigation with saline RW caused a nutrient imbalance and negatively affected some physiological parameters, although the plants still showed certain level of tolerance to salinity. The mixed substrate improved most of these parameters in our experiment, even in control irrigation conditions. The higher water-holding capacity of coconut fibre compared with traditional soil increased the overall substrate water content and nutrient availability. At the end of the experiment, it was seen that the use of AMF as a biofertilizer had only improved some physiological parameters due to the low percentage of colonization. This suggests that in commercial field conditions, the effectiveness of AMF is slower and dependent on several factors. The experiment highlights the importance of an in-depth evaluation of new sustainable cultural practices in commercial farms.

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