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Effect of halophyte-based management in physiological and biochemical responses of tomato plants under moderately saline greenhouse conditions

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ABSTRACT

Salinity, both in irrigation water and in soils, is one of the major abiotic constraints for agriculture activity worldwide. Phytodesalinization is a low-cost plant-based bioremediation strategy that can effectively amend salt-affected soils by cultivating salt tolerant plants. However, very few studies have evaluated the use of halophyte plants in crop management systems. In this work, we apply two different tomato crop management strategies involving the halophyte *Arthrocaulon macrostachyum* L. in a moderately saline soil: intercropping (mixed cultivation) and sequential cropping (cultivation of tomato where halophytes were previously grown). We investigated the effect of the different crop managements in some physiological and biochemical variables in tomato plants, including mineral nutrients content, photosynthesis, chlorophyll and flavonol contents, antioxidant metabolism and fruit production and quality. At soil level, both intercropping and sequential cropping decreased chloride content, sodium adsorption ratio and electrical conductivity, leading to reduced soil salinity. In tomato plants, halophyte-dependent management improved nutrient homeostasis and triggered a mild oxidative stress, whereas photosynthesis performance was enhanced by intercropping. In tomato fruits, the sequential cropping led to a 27% production increase and a slight decrease in the soluble sugar contents. We suggest the use of *A. macrostachyum*, and hence of halophyte plants, as an environmentally friendly phytoremediation strategy to improve plant performance while improving crop production, leading to a more sustainable agriculture and enhancing biodiversity.

1. Introduction

It is estimated that a 50% of crop production losses are consequence of environmental stresses (EL Sabagh et al., 2021). Among them, salt stress is one of the most important environmental challenges affecting plant productivity, especially in arid and semi-arid areas. In this sense, salinity affects over 20% of global cultivable land. This environmental constrain has been aggravated over the last 25 years due to anthropogenic activities, the increase in irrigation requirements in arid and semi-arid regions such as those found in the Mediterranean area, and the climate change. Reduced availability of freshwater resources because of climate change has also led to the use of low-quality water for crop irrigation. This is also accompanied by a reduction of arable lands, motivated by increasing urbanization and the continuous soils degradation. In addition, it is estimated that, by 2050, the world population will have increased 20%, reaching 9.7 billion (https://www.un.org/en/global-issues/population). This implies that crop yields will have to increase concomitantly to ensure food supply, especially in developing countries.

The use of salt tolerant plants to cope with soil and water salinity is an effective and low-cost phytoremediation strategy to improve production in salt-affected lands, due to the capacity of many halophyte species to accumulate salt in their tissues (Liang and Shi, 2021; Nanhapo et al., 2017; Wang et al., 2022). A growing body of research is dealing with the use of halophytic plants to remove salts from the soil in intercropping or sequential cropping systems (Ben Hamed et al., 2021; Maitra et al., 2021), while including a cash crop or a forage species to ensure economic sustainability (Dogliotti et al., 2004). The use of halophytes in intercropping has been successfully implemented to improve the growth and yield of plants of agronomic interest. Intercropping of

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watermelon with *Atriplex hortensis* L. or *Portulaca oleracea* L. increased watermelon yield, which correlated with a lower leaf Cl^- accumulation (Simpson et al., 2018). Intercropping of tomato and *Suaeda salsa* L. plants caused lower tomato leaf Na^+ accumulation, in parallel to reduced Na^+ content in the growing medium (Albaho and Green, 2000). High NaCl treatments (200–300 mM) inhibited the photosynthesis rate of cowpea plants. However, the observed suppression of photosynthesis was effectively mitigated by mixed cultivation with ice plant (Nanhapo et al., 2017). In turn, this response correlated with a lower Na^+ accumulation in cowpea plants, resulting in lower Na^+/K^+ ratios. However, there is less literature about the use of the sequential cropping strategy to improve plant performance under saline conditions. In this regard, several authors studied the soil desalination potential of the halophytes *Sesuvium portulacastrum* L. and *Arthrocnemum macrostachyum* L. previous to the cultivation of crop plants such as barley, spinach and wheat (Barcia-Piedras et al., 2019; Muchate et al., 2018; Rabhi et al., 2010), finding that salt-tolerant plants are of special interest for desalination strategies for salt-affected soils.

Tomato is one of the most important vegetable crops grown in the Mediterranean zone. The world tomato production in 2021 was 188 million tons, with Mediterranean countries production accounting for 20% of the total (https://fenix.fao.org/faostat/internal/es/#home). In 2022, 3.7 million tons of tomato were produced in Spain in 51,693 ha (https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/). Tomato is considered moderately tolerant to salinity, which could result in reduced crop yields in soils having an electrical conductivity (EC) over 2.5 dS m^{-1} (Hanson and May 2004).

In this work, we conducted intercropping and sequential cropping between the halophyte *A. macrostachyum* L. and tomato plants in a moderately saline soil. We investigated the effect of these crop systems in some physiological and biochemical parameters in tomato plants, including plant mineral nutrient, chlorophylls and flavonols contents, chlorophyll fluorescence parameters, antioxidant metabolism-related parameters and fruit production and quality.

2. Methods

2.1. Plant material, experimental design and sample collection

Field trials were conducted in greenhouse facilities at the Agricultural Demonstration Center "La Pilica" (Águilas, Murcia, Spain) (37.416253, −1.592437), from September 17, 2021 to January 24, 2022 (winter season). During this period, humidity ranged from 80 to 85%, photoperiod from 7.1 to 9.7 h, light intensity from 350 to 600 μmol photons m^{-2} s^{-1}, and mean monthly maximum/minimum temperatures from 25/14 °C to 30/22 °C. The EC of the irrigation water, from the desalination plant of Águilas/Guadalentín (Murcia, Spain), ranged between 0.3 and 0.5 dS m^{-1}. Before trial implementation, soils were characterized as moderately saline (Daliakopoulos et al., 2016; Richards, 1954) due to EC levels ranging 7.0–7.5 dS m^{-1} and the presence of Na^+, Cl^- and SO_4^{2-} at 11,770, 1,009 and 1,572 ppm, respectively.

Sodium adsorption ratio (SAR), an indicator of soil sodicity (Rabhi et al., 2010), was calculated following the equation: $SAR = Na^+/[(Ca^{2+} + Mg^{2+})/2]^{1/2}$, using soils samples extracted by an acid digestion.

Sixty-five days old tomato plants (*Solanum lycopersicum* cv. Sargento), provided by the farm association Coaguilas SCL (Aguilas, Murcia, Spain), and four months old *A. macrostachyum* L. plants, obtained from seeds in a plant nursery ("Viveros Muzalé", Abanilla, Murcia, Spain), were transplanted into the greenhouse. Plots were arranged in a randomized block design with three replicates. The experimental design included three types of plots (Fig. 1): tomato in monoculture (T_M), tomato in mixed cultivation with halophyte (T_H), and tomato cultivated in monoculture where halophyte plants were grown for six months before tomato cultivation (sequential cropping, T_S). Each plot consisted of a 10 m length row with 13 tomato plants. Additionally, in T_H plots two halophyte plants were located at both sides of each tomato plant. Two drippers per meter were arranged to provide ferti-irrigation according to the commercial production practices of Coaguilas SCL. Actively growing leaves on the third branch from the apex of the main stem were used for the different analyses. Tomato fruits were harvested at 95, 107 and 128

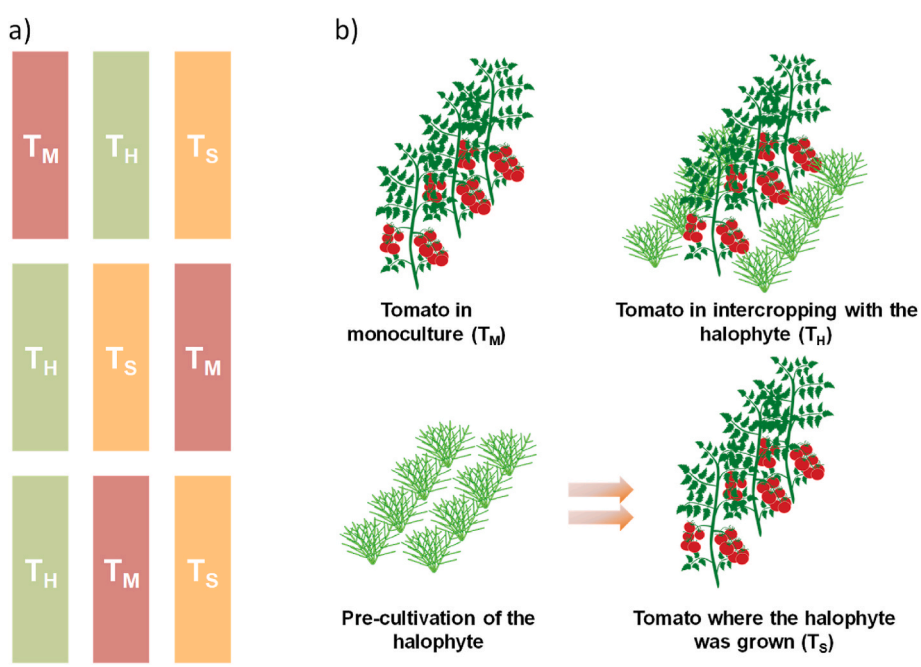


Fig. 1. Experimental design included tomato grown in monoculture (T_M), tomato grown in intercropping with *Artrhocaulon macrostachyum* (T_H) and tomato grown where the halophyte was previously cultivated (T_S). a) Randomized block design with three replicates. b) Schematical representation of the different crop managements.

days after planting (DAP). Plant roots (whole root) were taken at 128 DAP.

2.2. Mineral nutrient content

Analyses were done on samples taken at the end of the experiment (128 DAP). Soil samples were extracted with an auger (5 cm diameter), from 20 to 30 cm depth and air-dried for 48 h. Plant material (leaves and whole roots) was washed first with tap water and then with distilled water. The plant material was oven-dried at 60 °C for 4 days, and later the dry material was ground using a grinder. Leaf and root powder, as well as soil samples, were filtered through a sieve. Then, samples were digested using a high-performance microwave reaction with HNO_3/H_2O_2 (4/1, *v/v*) (Ultraclave; Milestone, Shelton, CT, USA), followed by macro- and micronutrient determination using inductively coupled plasma-optical emission spectrometry (ICP-OES) (ICAP 6000SERIES spectrometer, Thermo Scientific, Madrid, Spain). For anion analysis, leaf and root dry material was diluted in distilled water (1/10, *w/v*), heated at 75 °C for 3 h and incubated for 24 h at room temperature in continuous agitation. Subsequently, samples were centrifuged at 4000 rpm (Beckman Allegra X-22) in a swinging bucket rotor for 10 min. The generated supernatants were filtered through 0.45 μm Minisart filters (Sartorius, Goettingen, Germany) and anion concentrations were measured by ion-chromatography (Metrohm Ltd., Herisau, Switzerland).

2.3. Optical determination of chlorophylls, flavonols and anthocyanins

The assessment of flavonol, anthocyanin and chlorophyll contents in leaves was done using a portable leaf-clip optical sensor (Dualex Scientific, Force A, Orsay, France) at 18, 46, 73 and 95 DAP, between 9:00 and 11:00 h (GMT). Additionally, the nitrogen balance index (NBI) was calculated as the ratio chlorophylls/flavonols.

2.4. Chlorophyll fluorescence determination

Chlorophyll fluorescence data were determined in tomato leaves at 73 DAP between 9:00 and 11:00 h (GMT), using a portable modulated chlorophyll fluorimeter (FMS2, Hansatech Instruments, Pentney, UK). Previously, the leaves were dark adapted by using leaf clips, for at least 20 min. The different chlorophyll fluorescence parameters were obtained according to the manufacturer's instructions. Minimal fluorescence (Fo) and maximal fluorescence (Fm) values were measured. Then, the leaves were continuously illuminated with a white actinic light (200 μmol m^{-2} s^{-1}). The steady-state of fluorescence (Fs) was thereafter recorded and a second saturating pulse at 3,000 μmol m^{-2} s^{-1} was imposed to determine the maximal fluorescence level in light-adapted leaves (Fm'). Subsequently, the actinic light was removed and the minimal fluorescence level in the light-adapted state (Fo') was determined by illuminating the leaf with 1 s of far-red light. Using both light and dark fluorescence, we obtained the following parameters: the maximum quantum efficiency of PSII photochemistry (Fv/Fm), the quantum efficiency of PSII [Y(PSII)], the photochemical quenching coefficient (qP), the non-photochemical quenching (NPQ) and its coefficient (qN), and the electron transport rate (ETR).

2.5. Antioxidant enzymes extraction and analysis

Leaf samples (0.5 g) from 73 DAP tomato plants were snap-frozen in liquid nitrogen and stored at −80 °C until use. Samples were homogenized (1/4, *w/v*) in an ice-cooled extraction buffer (50 mM Tris-acetate buffer, pH 6.0) containing 0.1 mM EDTA, 2 mM cysteine and 0.2% (*v/v*) Triton X-100. For the APX activity, 20 mM sodium ascorbate was added to the extraction buffer. The extracts were centrifuged at 10,000 *g* for 15 min at 4 °C. The supernatant fraction was filtered on Sephadex NAP-10 columns (GE Healthcare, Chicago, IL, USA) equilibrated with 50 mM Tris-acetate buffer, pH 6.0. For the APX activity, 2 mM sodium ascorbate was added to the buffer. Ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), glutathione reductase (GR, EC 1.6.4.2), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase activities (POX, EC 1.11.1.7) were analyzed following routine protocols described in our laboratory (Cantabella et al., 2017; Hernández et al., 2021a, 2021b).

2.6. Histochemical staining of ROS

The histochemical staining to visualize superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) accumulation was carried out in tomato leaves at 73 DAP by incubation with 0.1 mg mL^{-1} Nitroblue tetrazolium (NBT) and 0.1 mg mL^{-1} 3,3′-diaminobenzidine (DAB), respectively, in darkness at 25 °C for 24 h. To assess the specificity of the staining, negative controls were performed with either 10 mM $MnCl_2$ (for $O_2^{\cdot-}$) or 10 mM ascorbate (for H_2O_2) (Hernández et al., 2001). Then the chlorophyll was extracted by 70% ethanol at 65 °C and photographed with an Olympus BX40 microscope (Olympus Medical Systems Corp., Tokyo, Japan).

2.7. Tomato fruit production and quality

Fully mature fruits were harvested at 95, 107 and 128 DAP. The number of fruits and mean fruit weight per plant were determined on ten plants per crop management. The data of the three time points were used to calculate the total production per plant. Twenty representative fruits from each crop management were squeezed at each harvest, and the juice filtered through a 1 mm sieve. The total soluble solid (TSS, expressed as °Brix) and the acidity (expressed as citric acid equivalents) of the extracts were determined using a PAL-BX/ACID refractometer (Atago Co. Ltd, Tokyo, Japan).

2.8. Statistical analysis

At least five biological replicates per treatment were included. Data were subjected to an analysis of variance (ANOVA), followed (when applicable) by Duncan's Multiple Range Test at the 5% probability level ($p \leq 0.05$), using SPSS® for Windows (IBM SPPS Statistics, version 27, Armonk, NY, USA).

3. Results

3.1. Effect of crop management on plant and soil mineral contents

The effect of crop management (monoculture, intercropping and sequential cropping) on the levels of macronutrients, micronutrients and anions in both tomato plants and soil samples was studied. In tomato leaves, the following concentration-based sequence for macronutrients was found, independently of the crop management: Ca^{2+}>K^+>S > P > Na^+>Mg^{2+}>Cl^- > Si^{4+} (Table 1). It must be taken into account that both Cl^- and Na^+, which are classified as micronutrients, were found at levels similar to that observed for macronutrients in tomato plants under our experimental conditions. In addition, Cl^- have been classified as an essential micronutrient in plants (Broyer et al., 1954).

The crop management strategies applied induced changes in the mineral nutrient contents in both tomato leaves and roots. In that regards, under intercropping (T_H) and sequential cropping (T_S) conditions, tomato leaves contained more P, Mg^{2+}, Na^+, NO_3^- and SO_4^{2-} than under monoculture (Tables 1 and 2). Moreover, T_S also increased the content of the macronutrients K^+ and S (Table 1). Regarding micronutrient contents, both management conditions led to a lower content in Fe, Al^{+3}, Cu^{2+} and Pb, whereas T_S showed increased Mn^{2+} levels (Table 3). The translocation factor (TF) from root to leaves showed that T_H and T_S leaves had higher potential to accumulate Na^+ than T_M leaves

Table 1
Effect of the halophyte-based crop management on the macronutrient contents (mg/Kg dry weight) in tomato plants and soil.

		Macronutrients						
		K	Ca	P	Mg	S	Na	Si
Leaf	T_M	17,633 ± 415b	43,975 ± 792 ab	3722 ± 98c	2383 ± 96b	5864 ± 432b	3162 ± 115b	351 ± 14
	T_H	18,296 ± 590b	42,749 ± 1076b	4370 ± 100b	2998 ± 137a	6356 ± 204b	4270 ± 139a	353 ± 6
	T_S	21,449 ± 436a	46,908 ± 888a	6163 ± 198a	2940 ± 189a	7750 ± 238a	3918 ± 320a	372 ± 30
Root	T_M	18,169 ± 766	20,181 ± 690a	3440 ± 169	1657 ± 66	2157 ± 90a	7258 ± 247a	257 ± 13c
	T_H	18,709 ± 593	16,419 ± 918b	3066 ± 199	1465 ± 59	1866 ± 63b	5538 ± 292b	394 ± 25b
	T_S	18,780 ± 890	18,868 ± 669 ab	3219 ± 160	1789 ± 157	1679 ± 62b	3689 ± 281c	498 ± 38a
Soil	T_M	11,290 ± 366a	29,666 ± 2524	6017 ± 597a	8242 ± 429 ab	8299 ± 1146	6116 ± 498	380 ± 24
	T_H	9575 ± 134b	28,338 ± 2393	6109 ± 584a	6921 ± 339 b	4850 ± 997	5625 ± 577	345 ± 7
	T_S	11,779 ± 558a	28,310 ± 2467	3315 ± 207b	9367 ± 869a	7470 ± 547	5350 ± 638	341 ± 18

Data represent the mean values ± SE of at least 4 different biological samples. Different letters indicate significant differences according the Tukey's Multiple Range Test ($p \leq 0.05$). T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

Table 2
Effect of the halophyte-based crop management on the anion contents (mg/Kg dry weight) in tomato plants and soil.

		Anions			
		Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Leaf	T_M	2129 ± 43	466 ± 40b	609 ± 48	1157 ± 68.0b
	T_H	2421 ± 116	671 ± 35a	552 ± 22	1471 ± 66a
	T_S	2294 ± 137	620 ± 27a	618 ± 37	1522 ± 106a
Root	T_M	1290 ± 26b	1775 ± 39a	1386 ± 24b	442 ± 11a
	T_H	1434 ± 15a	1815 ± 32a	1506 ± 60 ab	408 ± 6a
	T_S	1055 ± 13c	1552 ± 81b	1533 ± 28a	328 ± 26b
Soil	T_M	840 ± 44a	958 ± 78	54.4 ± 1.0a	1529 ± 238
	T_H	622 ± 55b	1010 ± 151	15.1 ± 1.94b	1117 ± 399
	T_S	486 ± 48b	581 ± 70	17.9 ± 0.7b	1754 ± 228

Data represent the mean values ± SE of at least 4 different biological samples. Different letters indicate significant differences according the Tukey's Multiple Range Test ($p \leq 0.05$). T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

(Supplementary Tables 1–3). Regarding the heavy metals Al^{3+} and Pb, the values of TF for T_H and T_S in roots showed a higher potential to accumulate them than in T_M, avoiding its transport to the leaves. In addition, T_H leaves accumulated more Mg^{2+}, S^{2+}, Mn^{2+}, NO_3^- and SO_4^{2-} than T_M leaves. On the other hand, T_S leaves accumulated more S, Cl^- and SO_4^{2-} than the other crop managements. Interestingly, T_H and T_S leaves generally accumulated lower heavy metals content than T_M leaves, especially Al^{3+}, Zn^{2+}, Cu and Pb.

In roots, lower Ca^{2+}, S and Na^+ as well as higher Si^{+4} contents were observed in T_S and T_H with respect to monoculture condition (Table 1). It is worth noting the 49% decrease of Na^+ observed in T_S when compared with T_M (Table 1). Regarding anion contents in roots, the intercropping only affected significantly the content in Cl^-, which slightly increased (Table 2). Major differences were observed in roots of T_S, which displayed lower content of Cl^-, NO_3^- and SO_4^{2-} as well as increased levels of PO_4^{3-} (Table 2). Concerning root micronutrients, T_H led to increased contents of Al^{3+}, Cu and Pb, whereas T_S roots accumulated more Fe, Al^{3+}, Cu, Pb and Ni^{+2} (Table 3).

In the soil, intercropping led to reduced contents of K^+ and Mg^{+2}, although no differences in micronutrient contents were observed (Tables 1 and 3). Both T_S and T_H showed reduced contents of Cl^- and PO_4^{3-}, the decrease in Cl^- being more noticeable in T_S (Table 2). Finally, strong decreases in P, Zn^{+2}, Cu and Pb were recorded in soils associated to sequential cropping (Tables 1 and 3).

From the total contents of Na^+, Ca^{2+} and Mg^{2+} obtained by acid digestions of soil samples, SAR values were calculated, observing a decline associated to intercropping [8.52 (mmol Kg)$^{1/2}$] and sequential cropping [10.31 (mmol Kg)$^{1/2}$] in comparison to T_M [12.50 (mmol Kg)$^{1/2}$]. The soil pH did not change significantly, neither with time nor among crop practices (data not shown). With respect to EC, a significant reduction occurred in all crop managements with respect to the initial values. Moreover, EC in T_S soils (4.31 dS m^{-1}) decreased in relation to T_M (5.28 dS m^{-1}) but not in relation to T_H soils (4.53 dS m^{-1}). These results correlate with lower Cl^- contents and SAR values observed in the soils.

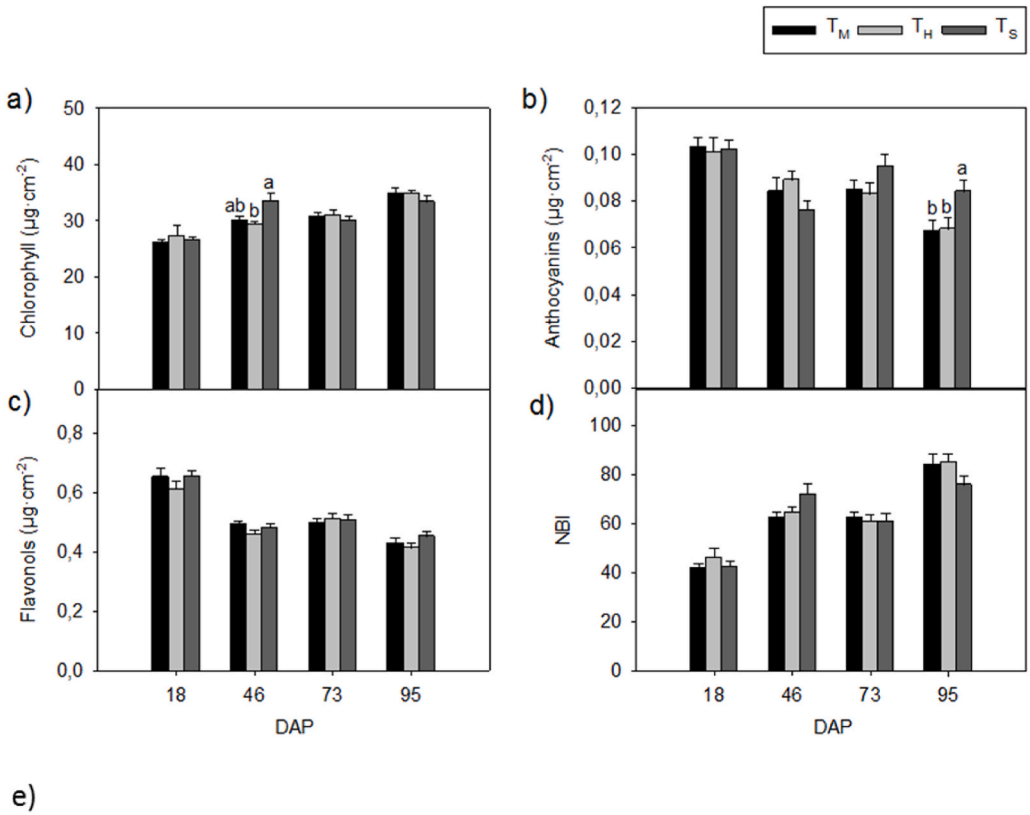
3.2. *Effect of crop management on the levels of chlorophyll, flavonols, anthocyanins and NBI*

No significant differences among the crop managements were observed in chlorophyll, flavonols and anthocyanins levels, or in NBI (Fig. 2). However, significant changes in these variables were observed during the evolution of the experiment (variable "Time"), and a significant interaction ("Crop management" X "Time") occurred for the anthocyanin levels (Fig. 2). Chlorophyll levels increased with time, reaching a maximum level at 95 DAP, whereas the levels of flavonoids and anthocyanins followed the opposite trend, displaying a progressive decrease with time (Fig. 2). As a consequence of increased chlorophyll and decreased flavonols over time, a progressive increase in the NBI was

Table 3
Effect of the halophyte-based crop management on the micronutrient contents (mg/Kg dry weight) in tomato plants and soil.

		MICRONUTRIENTS							
		Mn	Fe	Al	Zn	B	Cu	Pb	Ni
LEAF	T_M	48.5 ± 1.1b	236 ± 17a	309 ± 12a	11.7 ± 0.4	61.6 ± 2.6	61.0 ± 3.3a	1.12 ± 0.01a	1.64 ± 0.26
	T_H	49.2 ± 0.8b	142 ± 5b	151 ± 2b	10.9 ± 0.6	66.6 ± 1.3	45.9 ± 1.1b	0.69 ± 0.02b	1.78 ± 0.38
	T_S	56.3 ± 1.0a	143 ± 11b	129 ± 12b	11.4 ± 0.4	56.0 ± 5.2	46.1 ± 1.1b	0.77 ± 0.05b	1.06 ± 0.14
ROOT	T_M	35.2 ± 1.4 ab	206 ± 10b	179 ± 11c	46.0 ± 2.5	nd	17.0 ± 0.9b	0.76 ± 0.03c	1.94 ± 0.27b
	T_H	29.0 ± 1.6b	280 ± 21 ab	324 ± 25b	56.3 ± 5.3	nd	37.3 ± 2.9a	1.06 ± 0.04b	1.91 ± 0.25b
	T_S	38.6 ± 2.9a	319 ± 26a	452 ± 48a	59.6 ± 4.3	nd	22.0 ± 1.8b	1.43 ± 0.09a	3.66 ± 0.30a
SOIL	T_M	487 ± 5	32,189 ± 837	34,180 ± 787	342 ± 18a	30.7 ± 2.0	128 ± 10a	116 ± 5a	27.7 ± 0.15
	T_H	477 ± 6	28,097 ± 1568	32,126 ± 1597	306 ± 16a	26.3 ± 0.8	105 ± 4a	111 ± 5a	25.8 ± 1.2
	T_S	468 ± 31	29,273 ± 1446	37,820 ± 2262	219 ± 7b	26.5 ± 1.7	67 ± 5b	55.8 ± 5.4b	26.6 ± 1.2

Data represent the mean values ± SE of at least 4 different biological samples. Different letters indicate significant differences according the Tukey's Multiple Range Test ($p \leq 0.05$). nd, not detected. T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.



e)

	Chlorophyll	Flavonols	Anthocyanins	NBI
Crop management	0.879	0.242	0.33	0.803
Time	<0.001	<0.001	<0.001	<0.001
Crop management x Time	0.069	0.846	0.032	0.1

Fig. 2. Effect of the halophyte-based crop management on the contents of chlorophyll (a), anthocyanins (b) and flavonols (c), and in the nitrogen balance index (NBI) (d) in leaves from tomato plants grown in a moderately saline soil. Measurements were performed at 18, 46, 73 and 95 days after planting. e) p-values for two-way ANOVA test are showed. Data represent the mean values ± SE of at least 15 different biological samples. Different letters indicate significant differences according to the Tukey's Multiple Range Test ($p \leq 0.05$). T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

observed, reaching its maximum value at 95 DAP (Fig. 2).

3.3. Effect of crop management on chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were analyzed at 73 DAP. T_H plants displayed a slight decrease in Fv/Fm with respect to T_M and T_S leaves, accompanied by a strong increase in the non-photochemical quenching parameters qN and NPQ of about 33% and 88%, respectively (Fig. 3). However, in T_S plants, chlorophyll fluorescence parameters did not show significant changes in relation to monoculture, and only a significant decline in ETR occurred (Fig. 3).

3.4. Effect of crop management on the antioxidant metabolism

The effect of the crop management on the levels of POX, CAT, SOD and ASC-GSH cycle (APX, MDHAR, DHAR and GR) enzymes was analyzed in tomato leaves at 73 DAP. Fig. 4 shows the enzymatic antioxidant activities displaying significant differences. Regarding H_2O_2-scavenging enzymes, both T_H and T_S crop managements increased the APX activity, while a 26% increase in POX activity was recorded in T_S compared to T_H (Fig. 4). It is interesting to note that tomato plants mainly use the MDHAR activity for ASC recycling (Table 4), which is more efficient from an energetic point of view than the use of DHAR activity. In this sense, sequential cropping increased MDHAR activity, whereas DHAR decreased it in T_S and T_H (Fig. 4). Moreover, under T_S and T_H, the contribution of MDHAR to ascorbate recycling increased to 84–85%, while the involvement of DHAR activity declined to 15–16% (Table 4). Furthermore, GR activity, involved in GSH recycling, increased by 28% in T_S (Fig. 4). Finally, a 26% increase in POX activity was recorded in T_S compared to T_H (Fig. 4).

Histochemical staining of superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) was also performed at 73 DAP. An accumulation of $O_2^{\cdot-}$ and H_2O_2 in tomato leaves near the minor veins was observed in all crop managements, being more noticeable in T_H and T_S (Fig. 5). Taking into account the observed increase in APX activity and the more efficient ASC recycling activity in tomato leaves from T_H and T_S crop managements (Fig. 4 and Table 4), overall, our result suggests the establishment of a moderate oxidative stress, which in turn could trigger adaptation mechanisms.

3.5. Effect of crop management on plant production and quality

The total tomato production for the three consecutive harvests conducted reflected a statistically higher production, in terms of Kg of tomato/plant, under sequential cropping conditions (27% compared to monoculture) than in the other crop managements (Fig. 6A). This result

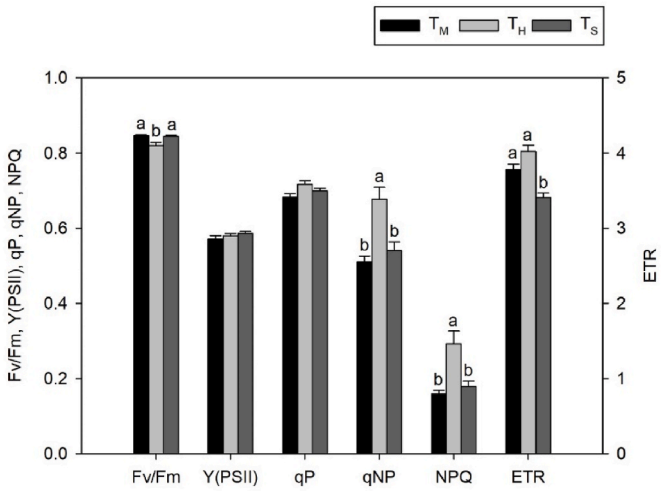


Fig. 3. Effect of the halophyte-based crop management on chlorophyll fluorescence parameters and electron transport rate (ETR) in leaves from tomato plants grown in a moderately saline soil. Measurements were performed at 73 days after planting. Data represent the mean values ± SE of at least 10 different biological samples. Different letters indicate significant differences according the Tukey's Multiple Range Test ($p \leq 0.05$). T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

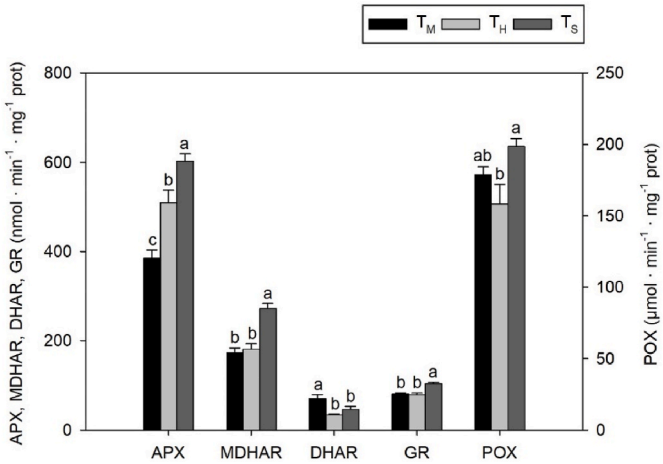


Fig. 4. Effect of the halophyte-based crop management on antioxidants enzymes in leaves from tomato plants grown in a moderately saline soil. Measurements were performed at 73 days after planting. Data represent the mean values ± SE of at least 5 different biological samples. Different letters indicate significant differences according the Tukey's Multiple Range Test ($p \leq 0.05$). T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

Table 4
Effect of the halophyte-based crop management on reduced ascorbate (ASC) recycling activity in leaves from tomato plants grown in a moderately saline soil. The values of the MDHAR and DHAR activities, responsible for ASC recycling, have been added and the percentage that each enzyme represents in the recycling capacity of the ASC has been calculated. Total ASC recycling activity is expressed in nmol min^{-1} mg^{-1} proteins.

	Total ASC recycling activity	% MDHAR	% DHAR
T_M	245	71	29
T_H	217	84	16
T_S	320	85	15

T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

was parallel with a 18% increase in the number of fruits/plant under T_S (Fig. 6B). Likewise, T_S significantly decreased the content of TSS in relation to the other crop managements (Fig. 6C). Regarding fruit acidity, none of the crop managements had an effect on this variable (Fig. 6D).

4. Discussion

In recent years, a growing body of research has dealt with phytoremediation of contaminated environments. While studies evaluating the use of halophytic plants in intercropping systems are well known, there is less information on the use of halophytic plants to cope with salinity in sequential cropping systems (Barcia-Piedras et al., 2019; Muchate et al., 2018; Rabhi et al., 2010). These strategies have potential for stress mitigation in areas where salt limits crop production. Halophytes can benefit salt-affected areas due to their ability to reduce salt contents, and therefore EC, in soils (Simpson et al., 2018), emerging as alternative cash crops and restoring biodiversity. Under our experimental conditions, decreases in EC and SAR were observed by the crop managements involving halophytes.

In previous research, tomato plants have been grown in intercropping with different halophyte plants, with contrasting results. The presence of the halophyte *S. salsa* L. as companion plant had no significant effects on tomato plant performance measured as shoots dry weight and fruit production (Albaho and Green, 2000). In intercropping experiments of tomato plants, the presence of the halophyte *P. oleracea* L. led to increases in plant growth and fruit production, whereas intercropping in the presence of *S. salsa* had negative effects in tomato plants in terms of plant growth and fruit yield, in relation to the saline control (Zuccarini, 2008). In other experiments, Karakas et al. (2016) reported that intercropping with *P. oleracea* or with *S. salsa* did not improved the salt-induced negative effects on tomato plant growth and fruit yield. However, under excess salinity conditions (EC 14–15 dS m^{-1}), the presence of *S. salsa* plants reduced the tomato plant susceptibility to salinity, improving the plant dry matter and fruit yield (Karakas et al., 2016). These results contrast with previous results described by Graifenberg et al. (2003), who reported that the presence of the same halophyte plants reduced the negative effect of salinity on tomato yield and growth.

In this study, both T_S and T_H led to enhanced leaf Na^+ accumulation while decreased Na^+ contents in the root (Table 1). Typically, glycophytes and halophytes show a deficit or imbalance of leaf nutrients due to competition with Na^+ and Cl^- (Ehtaiwesh, 2022; Flowers and Colmer, 2008). However, under our experimental conditions, Na^+ accumulation in leaves was accompanied by maintenance or increase in the contents of the other macronutrients analyzed, including K^+. On the other hand, the lower Na^+ ratio root/leaves found in the presence of the halophyte (T_H = 1.30; T_S = 0.94) compared with the monoculture (T_M = 2.29) indicates that there is a greater Na^+ translocation factor from root to leaves in T_H and T_S than in T_M (Supp. Table 1). In turn, this could be related to a higher Na^+ compartmentalization capacity in the vacuoles of T_H and T_S plants, facilitating osmotic adjustment (Acosta-Motos et al., 2017). Moreover, Na^+ shoot accumulation has been related to salt stress tolerance in plants (Liu et al., 2020; Venkataraman et al., 2021). In addition, mineral nutrient profile can be influenced by the growing season; in this regard, intercropping tomato with *S. salsa* plants led to decreased leaf Na^+ content in spring season whereas Na^+ levels remained unchanged in autumn trials (Albaho and Green, 2000). Our experiment was carried out in the autumn/winter season, and results regarding the effect of intercropping on leaf Na^+ contents were somewhat different from those found in the spring/summer season. In fact, in spring/summer trials we reported a decrease in Na^+ and Cl^- levels in tomato leaves (Jurado-Mañogil et al., 2023). In contrast to our results, intercropping tomato plants with the halophyte *P. oleracea* L. reduced Na^+ uptake and increased P and Ca^{2+} contents in the leaves (Graifenberg et al., 2003). Moreover, intercropping of tomato plants with the

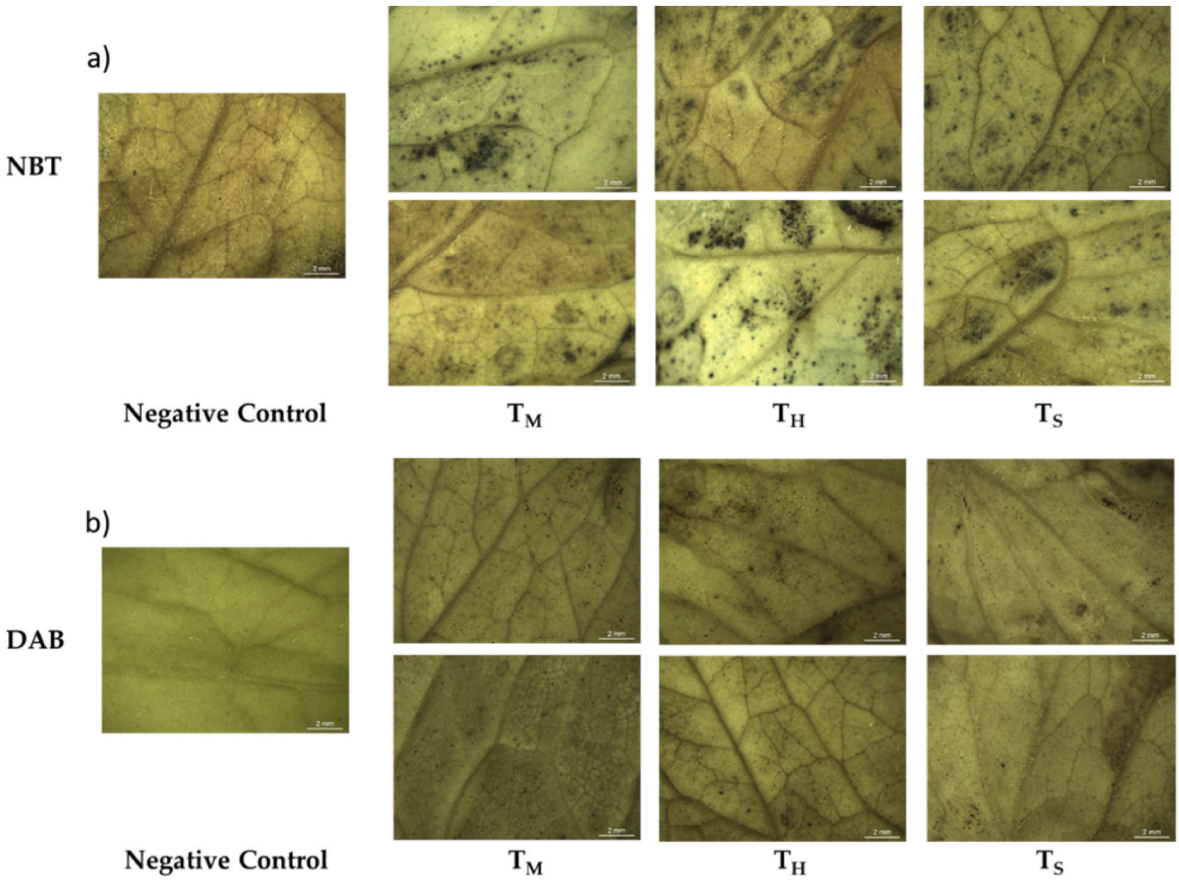


Fig. 5. Effect of the halophyte-based crop management on the accumulation of superoxide radical ($O_2^{\cdot}$) (a); and hydrogen peroxide (H_2O_2) (b), in leaves from tomato plants grown in a moderately saline soil. T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

halophytes *Salsola soda* L. or *P. oleracea* L. led to decreased Na^+ in leaves and roots, while improved the uptake of K^+ and Mg^{2+} in both organs as well as of Ca^{2+} in leaves (Karakas et al., 2016).

Regarding Cl^-, soils associated to T_H and T_S plants displayed lower content which, in the case of T_H plants, was reflected on higher Cl^- accumulation in the root than for T_M and T_S plants (Table 2). In contrast, Karakas et al. (2016) reported lower Cl^- accumulation in tomato roots in intercropping with halophytes. A decline in Na^+ and Cl^- levels was also reported in tomato leaves and roots by intercropping with the halophytes *P. oleracea* L. or *Atriplex hortensis* L. (Zuccarini, 2008). In other plant species, the halophyte-based intercropping also produced a similar effect on toxic ions accumulation. At a moderate salt stress (4.0 dS m^{-1}), the presence of *S. soda* reduced Na^+ and Cl^- accumulation in pepper leaves (Colla et al., 2006). In a more recent work, co-cultivation of strawberry plants with *P. oleracea* L. reduced the accumulation of Na^+ and Cl^- in leaves when grown in the presence of 90 mM NaCl (Karakas et al., 2021). However, less information is available on the effect of sequential cropping on the growth and/or mineral nutrient content of plants of agronomic interest. The halophyte *Sesuvium portulacastrum* L. reduced electric conductivity and Na^+ content in the first 10 cm of a saline soil, which helped improving growth of *Hordeum vulgare* plants cultivated afterwards, while increased K^+ and decreased Na^+ levels in the shoot (Rabhi et al., 2010). More recently, *A. macrostachyum* L. grown in a saline soil under non leaching conditions was able to decrease soil salinity by 31% after 30 days of cultivation, favouring the seed germination rate of glycophyte species such as barley and wheat (Barcia-Piedras et al., 2019). In addition, it is noteworthy that under our experimental conditions both crop managements favored the accumulation of some heavy metals in roots, such as Al^{+3}, Pb or Ni^{2+}, thus limiting its transport to the leaves. This could be related to halophyte-induced alterations of soil microbiota and hence nutrient availability (Y. Wang et al., 2022).

Moreover, both T_H and T_S, especially the latter, showed increased Si^{4+} in the roots (Table 1). This increase could have a beneficial effect on the response of tomato plants under saline stress. In fact, it has been reported that Si^{4+} supplement improved tomato plant performance under saline conditions in terms of plant growth and photosynthesis protection, and alleviated salt-induced oxidative stress (Muneer et al., 2014). In this sense, under our experimental conditions T_S plants displayed the highest Si^{4+} content in both leaves and roots, as well as K^+, Ca^{2+}, P and S contents in leaves, which could be related to the increased productivity observed for this crop management (Fig. 5), and supports a beneficial effect of halophytes in improving plant mineral nutrition in tomato plants, especially in T_S plants.

The levels of chlorophyll, flavonoids, anthocyanins and the NBI was statistically equivalent among treatments. Our results contrast with the data observed in other plant species, where intercropping improved the chlorophyll *a* and chlorophyll *b* contents in relation to monoculture (Ghaffarian et al., 2020; Karakas et al., 2021). Likewise, increased chlorophylls and decreased flavonols over time led to a rise in NBI, which is used for assessing the N nutrition of crops in precision agriculture (as an indicator of the N content), as described in wheat plants (Cartelat et al., 2005).

To our knowledge, this is the first report showing the effect of intercropping and/or sequential cropping on the chlorophyll fluorescence parameters. Intercropping produced a slight decrease in Fv/Fm value (Fig. 3), which yet was above 0.8, considered a normal value for most C3 species (Hernandez et al., 2006; Hernández et al., 2004a,b; Karpinski et al., 1997), while values below 0.8 are usually observed in response to stress conditions, indicating a phenomenon of

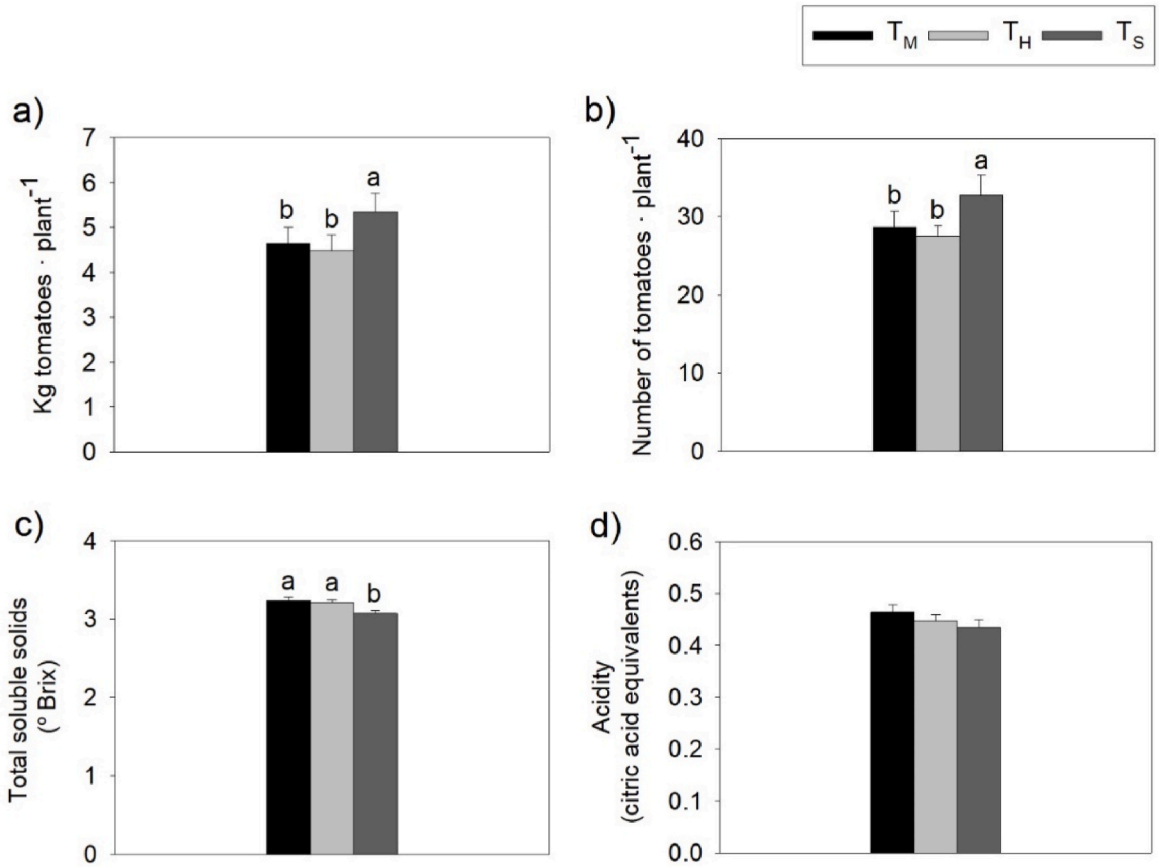


Fig. 6. Effect of the halophyte-based crop management on the production and quality of tomato fruits. a) Kg of tomato fruits per plant; b) number of tomatoes per plant; c) total soluble solids (TSS); d) acidity. T_M: tomato in monoculture; T_H: tomato in intercropping; T_S: tomato in sequential cropping.

photoinhibition (Hernández et al., 2004a,b; Hernandez et al., 2006; Karpinski et al., 1997). In fact, we have previously showed that sugar metabolism, which is closely linked to photosynthesis, is enhanced in T_H plants (Jurado-Mañogil et al., 2023). A possible season-dependent correlation between increased leaf Na^+ content and decreased Fv/Fm could also be proposed, since in a similar study, done in the summer season, tomato in intercropping displayed lower Na^+ content in the leaves with no changes in the Fv/Fm (Jurado-Mañogil et al., 2023). However, T_S leaves also presented an increased Na^+ content in leaves in relation to T_M plants, and no effects on Fv/Fm were observed. Taking into account the Fv/Fm values, the accumulation of Na^+ in T_H and T_S leaves did not affect the photosynthesis process, which suggests that Na^+ does not accumulate in chloroplasts. An opposite response was observed in leaves from salinity-sensitive plants, in which Na^+ accumulation in chloroplast was correlated with decreased PSII activity (Hernández et al., 1995). On the other hand, T_H leaves showed significant increases in NPQ and qN, reflecting a safer dissipation of excess energy as heat in the chloroplasts, avoiding a PSII damage in case of stress situation, whereas the photochemical quenching data [Y(PSII) and qP] were equivalent to that of T_M and T_S. Similar increases in the non-photochemical quenching parameters were also described in T_H plants during summer season (Jurado-Mañogil et al., 2023) and in other plant species, such as myrtle or Eugenia plants subjected to NaCl stress, this increase being related with a safe light energy excess dissipation (Acosta-Motos et al., 2015a,b).

Osmotic and ionic stresses caused by salinity are manifested as an oxidative stress mediated by reactive oxygen species (ROS) (Hernández et al., 2001). In this context, peroxidation of membrane lipids is an indicator of membrane damage and leakage under salinity stress (Hernández and Almansa, 2002). The levels of lipid peroxidation in tomato leaves did not show significant differences among agronomic managements (Supplementary Fig. 1). These results contrast with previous research in which intercropping with halophytes reduce lipid peroxidation levels in different plants (Ghaffarian et al., 2020; Karakas et al., 2016). In this sense, an inverse correlation between lipid peroxidation and thiamine diphosphate (vitamin B1) levels has been reported in tomato in intercropping conditions with *A. macrostachyum* (Jurado-Mañogil et al., 2023). This can be explained by a protective role of vitamin B1 against lipid peroxidation (Lukienko et al., 2000).

In all crop managements, an $O_2^{\cdot-}$ and H_2O_2 accumulation in leaf tissues localized near the minor veins was observed, similar to that described in leaves from pea plants treated with 90 mM NaCl (Hernández et al., 2001). In addition, in T_H and T_S changes in enzymes related to antioxidative metabolism were found, suggesting the establishment of a moderate oxidative stress in tomato plants induced by the presence of halophyte plants, either by intercropping or by sequential cropping managements, that could be involved in the described physiological and biochemical changes in tomato plants. Overall our result suggests the establishment of a controlled moderate oxidative stress which in turn could trigger adaptation mechanisms. This response could be linked with the increase in Na^+ levels detected in T_H and T_S leaves, since a correlation between leaf Na^+ accumulation and establishment of oxidative stress has been described (Hernández et al., 1995, 2001).

A common response observed to intercropping and sequential cropping was the increase in leaf APX activity in tomato in relation to monoculture, an enzyme responsible for the fine-tuning control of H_2O_2 levels in plant cells. In addition, sequential cropping increased MDHAR and GR activities, while reduced DHAR activity. As mentioned, in tomato leaves, the contribution of MDHAR activity for ASC recycling is higher to that of DHAR activity. In order to regenerate ASC, DHAR activity use GSH as a reducing power donor, and then NADPH is needed as

an electron donor to regenerate GSH via GR. Thus, the ASC recycling via MDHAR is energetically more efficient than via DHAR. Consequently, it can be suggested that T_H and T_S plants use less energy to enzymatically recycle ASC.

The increase in MDHAR activity as a salinity tolerance mechanism has also been observed in other plants such as *Eugenia myrtifolia* (Acosta-Motos et al., 2015a), *Myrtus communis* (Acosta-Motos et al., 2015b) and *Stevia rebaudiana* (Cantabella et al., 2017). Moreover, in *S. rebaudiana*, prolonged exposure to salinity led to a reduction in DHAR activity. On the other hand, the overexpression of a *MDHAR* gene from the halophytic mangrove *Avicennia marina* enhanced salt tolerance in transgenic tobacco plants, this response being correlated with increased APX and MDHAR activities as well as decreased DHAR activity (Kavitha et al., 2010).

The intercropping strategy had no effect on the production of tomato plants. In contrast, under sequential cropping conditions, the tomato production was statistically higher than in the other crop managements (Fig. 6A and B). These results differ from those described by other authors. Intercropping watermelon with *A. hortensis* L. plants increased yield, which correlated with a lower Cl^- accumulation (Simpson et al., 2018). In tomato plants, a yield increase of about 44% and increased fruit size and weight were recorded by intercropping with the halophytes *A. hortensis* L. or *P. oleracea* L. (Zuccarini, 2008). A similar result was reported in pepper plants when grown at a moderate salt stress in the presence of the halophyte *S. soda* L., which increased the total and marketable yield of fruits in comparison to those plants grown under monoculture conditions (Colla et al., 2006). In strawberry plants, fruit weight and yield decreased under salinity conditions, whereas the presence of *P. oleracea* increased the average and total fruit weight (Karakas et al., 2021).

Finally, we analyzed the effect of both crop managements on the acidity and TSS in tomato fruits, finding lower °Brix values in T_S fruits (Fig. 6C). The effect of intercropping and sequential cropping on tomato fruit quality was very scarce. In this sense, the growth of pepper plants with *S. soda* decreased °Brix in fruits (Colla et al., 2006). The co-culture of tomato plants with the halophytes *S. soda* or *P. oleracea* under salinity conditions produced in the fruit a decline in vitamin C content under high salinity (14.05 dS m^{-1} NaCl) and an increase in lycopene at medium salinity (7.22 dS m^{-1} NaCl) (Karakas et al., 2016). These authors suggested that intercropping may alleviate salt-induced stress, allowing tomato plants to use more energy in the biosynthesis of organic compounds, such as lycopene, rather than in the biosynthesis of defense-related compounds, such as vitamin C (Karakas et al., 2016).

Fig. 7 summarizes the main changes observed in the tomato plant in T_H and T_S conditions in comparison to tomato plants grown in monoculture.

5. Conclusions

Salinity in soils and irrigation water is one of the most important threats for agriculture in arid and semi-arid regions, as the Mediterranean basin. This study shows that both intercropping and sequential cropping between the halophyte *A. macrostachyum* and tomato plants enhanced crop performance while reduced soil SAR and EC values under moderately saline conditions. Sequential cropping improved nutrient homeostasis and triggered a mild oxidative stress in the tomato plant, which was reflected on an increased fruit production and a slight decrease in TSS, while intercropping enhanced the non-photochemical quenching parameters in tomato chloroplasts. This research supports the introduction of *A. macrostachyum* in the farming systems and highlights the sustainability of practices based on the cultivation of halophytes.

Contributions

JAH, PDV, GBE, conceptualized the idea of the experiment; JAH, PDV, GBE, JRAM, CJM conducted the experiment; JAH, PDV, GBE carried out the evaluation of the experiment; all the authors performed the statistical analysis of the experiment; JAH, CJM wrote the first version of the manuscript. All authors contributed in writing corrections

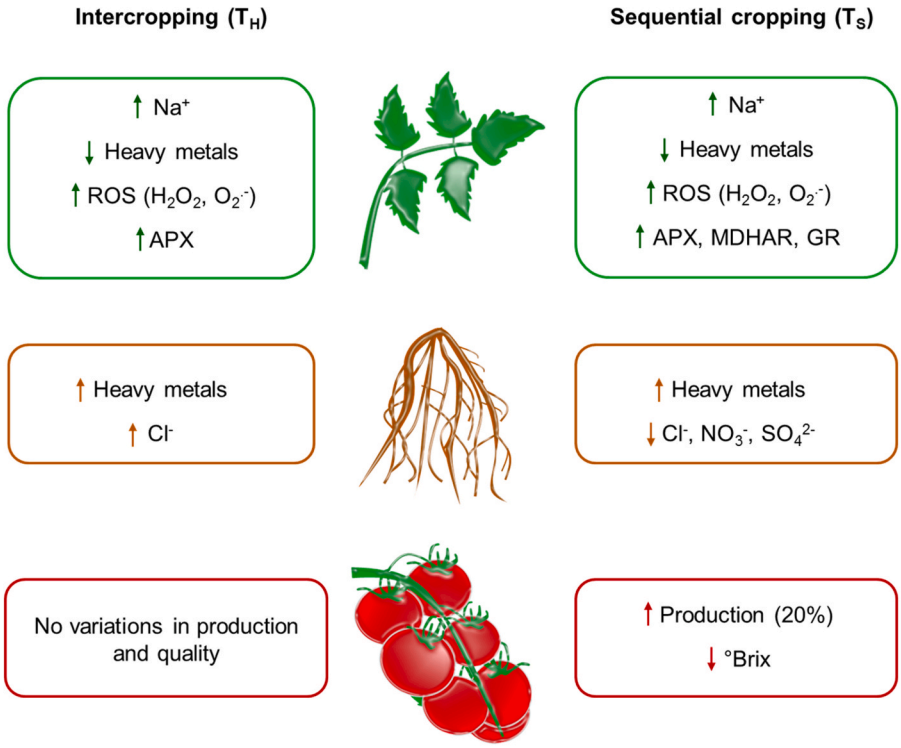


Fig. 7. Principal changes in tomato leaf, root and fruit in intercropping (TH) and sequential cropping (TS) conditions in comparison to tomato plants grown in monoculture.

to the manuscript. All authors revised and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2023.108228.

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