Environmental and Experimental Botany 228 (2024) 106013

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Intercropping salt-sensitive *Solanum lycopersicum* L. and salt-tolerant *Arthrocaulon macrostachyum* in salt-affected agricultural soil under open field conditions: Physiological, hormonal, metabolic and agronomic responses

Tarek Slatni[a,b,*,1], Aida Selmi[a], Nesrine Kalboussi[c], Hassène Zemni[d], Adel Echadly[e], Gregorio Barba Espin[f], José Antonio Hernandez[f], Hamza Elfil[c], Luísa Custódio[g], Tiago Braga[g], Pedro Diaz-Vivancos[f], Karim Ben Hamed[a]

[a] *Laboratory of Extremophile Plants (LPE), Centre of Biotechnology of Borj Cedria (CBBC) BP 901, Hammam-Lif 2050, Tunisia*
[b] *Faculty of Sciences of Tunis (FST), University of Tunis El Manar (UTM), Tunis 1060, Tunisia*
[c] *Laboratory of Natural Water Treatment (LADVEN), Water Researches and Technologies Center, Techno-Park Borj-Cedria, University of Carthage, BP 273, Soliman 8020, Tunisia*
[d] *Laboratory of Plant Molecular Physiology (LPMP), Centre of Biotechnology of Borj Cedria (CBBC) BP 901, Hammam-Lif 2050, Tunisia*
[e] *AGRILAP, SUARL, Manar I 2092, Tunisia*
[f] *Group of Fruit Trees Biotechnology, Department of Plant Breeding, CEBAS-CSIC, Murcia, Spain*
[g] *Centre of Marine Sciences, Faculty of Sciences and Technology, University of Algarve, Ed. 7, Campus of Gambelas, Faro 8005-139, Portugal*

ARTICLE INFO

Keywords:
Arthrocaulon macrostachyum
Intercropping
Salt-affected soil
Tomato
Yield

ABSTRACT

Salinity is one of the important environmental risks affecting agricultural production in the world. Under this condition and with the conventional cultivation methods, glycophyte plants, like tomato, are subjected to many stresses, such as ion toxicity, osmotic stress, nutritional disturbance, oxidative damage and metabolic disorders, which cause growth inhibition and yield reduction. In this context, the main objective of our study was to compare the physiological, hormonal, metabolic and agronomic responses of tomato plants (*Solanum lycopersicum* L.) grown in monoculture (TM) or intercropping (TH) with the halophytic species *Arthrocaulon macrostachyum* in a salt affected soil. The results showed that the intercropping system (TH) reduced the soil electrical conductivity, and Na^+ and Cl^- contents, improving mineral nutrition in tomato plants compared to TM. In addition, TH decreased the osmotic stress, improved water potential and increased water use efficiency in tomato plants, whereas the integrity of gas exchange parameters were maintained; as a consequence, an increase in tomato yield was achieved. Moreover, the ratio of stress hormones (ABA, SA and JA) to growth regulating hormones (GA, auxins and cytokinins) decreased under TH. Metabolomic analysis showed clear defined patterns of differentially accumulated metabolites. Some of the metabolites with higher abundance in TH were linked to phenylpropanoid biosynthesis and phenylalanine metabolism, whereas alanine, aspartate and glutamate metabolism, monoterpenoid biosynthesis and butanoate metabolism pathways were downregulated. Our results support the importance of *A. macrostachyum* in the desalination of salt-affected soils and in the improvement of tomato yield in mixed culture. Indeed, this intercropping system offers farmers a low-cost biosolution that improves yields while respecting the environment.

1. Introduction

Global agriculture faces environmental constraints such as climate change, soil erosion, salinization and water shortages. In fact, this imbalance in food security remains a major challenge for the world, especially in the Mediterranean region. Globally, more than 8.7 % of the

---

* Correspondence to: Department of Biology, Faculty of Sciences of Tunis (FST), University of Tunis El Manar (UTM), Tunis 1060, Tunisia.
E-mail address: tarek.slatni@fst.utm.tn (T. Slatni).

[1] Orcid.org/0000–0002-0937–440X

https://doi.org/10.1016/j.envexpbot.2024.106013
Received 28 April 2024; Received in revised form 30 September 2024; Accepted 16 October 2024

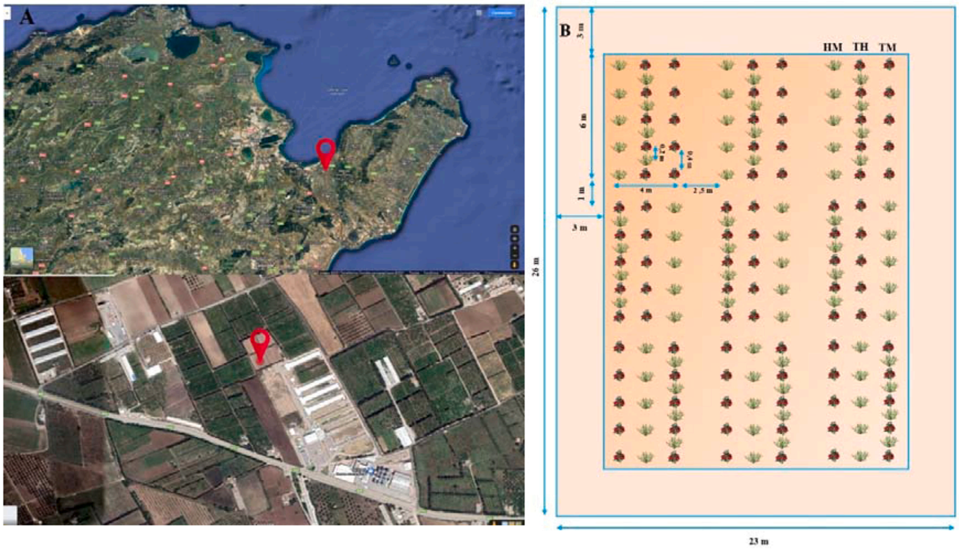

Fig. 1. A: Demonstration site in Tunisia (frontal and lateral view of the field) and B: field experiment design. HM: *A. macrostachyum* plants in monoculture, TH: tomato and halophyte plants in intercropping, TM: tomato in monoculture.

land area is affected by salinization (FAO, 2011), and salt stress affects about 20 % of the world's arable land (Negacz et al., 2019). It is estimated that by 2050, 50 % of the world's irrigated land will be salinized (Wang et al., 2022a, 2022b).

Most of the arable land in Mediterranean countries is located in arid and semi-arid areas, where soil and water salinization, water shortages and lack of soil nutrients are the biggest constraints to food and feed production. In Tunisia, 10 % of the total area and 20 % of irrigated area are salinized (Ben Hamed et al., 2021). This situation is exacerbated by intensive cultivation and excessive use of mineral fertilizers, which lead to soil and water pollution. Furthermore, in arid or semi-arid climates, fresh water is an increasingly scarce agricultural resource due to the increasing salinization of available water and the increasing demand of water for urban and industrial use.

Moreover, smallholder farmers have limited knowledge of how to improve traditional agriculture under drought and saline conditions. In fact, most crops used in traditional agriculture are sensitive to salt. In contrast, halophytes can grow in conditions where other glycophytes cannot, particularly in farming systems that utilize saline irrigation water resources and/or saline soils. Despite recent interest in the commercial potential of halophytes, doubts remain about their suitability as replacement or feed crops.

There is little scientific literature on large-scale halophyte production and no cultivation management options as for conventional glycophyte plants. In this sense, there is a need to develop economically, socially and ecologically viable halophyte production systems. It is also important to optimize and make available to farmers innovative production and cultivation systems, that rely on these halophyte crop, addressing soil and water salinization issues while contributing to food security and environmental, economic and social sustainability.

To cope with climate change, farmers must develop new agricultural systems that increase food production and build resilience to extreme environmental conditions. In fact, sustainable agriculture requires a strategic approach than simplifying production systems (monoculture). For that, intercropping can strengthen and stabilize agricultural ecosystems in the context of climate change and environmental constraints by improving resource efficiency, enhancing soil water holding capacity, and increasing tolerance to biotic and abiotic constraints. This system represents a low-cost ecological solution for farmers. This work is particularly important because, on the one hand, it aims to address global agricultural challenges by proposing nature-based solutions to stop soil erosion or convert low-value lands, diversify cropping to grow a range of traditional food crops, traditional cash crops and new high-value crops, and contribute to the conservation of global biodiversity and natural resources. On the other hand, it aims to understand the mechanisms of response of tomato to salt stress under this halophyte-based intercropping system

In this context, the present work aims to optimise sustainable and profitable agricultural and production systems, able to cope with soil and water salinization through the use of halophytes. In this way, we studied the responses of tomato plants grown in monoculture or intercropped with *A. macrostachyum* plants in salt-affected agricultural soils at the physiological, hormonal, metabolic and agronomic levels. The use of these halophytes will reduce salinity in the rhizosphere of sensitive plants and increase their yield (Grafienberg et al., 2003; Ashraf et al., 2010). This phytoremediation/ phytodesalination approach is considered a novel and cost-effective biological approach to control soil and water salinization while increasing the productivity of salt-sensitive crops such as tomato (Zuccarini, 2008; Karakas et al., 2016; Jurado et al., 2024). It has been demonstrated that the use of halophytes species in crop rotations or intercropping increases the productivity of glycophyte species. This rise was linked to the halophyte biomass's removal of salt from salt-affected soils (Hasanuzzaman et al., 2014; Agnihotri and Kumar, 2015). Our study's primary goal was to determine how *A. macrostachyum* based intercropping system improve the physiological, hormonal, metabolic and agronomic responses of tomato to salt stress.

2. Materials and methods

2.1. Field experiment and crop management

The experimental field design consisted of three types of plots arranged in a randomised block design with three replications repeated three times an area of approximately 600 m^2 (Fig. 1): the first contained *A. macrostachyum* plants in monoculture (HM), the second contained tomato (var. Sabra) and halophyte plants in intercropping (TH), and the third contained tomato in monoculture (TM). Each plot consisted of a 6 m length row with 15 plants (40 cm apart). Tomato plants were obtained from a Tunisian nursery and transplanted in mid-March, whereas the halophyte plants were obtained from a CBBC nursery and transplanted at the same time. The plants were grown on a farm located in the Soliman region, northeast Tunisia, position N43°40'30.7", E10° 18' 38.6" (Fig. 1). The aerial part of the plants was harvested for the different analysis, during the vegetative period.

2.2. Mineral content

Aerial parts of plants were washed with tap water and distilled water and then dried in an oven at 65 ºC. Dried material was ground to a fine powder. In sterile vials, 25 mg of leaves powder was mixed with 10 ml of H_2SO_4 and incubated one hour in a water bath at 80 °C. Then, these vials were left to precipitate for 24 hours. The supernatant obtained was then filtered using sterile filter paper, the pellet removed and the filtrate retained for assay (Zorrig et al., 2010).

2.3. Gas exchange measurements

Gas exchange parameters, including the net CO_2 assimilation rate (A), leaf transpiration (E), stomatal conductance (gs), internal CO_2 concentration (Ci), instantaneous water use efficiency (WUE = A/E) and intrinsic water use efficiency (iWUE = A/gs) were determined using a portable photosynthetic meter (CI-430, CID Bio-Science, WA, USA). Measurements were carried out in the morning between 10:00 and 12:00, on mature leaves. These parameters were registered at saturating light (Faize et al., 2011; Liang et al., 2023; Idoudi et al., 2024).

2.4. Chlorophyll content

Chlorophyll levels were measured on young and mature leaves with a portable SPAD chlorophyll meter (SPAD-502Plus KONICA MINOLTA). SPAD stands for Soil and Plant Analyze Developments. The SPAD chlorophyll meter was used for a fast, precise and non-destructive estimation of chlorophyll levels in leaves (Shah et al., 2017).

2.5. Leaf water potential and water content

Leaf water potential (Ψw) was measured using a pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA, USA) (Scholander et al., 1965) on five fully expanded young leaves per treatment.

2.6. Total phenolic (TPC), flavonoid (TFC) and tannin contents (TTC)

To prepare ethanol extracts, dried biomass from tomatoes leaves were mixed with 96 % ethanol (1:40 w/v), stirred continuously overnight at room temperature (RT, approximately 25°C). The mixture was subsequently filtered, and the ethanol was evaporated using a rotary evaporator under controlled conditions. The resultant dry extract was redissolved in ethanol to achieve a 10 mg/ml concentration and stored at 4°C until use in the determination of secondary metabolites, and antioxidant properties.

TPC was determined by the Folin–Ciocalteu method (Velioglu et al., 1998), and expressed as gallic acid equivalents (GAE) in milligrams per gram of extract (dry weight, DW). TFC was estimated by the aluminium chloride ($AlCl_3$) colorimetric method adapted to 96-well microplates (Zou et al., 2011) and expressed as rutin equivalents (RE) in milligrams per gram of DW. TTC was analyzed by the 4- dimethylaminocinnamaldehyde- hydrochloric acid (DMACA–HCl) colorimetric method (Li et al., 1996) adapted to 96-well microplates and expressed as catechin equivalents (CE) in milligrams per gram of DW.

2.7. Determination of in vitro antioxidant activity

The *in vitro* antioxidant properties of the extracts were evaluated on samples at the concentration of 10 mg/ml. Radical scavenging activity (RSA) was evaluated against 1-diphenyl-2-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995) and 2,2′-azino-bis(3-ethylbenzthiazoline-6- sulphonic acid (ABTS) radicals (Wang et al., 2008). The absorbance was measured at 517 nm and RSA were expressed as percent inhibition relative to a blank containing methanol.

The ferric reducing antioxidant power (FRAP) was assayed by the method of Oyaizu (1986) modified by Megías et al. (2009). Samples (50 µl), distilled water (50 µl) and 1 % potassium ferricyanide (50 µl) were mixed and incubated at 50 °C for 20 min. Then, 50 µl of 10 % trichloroacetic acid (*w/v*) and ferric chloride solution (0.1 %, *w/v*) were added, and absorbance was measured at 700 nm (EZ read 400, Biochrom, Cambridge, UK).

The iron chelating activity (ICA) was determined by measuring the formation of the Fe^{2+} ferrozine complex according to Megías et al. (2009). Thirty µl of the samples were mixed with 200 µl of distilled water and 30 µL of a $FeCl_2$ solution (0.1 mg/ml in water) in 96- well microplates. After 30 min, 12.5 µl of ferrozine solution (40 mM in water) was added. Absorbance was measured at 562 nm using a microplate reader (EZ read 400, Biochrom). EDTA (1 mg/ml) was used as the positive control. Increased absorbance of the reaction mixture indicated increased reducing power. Results were expressed as percentage of inhibition, relative to the positive control (FRAP) and to the negative control (ICA).

2.8. Analysis of plant hormones

Phytohormones including gibberellins, cytokinins, auxines, abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) were analyzed in leaves. Approximately 50 mg of leaf samples were homogenized in liquid nitrogen and incubated in 1 ml of cold (–20 °C) extraction mixture (methanol/water, 80/20 *v/v*). The extraction procedure was performed as previously described (Albacete et al., 2008; Jurado-Mañogil et al., 2024) and phytohormones were determined using a U-HPLC-HRMS system comprising an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific) with a heated electrospray ionisation interface. Xcalibur software version 4.3 (ThermoFisher Scientific) was used to obtain mass spectra and for each analysed component calibration curves (1, 10, 50, and 100 µg l^{-1}) were constructed (Jurado-Mañogil et al., 2024).

2.9. Metabolomic analysis

Plant material consisting in mature tomato leaves was snap-frozen in liquid nitrogen and later freeze-dried. Four samples of both tomato in monoculture (TM) and in intercropping with the halophyte (TH) were analysed. For the extraction, 70–90 mg of freeze-dried material was homogenised with 50 % methanol (HPLC grade, Sigma-Aldrich) in a ratio of 1/20 (w/v) and then extracts were vigorously vortexed, centrifuged for 10 min at 13,500× g and the supernatants filtered through PTFE 0.45 µm filters (Agilent Technologies). Ultra-performance liquid chromatography–quadrupole-time-of-flight mass spectrometry (UPLC–QToF–MS/MS) and MS data acquisition was performed at the Metabolomics Platform at CEBAS-CSIC, Murcia, Spain (Jurado-Mañogil et al., 2023; Barba-Espin et al., 2024).

The metabolomic data analyses were performed using the MetaboAnalyst 5.0 software (https://www.metaboanalyst.ca), with data subjected to the following sample normalization: data logarithmic (base 10) transformation and Pareto scaling.

For data organization, visualization, and classification, a partial least square discriminant analysis (PLS-DA) and a hierarchical clustering heatmap for sample averages displaying the top 25 variable features (criteria: distance measure, Euclidean; clustering algorithm, Ward) were performed. Finally, the Mummichog algorithm, allowing direct mapping of mass spectra to existing pathway databases was used to identify those metabolic pathways differentially affected in the pairwise comparison TH vs. TM. In this analysis, the molecular weight tolerance was setup at 5 ppm, the primary ions enforced, and *Arabidopsis thaliana* (Kyoto Encyclopedia of Genes and Genomes, KEGG) selected as pathway library (Jurado-Mañogil et al., 2023).

Table 1

Electrical conductivity, Na$^+$ and Cl$^-$ contents (mg/ kg) of the soil before and after planting tomato (TM) and halophyte (HM) in monoculture or in intercropping system (TH). Error bars represent the standard error of the mean.

plot	EC (dS/ m) before	EC (dS/ m) after	Na$^+$ (mg/ kg) before	Na$^+$ (mg/ kg) after	Cl$^-$ (mg/ kg) Before	Cl$^-$ (mg/ kg) after
HM	2.8	1.1	555.8 ±5.6	279 ±2.8	1229 ±12.3	281.2 ±2.8
TM	3.1	11.2	559.4 ±5.6	466.2 ±4.7	1262.4 ±12.6	1832.8 ±18.3
TH	3.2	1.3	553.6 ±5.5	303 ±3	1232.8 ±12.3	426.8 ±4.3

Table 2

Effect of the halophyte-based intercropping management on the cation contents (mg/g dry weight) in tomato plants. TM: tomato in monoculture; TH: tomato in intercropping. Significant differences between the means ± SE ($n = 3$) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at $P < 0.05$. Error bars represent the standard error of the mean.

	Na$^+$	K$^+$	Mg^{2+}	Ca^{2+}
TM	64.5 ±3.2b	128.7 ±6.4a	63.5 ±3.2a	110.9 ±5.5a
TH	28.1 ±1.4a	148.9 ±7.4b	76.1 ±3.8b	152.7 ±7.6b

Table 3

Effect of the halophyte-based intercropping management on the anion contents (mg/g dry weight) in tomato plants. TM: tomato in monoculture; TH: tomato in intercropping. Significant differences between the means ± SE ($n = 3$) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at $P < 0.05$. Error bars represent the standard error of the mean.

	SO$_4^{2-}$	NO$_3^-$	Cl$^-$
TM	181.6 ±9.2a	183.8 ±9.9a	185.4 ±9.3b
TH	401.8 ±20.1b	224.4 ±10.8b	158.1 ±7.9a

2.10. Tomato fruit production and quality

The number of mature fruits and mean fruit weight per plant were determined. Five harvests (one harvest/week) were used to calculate the total production of tomato. Ten representative fruits from each crop management were squeezed at each harvest, and the juice filtered to determine the quality of tomato. The total soluble solid (TSS, expressed as Brix), the acidity and conductivity of the tomato extracts were determined.

2.11. Statistical analysis

Significant differences between the means ± SE ($n = 5$) were determined by means of Tukey's test (HSA) using the SPSS 16.0 software. The means followed by the same letters are not significantly different at $P \leq 0.05$. Error bars represent the standard error of the mean.

3. Results

3.1. Soil mineral contents

Soil analysis before and after field trial showed that the intercropping of tomatoes with halophytes (TH) reduced the electrical conductivity by 58.3 % (Table 1). This decrease was related especially to a significant decrease on Na$^+$ and Cl$^-$ content in the soil in TH of 45.3 % and 65.4 %, respectively. On the other hand, TM showed increased soil EC by 268 %. This rise was associated to increase of Cl$^-$ contents in the soil by 45.2 % despite of the reduction observed in Na$^+$ levels (16.7 %). Concerning the halophyte in monoculture (HM), our results showed a significant decrease on Na$^+$ and Cl$^-$ contents in the soil of 49.8 % and 77.1 %, respectively, leading to a 62.4 % decrease in soil EC (Table 1).

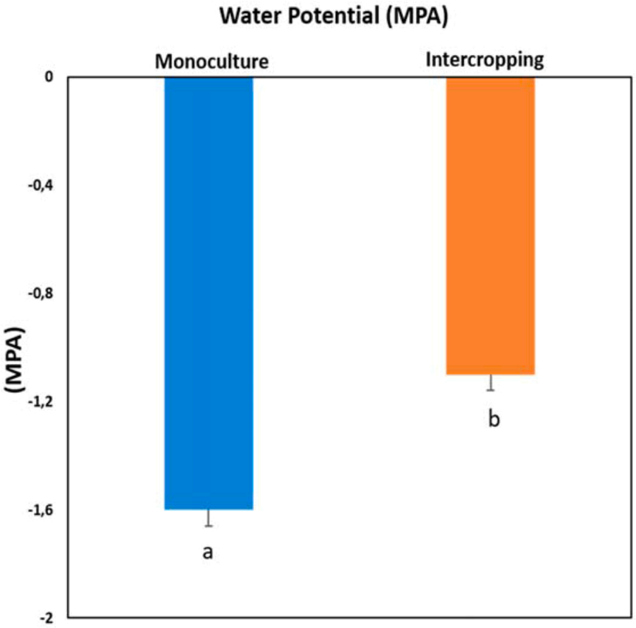

Fig. 2. Water Potential in the leaves of Tomato in monoculture (TM) or intercropping systems (TH): Significant differences between the means ± SE (n = 6) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at P < 0.05. Error bars represent the standard error of the mean.

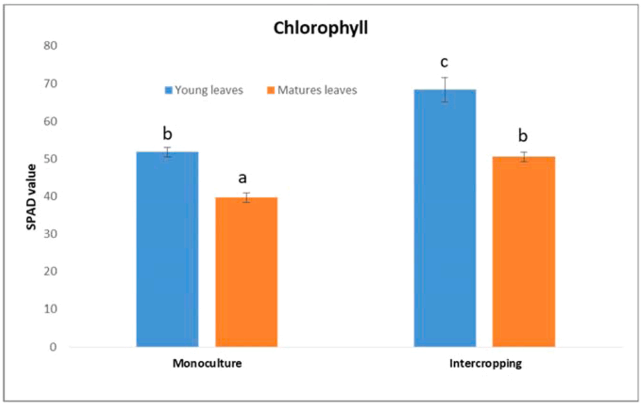

Fig. 3. Chlorophyll content (SPAD values) in young and matures leaves of tomato in monoculture (TM) or intercropping systems (TH). Significant differences between the means ± SE (n = 6) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at P < 0.05. Error bars represent the standard error of the mean.

3.2. Mineral contents and water potential

The intercropping system of tomatoes with halophytes changed the cation (Table 2) and anion (Table 3) contents of the tomato plants. In this regard, TH reduced sodium levels by 56.5 % compared with TM. In addition, intercropping improved mineral nutrition in tomatoes. In this sense, potassium, magnesium and calcium levels significantly increased by 15.7 %, 19.8 % and 37.6 %, respectively, in TH compared to TM.

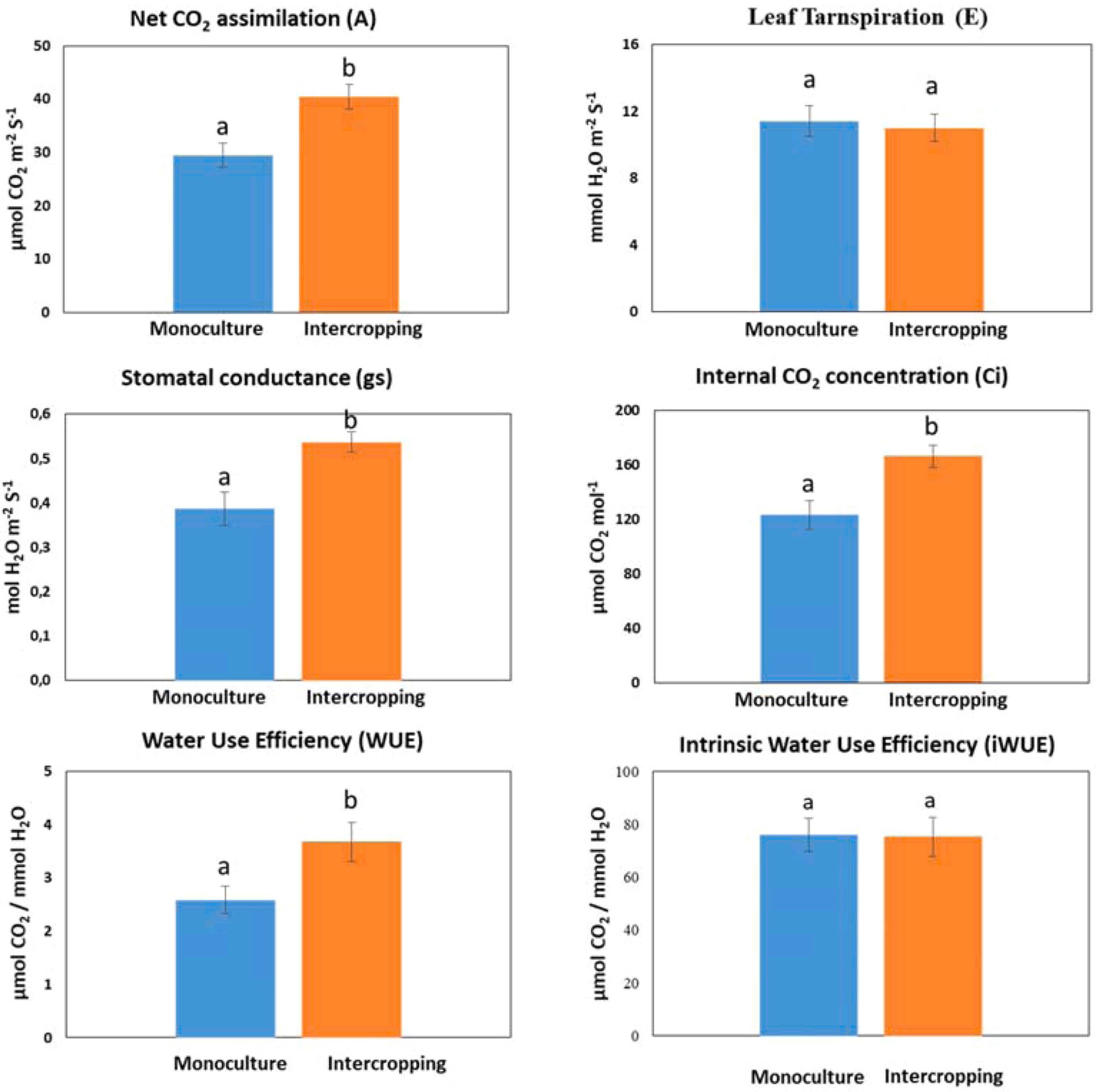

Fig. 4. Gas exchange parameters in tomato in monoculture (TM) or intercropping systems (TH): stomatal conductance (gs), internal C0$_2$ concentration (Ci), CO_2 uptake (A), leaf transpiration (E), water use efficiency (WUE = A/E) and intrinsic water use efficiency (iWUE = A/gs). Significant differences between the means ± SE (n = 6) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at $P < 0.05$. Error bars represent the standard error of the mean.

Table 3 also shows that Cl$^-$ content decreased in intercropped tomatoes by 14.7 %, while SO_4^{2-} and NO_3^- content increased by 121 % and 22.1 %, respectively. These results clearly show the improvement in nutrient uptake in intercropped tomatoes compared with monoculture, concomitantly with the reduction of Na$^+$ and Cl$^-$ contents. Intercropping improved the water potential in tomato by 31.3 % and, consequently, decreased the osmotic stress (Fig. 2).

3.3. Chlorophyll and gas exchange

Intercropping increased the chlorophyll contents in young and mature leaves of tomato by 32.0 % and 27.4 % respectively compared to tomato in monoculture (Fig. 3). In addition, intercropping improved photosynthesis activity of tomato, as reflected by the increase of net assimilation of CO_2 (37.4 %), stomatal conductance (38.7 %) and internal CO_2 concentration (35.2 %) compared to tomato in monoculture, whereas no significant change was observed for transpiration. In addition, intercropping increased the instantaneous water use efficiency by 42.5 %, while the intrinsic water use efficiency did not show any significant changes (Fig. 4). This is line with the CO_2 assimilation and stomatal conductance levels, which increased similarly under intercropping (Fig. 4).

Table 4
Effect of the halophyte-based intercropping management on the antioxidant properties: the DPPH, iron-chelating activity (ICA) and ferric reducing antioxidant power (FRAP) in tomato plants. TM: tomato in monoculture; TH: tomato in intercropping; n.a. no activity. Significant differences between the means ± SE ($n = 3$) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at $P < 0.05$. Error bars represent the standard error of the mean.

	DPPH	ICA	FRAP
TM	31.5 ± 1.2a	n.a.	n.a.
TH	35.3 ± 0.7a	10.7 ± 2.0	n.a.

Table 5
Effect of the halophyte-based intercropping management on hormones concentration in tomato after 57 days of planting. TM: tomato in monoculture; TH: tomato in intercropping. Significant differences between the means ± SE ($n = 3$) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at $P < 0.05$. Error bars represent the standard error of the mean.

Hormone concentration (ng g^{-1})		TM	TH
Stress hormones	ABA + SA + JA	5994 ± 263b	4809 ± 120a
Gibberellins	GA1 + GA3 + GA4	93.5 ± 14.6a	149.5 ± 11.9b
Cytokinins	tZ + ZR + iP	10844 ± 435a	11661 ± 433a
Auxins	IAA + OxIAA + OxIAA + IAA-Ala	6248 ± 523a	9148 ± 731b
Stress hormones/growth regulating hormones	SH/GH	0.349 ± 0.004b	0.229 ± 0.07a
ABA/GA	ABA/ GA	3.92 ± 1.1b	3.29 ±0.04a

3.4. *Antioxidant compounds and in vitro antioxidant properties*

No significant changes were detected in the TPC between monoculture (TM) and intercropping (TH) treatments, with values of 53.3 mg GAE/mg extract and 50.6 mg GAE/mg extract, respectively. Similarly, TFC levels were comparable between TM (36.9 mg RE/mg extract DW) and TH (37.5 mg RE/mg extract DW). However, there was a significant reduction in the TC) from TM (76.0 mg CE/mg extract DW) to TH (61.0 mg CE/mg extract DW). Antioxidant properties of the extracts appeared diminished, with intercropping showing no significant effect on these measures (Table 4).

3.5. *Hormones levels*

In the intercropping system, tomato plants exhibited a 19.8 % reduction in the combined levels of stress hormones, including abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) (Table 5). Furthermore, under TH conditions, the levels of gibberellins (GA1, GA3, and GA4) and auxins (IAA, OxIAA, and IAA-Ala) surged by 59.8 % and 46.4 %, respectively. Concurrently, the ABA to GA ratio was reduced by 16.9 % (Table 5). To elucidate the phytohormonal interplay in response to TM and TH conditions, the ratio of stress hormones to growth hormones was calculated, revealing a 34.2 % decline under TH compared to TM (Table 5)

3.6. *Metabolomic analysis*

The multivariate separation method PLS-DA displayed a clear segregation between TH and TM samples (Fig. 6A). In addition, according to the heatmap analysis, clearly defined patterns of differentially accumulated metabolites were observed (Fig. 6B). Overall, among the top 25 variable features, intercropped tomato displayed 15 features with higher intensity than TM (Fig. 6B). Some of these metabolites with higher abundance were "Phenylpropanoid biosynthesis"-related compounds according to the metabolic pathway analysis (Table 6). Knowledge about pathway modulation is essential to understand how biological systems adapt to the surrounding conditions. In TH plants, a stimulation of "Phenylpropanoid biosynthesis" and "Phenylalanine metabolism" pathways took place, whereas intercropping induced a downregulation of "Butanoate metabolism", "Monoterpenoid biosynthesis" and "Alanine, aspartate and glutamate metabolism" pathways (Table 6, Fig. 6C). Other metabolic pathways affected by the intercropping condition were the "Citrate cycle (TCA cycle)" and the "Riboflavin metabolism" (Table 6, Fig. 6C).

3.7. *Yield and quality of tomato*

A significant increase (15.8 %) was observed in tomato yield under TH conditions compared to monoculture cultivation, while fruit quality, as measured by acidity, conductivity, and Brix parameters, remained unaffected (Table 7).

4. Discussion

4.1. *Intercropping restored soil quality and improved mineral nutrition in tomato compared to monoculture under salt stress*

Our findings show that intercropping tomato plants with the halophyte *A. macrostachyum* significantly reduced soil salinity, as reflected

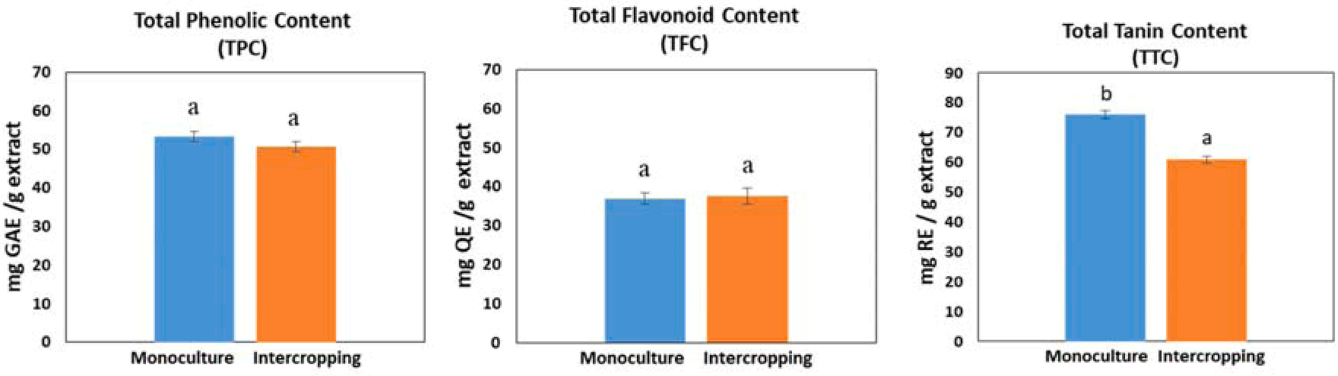

Fig. 5. Antioxydant compounds in tomato in monoculture (TM) or intercropping systems (TH): total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC). Significant differences between the means ± SE (n = 3) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at P < 0.05. Error bars represent the standard error of the mean.

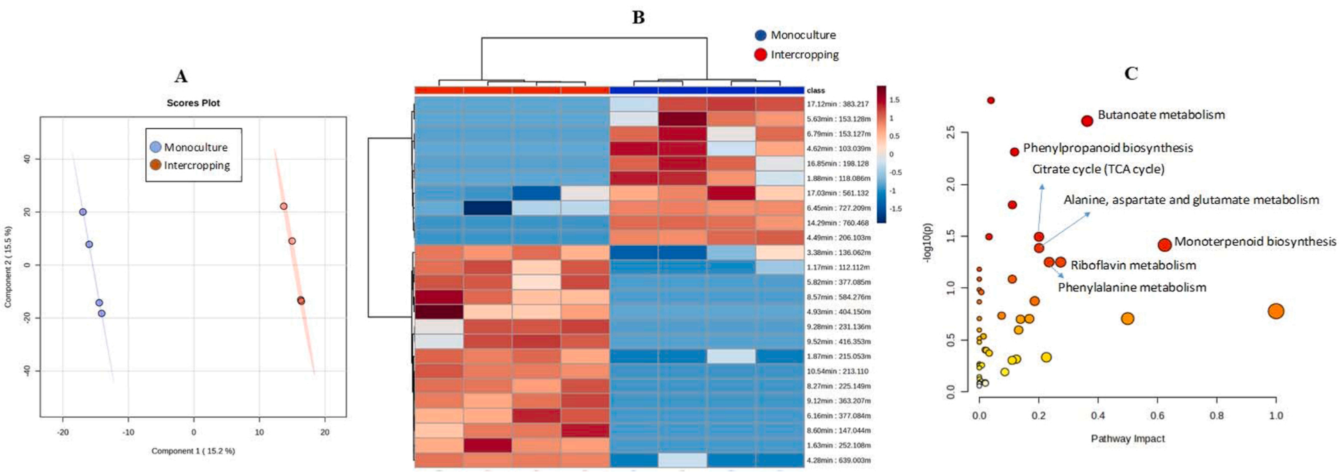

Fig. 6. A: Partial least square discriminant analysis (PLS-DA) of tomato samples. B: Hierarchical clustering heatmap for sample averages displaying the top 25 variable features (according to t-test/ANOVA). C: Metabolic pathways analysis using the Mummichog algorithm. Comparison tomato in intercropping versus tomato in monoculture.

Table 6
The most affected metabolic pathways in tomato plants according to Mummichog algorithm. Comparison: intercropping *vs*. monoculture.

	Total	Hits	Impact	KEEG	Compound name	*m/z*	log2(FC)
Butanoate metabolism	17	4	0.364	C00232	Succinic acid semialdehyde	103,039	−8,1
				C00164	Acetoacetic acid	103,039	−8,1
				C00334	Gamma-Aminobutyric acid	86,060	−2,6
				C00042	Succinic acid	101,023	−2,1
Phenylpropanoid biosynthesis	46	6	0.119	C00482	Sinapic acid	225,076	−2,1
				C00852	Chlorogenic acid	377,084	2,9
				C00811	4-Hydroxycinnamic acid	147,044	3,3
				C10945	Caffeic aldehyde	147,044	3,3
				C05838	Coumaric acid	147,044	3,3
				C18069	Tricoumaroyl spermidine	584,276	3,3
Citrate cycle (TCA cycle)	20	3	0.201	C00042	Succinic acid	101,023	−2,1
				C00158	Citric acid	193,035	3,9
				C00311	Isocitric acid	193,035	3,9
Monoterpenoid biosynthesis	9	2	0.625	C00341	Geranyl diphosphate	337,056	−3,5
				C17621	10-Hydroxygeraniol	153,127	−5,4
Alanine, aspartate and glutamate metabolism	22	3	0.493	C00232	Succinic acid semialdehyde	103,039	−8,1
				C00334	Gamma-Aminobutyric acid	86,060	−2,6
				C00042	Succinic acid	101,023	−2,1
Phenylalanine metabolism	11	2	0.235	C00166	Phenylpyruvic acid	147,044	3,3
				C02505	2-Phenylacetamide	136,076	4,1
Riboflavin metabolism	11	2	0.274	C01268	5-Amino−6-(5′-phosphoribosylamino)uracil	337,056	−3,5
				C04454	5-Amino−6-(5′-phosphoribitylamino)uracil	357,082	2,2

Table 7
Effect of the halophyte-based intercropping management on the production and quality of tomato fruits. TM: tomato in monoculture; TH: tomato in intercropping. Significant differences between the means ± SE ($n = 3$) were determined using Tukey's test (HSA). The means followed by the same letters are not significantly different at $P < 0.05$. Error bars represent the standard error of the mean.

	TM	TH
Yield (T/plot)	11 ±0.6a	12.8 ±0.64b
Estimated Yield (T/ha)	79.3 ±4a	91.9 ±4.6b
Acidity (pH)	4.1 ±0.2a	4.1 ±0.2a
Conductivity	4.4 ±0.22a	4.6 ±0.23a
TSCC (Brix)	5.7 ±0.29a	5.8 ±0.29a

by decreased soil electrical conductivity and reduced sodium (Na^+) and chloride (Cl^-) concentrations in the soil and, as well as in plant tissues. These results are consistent with those of Barcia-Piedras et al. (2023), who documented a 31 % reduction in soil salinity due to the presence of *A. macrostachyum* for more than 30 days, alleviating the negative effects of salinity on germination in barley and wheat. Similarly, Jurado-Mañogil et al. (2023) reported a decrease in soil Cl^-levels when they applied a halophyte/tomato intercrop in a greenhouse. This is consistent with the successful remediation of saline soils by Wang et al. (2020) planting the euhalophyte *Suaeda salsa* using drip irrigation technology, as shown in their three-year field experiment.

Furthermore, studies using halophytes (*Portulaca oleracea* L., *Salsola soda* L., *Atriplex hortensis* L.) in tomato intercropping systems under salinity conditions have shown that Na^+ content decreased in tomato roots and leaves, while K^+, Ca^{2+}, P and Mg content increased (Zuccarini, 2008; Albaho and Green, 2000; Karakas et al., 2019). Our study showed that TH intercropping had a positive effect on improving the mineral supply of tomato plants, as evidenced by the increase in K^+ Ca^{2+}, Mg^{2+}, SO_4^{2-} and NO_3^- levels in tomato plants. Similar results have been found in other glycophyte species such as maize, barley, watermelon, and rice, grown in intercropping systems with halophytes such as *Sesuvium portulacastrum, Spinacia oleracea, Bacopa monnieri*, *Sesuvium verrucosum, Salsola komarovii* and *Atriplex canescens* (Rabhi et al., 2010; Muchate et al., 2018; Simpson et al., 2018; Lastiri-Hernandez et al., 2020). These findings highlight the significance of intercropping with halophytes in maintaining ionic balance in glycophytes and in reducing soil Na^+ levels.

Many studies have shown that intercropping systems influence rhizosphere microbial communities and provided insight into the roles of microbial biodiversity and ecological performance in improving crop production under environmental constraints (Ichihashi et al., 2020; Mukhtar et al., 2021; Zhang et al., 2021). Gong et al. (2019) found that intercropping improved soil fertility and rhizosphere enzyme activity by altering rhizosphere microbial communities, which differed significantly from those in monoculture systems. In our study, the enhanced nutrient levels in tomatoes may be attributed to increased soil availability of these nutrients, facilitated by microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), within the intercropping system. Moreover, many works showed that the root-associated microbiota contribute to improving plant tolerance to abiotic stresses (Soussi et al., 2016; Mukhtar et al., 2021; Slatni et al., 2024).

4.2. *Intercropping system decreased the osmotic stress in tomato and maintained the integrity of photosynthesis machinery under salt stress*

Salinity disrupts plant cell function in multiple ways. In glycophyte plants such as tomatoes, it causes ionic stress through the accumulation of Na^+ and Cl^-, induces osmotic stress, leads to nutrient imbalance, and triggers oxidative stress (Zhu, 2002). In fact, the relatively tolerant tomato varieties are characterized by significant Na^+ compartmentalization, selective uptake and redistribution of K^+, (especially in young leaves), and high antioxidant enzyme activity (Yang et al., 2020). Besides, high concentrations of Na^+ disrupt osmotic balance and cause physiological drought, which prevents plants from absorbing water (Rodríguez-Rosales et al., 2008). Our results showed that tomatoes grown under TM conditions had more negative water potential than those grown in intercropping systems. This suggests that tomatoes were less osmotically stressed during intercropping, suggesting that intercropping may have a mitigating effect on water stress. In fact, planting *A. macrostachyum* can reduce soil salinity, thus alleviating the harsh conditions for tomato cultivation. In contrast, intercropping *A. macrostachyum*, leads to a decrease in water potential, resulting in better absorption of water and ions, including Na^+ and Cl^- ions (data not shown). In addition, halophytes have been shown to tolerate Na^+ toxicity mainly through their compartmentalization at the vascular level (Flowers and Colmer, 2008; Koyro et al., 2011). Therefore, osmotic stress can explain the main cause of plant growth inhibition under severe salt stress (Rodríguez-Rosales et al., 2008).

Photosynthetic parameters and chlorophyll content are generally considered as indicators of plant stress (Qaseem et al., 2019). Under intercropped conditions, chlorophyll content of tomato leaves was higher than that of monoculture, especially in young leaves, which was associated with a reduced leaf transpiration rate, which corrected the CO2 influx. These findings suggest enhanced recovery of gas exchange parameters, particularly net assimilation and internal CO_2 concentration, toward levels observed under non-stress conditions, thereby preserving photosynthetic function.

Rocha and Morales (1997) showed that an increase in iWUE (A/gs) was associatedwith a decrease in stomatal conductance, while in our study, the increase in stomatal conductance of intercropped tomato leaves was similar to the net assimilation of CO_2, which explains why iWUE remained constant between TM and TH. However, the increase in WUE (A/E) was associated with an increase in A under TH, while transpiration remained constant. Jurado et al. (2024) found that tomato intercropping with halophytes triggers protective mechanisms that safeguard photosynthesis under salt stress, specifically by stabilizing the dissipation of excess light energy and boosting the efficiency of photosystem II (Y(PSII)).

4.3. *Intercropping system decreased stress hormones and increased plant growth regulating hormones in tomato compared to monoculture under salt stress*

Plant hormones regulate various aspects of plant growth and development, including pathogen defense and stress tolerance (Fahad et al., 2015; Cho et al., 2015; Castro-Camba et al., 2022). This study found that the stress-to-growth hormone ratio was reduced by 34.2 % in intercropped tomatoes, indicating lower salt stress compared to monoculture. This is in agreement with previous results showing that Na^+ content in soil and tomato leaves was reduced when halophytes were intercropped with tomato (Albaho and Green, 2000; Zuccarini et al., 2008, Simpson et al., 2018). In addition, similar results have been obtained with Medicago species grown with Atriplex (Kurdali, 2010).

Beyond their effects on important plant physiological mechanisms, GA_S also play a major role in regulating stress tolerance (Yang et al., 2014; Shabala et al., 2016; Yu et al., 2020). Several studies have analysed their interactions with stress hormones such as ABA to understand plant response strategies to environmental constraints (Achard et al., 2006; Colebrook et al., 2014; Castro-Camba et al., 2022). The nature of the interaction between GA and stress hormones is quite complex, although it is mainly antagonistic. Other plant growth-promoting plant hormones, such as cytokinins and auxins, can improve plant salt tolerance (Wang et al., 2009, 2011; Verma et al., 2016). In this work, GA in tomato under intercropping conditions increased significantly by 59.8 % compared with monoculture, suggesting that TH plants are less affected by salinity. In fact, several studies have shown that salt exposure leads to a decrease in endogenous GA in *A. thaliana* seedlings (Achard et al., 2006; Magome et al., 2008; Colebrook et al., 2014).

Studies have shown that the involvement of stress-related plant hormones in plant responses to salinity is crucial and varies depending on the stress intensity, plant genus and species, and developmental stage (Ku et al., 2018; Yang et al., 2019; Yu et al., 2019, 2020). In this work, the ratio of stress hormones (ABA, JA, and SA) to growth hormones (GA, auxin, and cytokinin) was lower under TH conditions, suggesting that tomatoes were less stressed. Yu et al. (2020) stated that plant adaptation to salt stress depends on dynamic hormone regulation, both stress and growth hormones crucially mediate the response to salt stress, and the complex interaction of various hormones promotes plant growth adaptation to salt conditions (Yang et al., 2019; Yu et al., 2019, 2020). Salt response in tomato is associated with activation of crucial genes responsible for biosynthesis and metabolism of stress hormones, especially ABA, which is an important factor in the salt tolerance mechanism of plants (Yang et al., 2014). ABA induces the expression of genes encoding ion transporters, thereby improving the selective uptake of ions such as K^+ and facilitating the transfer of Na^+ from the cytoplasm to the vacuole or removal from the plant to maintain a normal K^+/Na^+ ratio (Zhao et al., 2009, Uertas et al., 2012, Yarra et al., 2012). ABA is also essential for reducing stomatal conductance and inducing the expression of genes responsible for the synthesis of various osmolytes such as proline (Antoni et al., 2011). The present work showed that under the intercropping system, stress hormones were reduced, especially SA (data not shown), suggesting that tomato plants were less stressed when intercropped with *A. macrostachyum* on salt-affected soil. In fact, the ABA/GA ratio decreased by 16.9 % in tomatoes intercropped with halophyte species, while ABA levels remained higher in the leaves (data not shown) to further support tomato tolerance to salinity.

4.4. *Intercropping system increased phenylpropanoid biosynthesis, phenylalanine metabolism and maintained antioxidant properties in tomato under salt stress*

The metabolic pathways analysis using the Mummichog algorithm showed that, under our experimental conditions, the intercropping conditions mainly led to a stimulation of the “Phenylpropanoid biosynthesis” and “Phenylalanine metabolism”, whereas the abundance

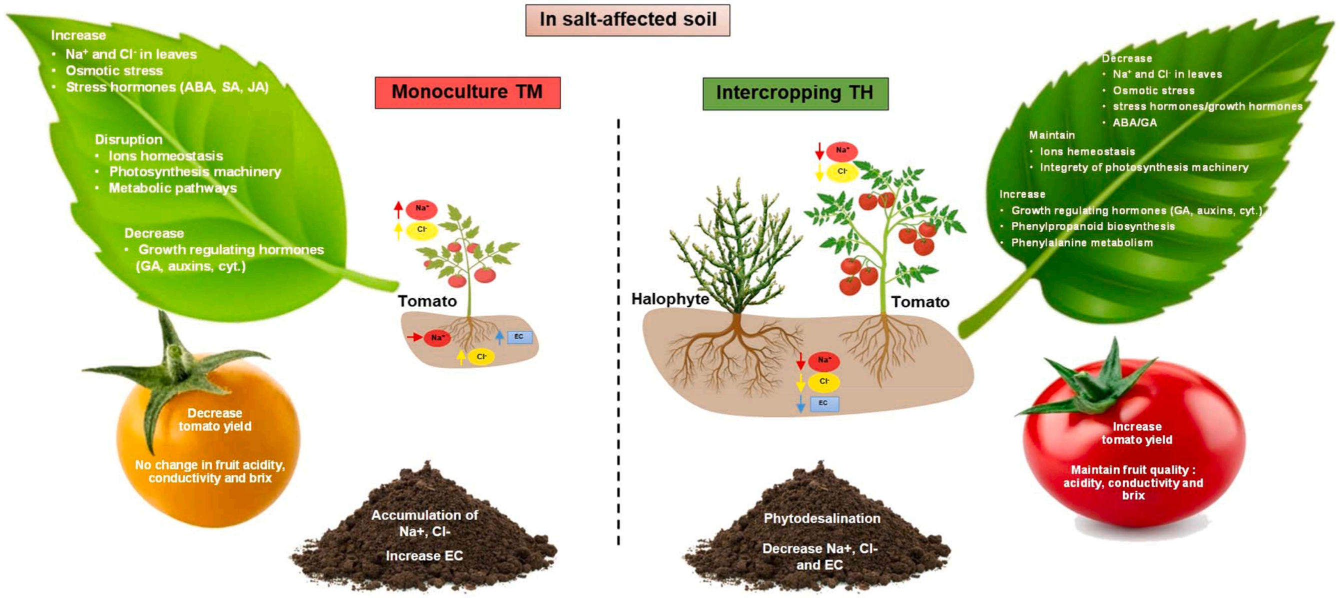

Fig. 7. The main changes of tomato cultivated in monoculture (TM) or in intercropping system (TH) in salt-affected soil.

of metabolites related to "Butanoate metabolism", "Alanine, aspartate and glutamate metabolism" and "Monoterpenoid biosynthesis" decreased in TH plants compared to TM. Many secondary metabolites that play key roles in plant growth, development and stress responses to environmental stresses are produced through the phenylpropanoid biosynthetic pathway (Robe et al., 2021). Moreover, phenylpropanoid-related compounds have been implicated in the elimination of reactive oxygen species under salt stress in tomato plants (Jia et al., 2022). Phe metabolism also confers salt tolerance to tomatoes through targeted regulation of metabolites and antioxidants (Almas et al., 2021). These authors showed that exogenous application of Met and/or Phe improved salt tolerance in tomato. The same work suggests that foliar application of Met and/or Phe alleviated the harmful effects of salinity on growth and yield of tomato plants by enhancing plasma membrane stability, increasing osmoticum content, and antioxidant enzyme activity (Almas et al., 2021). Furthermore, metabolome changes in tomato plants intercropped with *A. macrostachyum* under greenhouse conditions were recently proposed to be associated with adaptive mechanisms against moderate oxidative stress (Jurado-Mañogil et al., 2023).

The intercropping condition lead to a downregulation of the "Butanoate metabolism". The non-protein amino acid, gamma-aminobutyric acid (GABA) regulates plant growth and accumulates in plant tissues in response to biotic and abiotic stresses (Ramesh et al., 2015). The butanoate metabolism, among other reactions, includes L-glutamate degradation into the signal molecule GABA followed by subsequent reactions to produce further products. Although the role of GABA in mitigating abiotic stresses has been largely described, a holistic assessment of the involvement of GABA-related metabolic pathways in salt tolerance has not been addressed (Dabravolski and Isayenkov, 2023). Therefore, the downregulation of the "Butanoate metabolism" observed in TH plants could be related to changes in other metabolic pathways leading to the adaptive mechanisms of tomato plants. In this sense, alternative pathways for GABA biosynthesis from polyamines have been reported, GABA metabolism also being related to TCA cycle (Dabravolski and Isayenkov, 2023); citrate cycle was also affected in TH plants. Likewise, intercropping leads to the downregulation of metabolites related to "alanine, aspartate and glutamate metabolism". Thus, recent work based on integrated analyses (metabolomics and transcriptomics) found that alanine, aspartate and glutamate metabolism in tomato plants may respond negatively to salt stress (Liu et al., 2024).

Ethanol extracts were prepared from dried tomato leaves and used to evaluate the influence of intercropping on the levels of phenolics (TPC), flavonoids (TFC), and condensed tannins (TCT) key bioindicators of plant stress responses as well as the oxidative status in tomato leaves, inferred from their in vitro antioxidant properties. Ethanol is an effective solvent often resulting in the extraction of relevant levels of plant secondary metabolites, which are integral to the plant's antioxidant defenses (Stalikas, 2007). The TPC and TFC values observed for both TM and TH fall within the range reported by Vallverdú-Queralt et al. (2013), who noted that the phenolic content in tomatoes varies greatly widely depending on the cultivar and environmental factors. The lack of significant differences between TM and TH suggests that intercropping with *A. macrostachyum* does not affect the synthesis of phenolic and flavonoid compounds, which are essential for plant defense against stress and for human health due to their antioxidant properties (Stewart et al., 2000). However, the decrease in TTC observed in TH samples compared to TM suggests that there may be a trade-off in the production of secondary metabolites when tomatoes are intercropped with halophytes. This can be explained by resource allocation theory, which suggests that plants may prioritize certain pathways depending on the biotic and abiotic interactions they are exposed to (Bazzaz et al., 1987). The lack of significant effects of intercropping on TPC and TFC, despite changes in TTC, may be related to a specific adaptive response of tomatoes to altered microclimate and soil conditions induced by to the presence of *A. macrostachyum*, which may have different effects on biosynthetic pathways (Isah, 2019).

4.5. Intercropping system increased tomato yield in salt-affected soil compared to monoculture

In our study, Tomato yields were higher in intercropping tomatoes with *A. macrostachyum* than in monoculture, which may be due to reduced soil salinization and reduced accumulation of toxic Na^+ and Cl^- ions in the tomato leaves. The increase in tomato yield under the intercropping system may be attributed to increased nutrient accumulation in tomatoes, suggesting that intercropping promotes more efficient absorption and utilization of nutrients. Our findings are consistent with previous studies that have shown a positive correlation between increased yield and reduced N^+ and Cl^- accumulation in plants, supporting the idea that effective nutrient management can improve crop performance (Zuccarini, 2008; Simpson et al., 2018). In some cases, the increase in yield was associated with an increase in tomato size and weight rather than an increase in total yield (Zuccarini, 2008; Karakas et al., 2021). In this work, no significant differences were found in the quality of tomatoes, with acidity and Brix values remaining constant throughout the trials. In fact, our field results indicate that salinity affects the color of tomatoes, and we identified color difference between monoculture and intercropped tomatoes with the naked eye (data not shown). Fig. 7 summarizes the main changes in tomatoes grown in monoculture (TM) or intercropping (TH) systems in salt-affected soils.

5. Conclusion

In conclusion, this work shows that intercropping tomatoes with salt-tolerant halophytes *A. macrostachyum* improves the performance of tomatoes on moderately saline soils. Intercropping notably reduced soil salinity and the accumulation of Na^{+} and Cl^{-} in tomato leaves, while concurrently increasing tomato yield. These outcomes underscore the value of halophytes in remediating salt-affected soils. Furthermore, our findings indicate that intercropping contributes to the tolerance and improved mineral nutrition of tomatoes under saline conditions, which is corroborated by the maintained levels of total phenolic and flavonoid contents (TPC and TFC) in tomato leaves, like those in monoculture. This approach signifies a practical and eco-friendly strategy that could enhance food security in salt-impacted regions globally by providing an economical method to boost yields and crop quality without negatively affecting the environment.

CRediT authorship contribution statement

Tarek Slatni: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aida Selmi**: Methodology, Data curation, Conceptualization. **Nesrine Kalboussi**: Methodology, Data curation, Conceptualization. **Hassène Zemni**: Methodology, Conceptualization. **Adel Echadly**: Methodology. **Gregorio Barba Espin**: Writing – review & editing, Methodology, Data curation, Conceptualization. **José Antonio Hernandez**: Writing – review & editing, Data curation, Conceptualization. **Hamza Elfil**: Data curation, Conceptualization. **Luísa Custódio**: Writing – review & editing, Methodology, Data curation, Conceptualization. **Tiago Braga**: Methodology, Data curation. **Pedro Diaz- Vivancos**: Writing – review & editing, Methodology, Data curation, Conceptualization. **Karim Ben Hamed**: Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization.

Declaration of Competing Interest

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

Acknowledgments

The authors thank the financial support of the project HaloFarMs by PRIMA, a program supported by the European Union, and by the Tunisian Ministry of Higher Education and Scientific Research (MHESR, LR15CBBC02). In addition, this work was supported by ICOOP program (Ref. ICOOPB20631). We also acknowledge the support of the Portuguese FCT, Foundation for Science and Technology, for financing research at CCMAR (Algarve University, Portugal).

References

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J., Harberd, N.P., 2006. Integration of plant responses to environmentally activated phytohormonal signals. Science 311, 91–94.

Agnihotri, P., Kumar, A., 2015. Halophytes for saline lands, their economic potential and demonstration of salt tolerance. Am. J. Pharm. Health Res 3, 279–283.

Albacete, A., Ghanem, M.E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Pérez-Alfocea, F., 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. Journal of Experimental Botany 59, 4119–4131. https://doi.org/10.1093/jxb/ern251.

Albaho, M.S., Green, J.L., 2000. Suaeda salsa, A desalinating companion plant for greenhouse tomato. Hort. Sci. 35 (26), 620–623.

Almas, H.I., Un-Nisa, Z., Anwar, S., et al., 2021. Exogenous application of methionine and phenylalanine confers salinity tolerance in tomato by concerted regulation of metabolites and antioxidants. J. Soil Sci. Plant Nutr. 21, 3051–3064. https://doi.org/10.1007/s42729-021-00588-9.

Antoni, R., Rodriguez, L., Gonzalez-Guzman, M., Pizzio, G.A., Rodriguez, P.L., 2011. News on ABA transport, protein degradation, and ABFs/WRKYs in ABA signalling. Curr. Opin. Plant Biol. 14 (5), 547–553. https://doi.org/10.1016/j.pbi.2011.06.004.

Ashraf, M.Y., Ashraf, M., Mahmood, K., Akhter, J., Hussain, F., Arshad, M., 2010. Phytoremediation of saline soils for sustainable agricultural productivity. In: Ashraf, M., Ozturk, M., Ahmad, M.S.A. (Eds.), Plant Adaptation and Phytoremediation. Springer, Berlin, Germany, pp. 335–3355.

Barba-Espin, G., Diaz-Vivancos, P., Pérez-Caselles, C., Faize, L., Hernandez, J.A., Pedreño, M.A., Alburquerque, N., 2024. Tomato plants expressing a stilbene synthase gene display genotype-depending alterations in metabolome profile and antioxidant system. Physiol. Plant. 176 (1), e14147.

Barcia-Piedras, J.M., Pérez-Romero, J.A., Mateos-Naranjo, E., Camacho, M., Redondo-Gómez, S., 2023. Effect of prior salt experience on desalination capacity of the halophyte *Arthrocnemum macrostachyum*. Desal 463 (7), 50–54.

Bazzaz, F.A., Chiariello, N.R., Coley, P.D., Pitelka, L.F., 1987. Allocating resources to reproduction and defense. BioScience 37 (1), 58–67.

Ben Hamed, K., Castagna, A., Ranieri, A., García-Caparrós, P., Santin, M., Hernandez, J. A., Espin, G.B., 2021. Halophyte based Mediterranean agriculture in the contexts of food insecurity and global climate change. Environ. Exp. Bot. 191, 104601. https://doi.org/10.1016/j.envexpbot.2021.104601.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Leb. Wiss. Technol. 28, 25–30.

Castro-Camba, R., Sánchez, C., Vidal, N., Vielba, J.M., 2022. Plant development and crop yield: the role of gibberellins. Plants *11*, 2650. https://doi.org/10.3390/plants11192650.

Cho, S.T., Chang, H.H., Egamberdieva, D., Kamilova, F., Lugtenberg, B., Kuo, C.H., 2015. Genome analysis of Pseudomonas fluorescens PCL1751: a rhizobacterium that controls root diseases and alleviates salt stress for its plant host. PLOS ONE 10, e0140231. https://doi.org/10.1371/journal.pone.0140231.

Colebrook, E.H., Thomas, S.G., Phillips, A.L., Hedden, P., 2014. The role of gibberellin signalling in plant responses to abiotic stress. J. Exp. Biol. 217, 67–75. https://doi.org/10.1242/jeb.089938.

Dabravolski, S.A., Isayenkov, S.V., 2023. The Role of the γ-Aminobutyric Acid (GABA) in Plant Salt Stress Tolerance. Horticulturae 9, 230.

Fahad, S., Nie, L., Chen, Y., Wu, C., Xiong, D., Saud, S., Hongyan, L., Cui, K., Huang, J., 2015. Crop plant hormones and environmental stress. Sustain. Agric. Rev. 15, 371–400.

Faize, M., Burgos, L., Faize, L., Piqueras, A., Nicolas, E., Barba-Espin, G., Clemente-Moreno, M.J., Alcobendas, R., Artlip, T., Hernandez, J.A., 2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. J. Exp. Bot. 62 (8), 2599–2613. https://doi.org/10.1093/jxb/erq432.

FAO, 2011. The State of the World's Land and Water Resources for Food and Agriculture (SOLAW) – Managing Systems at Risk. Food and Agriculture Organization of the United Nations, Rome and Earth scan, London.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. N. Phytol. 179 (4), 945–963.

Gong, X., Liu, C., Li, J., Luo, Y., Yang, Q., Zhang, W., Feng, B., 2019. Responses of rhizosphere soil properties, enzyme activities and microbial diversity to intercropping patterns on the Loess Plateau of China. Soil and Tillage Research 195, 104355.

Grafienberg, A., Botrini, L., Giustiniani, L., Filippi, F., Curadi, M., 2003. Tomato growing in saline conditions with biodesalinating plants: salsola soda and portulaca oleracea. Acta Hortic. (609), 301–305.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Bhowmik, P.C., Hossain, M.A., Rahman, M. M., Prasad, M.N.V., Ozturk, M., Fujita, M., 2014. Potential use of halophytes to remediate saline soils. Bio. Med. Res. Int. https://doi.org/10.1155/2014/589341.

Ichihashi, Y., Date, Y., Shino, A., Shimizu, T., Shibata, A., Kumaishi, K., Funahashi, F., Wakayama, K., Yamazaki, K., Umezawa, A., Sato, T., Kobayashi, M., Kamimura, M., Kusano, M., Che, F.S., O`Brien, M., Tanoi, K., Hayashi, M., Nakamura, R., Shirasu, K., Kikuchi, J., httand Nihei, N. 2020. Multi-omics analysis on an agroecosystem reveals the significant role of organic nitrogen to increase agricultural crop yield. 117(25) 14552-14560.

Idoudi, M., Slatni, T., Laifa, I., Rhimi, N., Rabhi, M., Hernández-Apaolaza, L., Zorrig, W., Abdelly, C., 2024. Silicon (Si) mitigates the negative effects of iron deficiency in common bean (*Phaseolus vulgaris* L.) by improving photosystem activities and nutritional status. Plant Physiol. Biochem. 206, 108236. https://doi.org/10.1016/j.plaphy.2023.108236.

Isah, T., 2019. Stress and defense responses in plant secondary metabolites production. Biol. Res. 52 (1), 39.

Jia, C., Guo, B., Wang, B., Li, X., Yang, T., Li, N., Wang, J., Yu, Q., 2022. Integrated metabolomic and transcriptomic analysis reveals the role of phenylpropanoid biosynthesis pathway in tomato roots during salt stress. Front. Plant Sci. 13, 1023696. https://doi.org/10.3389/fpls.2022.1023696.

Jurado-Mañogil, C., Martínez-Melgarejo, P.A., Martínez-García, P., Rubio, M., Hernández, J.A., Barba-Espín, G., Martínez-García, P.J., 2024. Comprehensive study of the hormonal, enzymatic and osmoregulatory response to drought in Prunus species. Scientia Horticulturae 326, 112786.

Jurado, C., Díaz-Vivancos, P., Barba-Espín, G., José Ramón, A.-M., Hernández, J.A., 2024. Effect of halophyte-based management in physiological and biochemical responses of tomato plants under moderately saline greenhouse conditions. Plant Physiol. Biochem. 206, 108228.

Jurado-Mañogil, C., Barba-Espín, G., Hernańdez, J.A., Diaz-Vivancos, P., 2023. Comparative metabolomic analysis between tomato and halophyte plants under intercropping conditions. Physiol. Plant. 175, e13971. https://doi.org/10.13039/501100011033.

Karakas, S., Bolat, I., Dikilitas, M., 2021. The Use of Halophytic Companion Plant (*Portulaca oleracea* L.) on Some Growth, Fruit, and Biochemical Parameters of Strawberry Plants under Salt Stress. Horticulturae 7, 63. https://doi.org/10.3390/horticulturae7040063.

Karakas, S., Cullu, M.A., Kaya, C., Dikilitas, M., 2016. Halophytic companion plants improve growth and physiological parameters of tomato plants grown under salinity. Pak. J. Bot. 48 (1), 21–28.

Karakas, S., Dikilitas, M., Aslan, M., Güze, A.N., 2019. Evaluation of biochemical and physiological responses of salsola spp at their natural habitats. – Harran. Journal of Agricultural and Food Science 23, 226–233.

Koyro, H.W., Khan, M.A., Lieth, H., 2011. Halophytic crops: a resource for the future to reduce the water crisis? Emir. J. Food Agric. 23 (1), 1–16.

Ku, Y.S., et al., 2018. Plant hormone signaling crosstalks between biotic and abiotic stress responses. Int. J. Mol. Sci. 19, 3206.

Kurdali, F., 2010. Growth and N 2 fixation in mixed cropping of Medicago arborea and Atriplex halimus grown on a salt-affected soil using a 15 N tracer technique. J. Plant Interact. 5, 37–44.

Lastiri-Hernandez, M.A., Alvarez-Bernal, D., Ochoa-Estrada, S., Contreras-Ramos, S.M., 2020. Potential of *Bacopa monnieri* (L.) Wettst and *Sesuvium verrucosum* Raf. As an agronomic management alternative to recover the productivity of saline soils. Intern. J. Phytoremed. 22 (4), 343–352.

Li, Y.G., Tanner, G., Larkin, P., 1996. The DMACA-HC1 protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. J. Sci. Food Agric. 70, 89–101.

Liang, J., Krauss, K.W., Finnigan, J., Stuart-Williams, H., Farquhar, G.D., Ball, M.C., 2023. Linking water use efficiency with water use strategy from leaves to communities. N. Phytol. 240, 1735–1742. https://doi.org/10.1111/nph.19308.

Liu, Y., Zheng, J., Ge, L., Tang, H., Hu, J., Li, X., Wang, X., Zhang, Y., Shi, Q., 2024. Integrated metabolomic and transcriptomic analyses reveal the roles of alanine, aspartate and glutamate metabolism and glutathione metabolism in response to salt stress in tomato. Sci. Hortic. 328, 112911. https://doi.org/10.1016/j.scienta.2024.112911.

Magome, H., Yamaguchi, S., Hanada, A., Kamiya, Y., Oda, K., 2008. The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in Arabidopsis. Plant J. 56, 613–626.

Megías, C., Pastor-Cavada, E., Torres-Fuentes, C., Girón-Calle, J., Alaiz, M., Juan, R., Pastor, J., Vioque, J., 2009. Chelating, antioxidant and antiproliferative activity of Vicia sativa polyphenol extracts. Eur. Food Res. Technol. 230, 353–359.

Muchate, N.S., Rajurkar, N.S., Suprasanna, P., Nikam, T.D., 2018. Evaluation of *Spinacia oleracea* (L.) for phytodesalination and augmented production of bioactive metabolite, 20-hydroxyecdysone. Int. J. Phytoremediat. 20, 981–994. https://doi.org/10.1080/15226514.2018.1452184.

Mukhtar, S., Mehnaz, S., Malik, K.A., 2021. Comparative study of the rhizosphere and root endosphere microbiomes of cholistan desert plants. Front. Microbiol. 12, 618742. https://doi.org/10.3389/fmicb.2021.618742.

Negacz, Katarzyna, Pier, Vellinga, Arjen, de Vos, 2019. Saline Degraded Lands Worldwide: Identifying Most Promising Areas for Saline Agriculture. Paper Presented at the Saline Futures Conference.

Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 103, 413–419.

Qaseem, M.F., Qureshi, R., Shaheen, H., 2019. Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. Sci. Rep. 9, 6955. https://doi.org/10.1038/s41598-019-43477-z.

Rabhi, M., Ferchichi, S., Jouini, J., Hamrouni, M.H., Koyro, H.W., Ranieri, A., Abdelly, C., Smaoui, A., 2010. Phytodesalination of a salt-affected soil with the halophyte *Sesuvium portulacastrum* L. to arrange in advance the requirements for the successful growth of a glycophytic crop. Bioresour. Technol. 101, 6822–6828. https://doi.org/10.1016/j.biortech.2010.03.097.

Ramesh, S., Tyerman, S., Xu, B., et al., 2015. GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. Nat. Commun. 6, 7879.

Robe, K., Izquierdo, E., Vignols, F., Rouached, H., Dubos, C., 2021. The coumarins: secondary metabolites playing a primary role in plant nutrition and health. Trends Plant Sci. 26, 248–259.

Rocha, A., M.S., Morales, J.A.P.V., 1997. Influência do estresse hídrico sobre as trocas gasosas em plantas jovens envasadas de Stryphnodendron adstringens (Mart.) Coville. Rev. Bras. De. Fisiol. Veg., Camp. 9 (1), 41–46.

Rodríguez-Rosales, M.P., Jiang, X.J., Gálvez, F.J., Aranda, M.N., Cubero, B., Venema, K., 2008. Overexpression of the tomato K+/H+ antiporter LeNHX2 confers salt tolerance by improving potassium compartmentalization. N. Phytol. 179, 366–377. https://doi.org/10.1111/j.1469-8137.2008.02461.x.

Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A., Hammel, H.T., 1965. Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Sci. (N. Y., N.Y.) 148, 339–346.

Shabala, S., Bose, J., Fuglsang, A.T., Pottosin, I., 2016. On a quest for stress tolerance genes: membrane transporters in sensing and adapting to hostile soils. J. Exp. Bot. 67, 1015–1031.

Shah, S.H., Houborg, R., McCabe, M., 2017. Response of chlorophyll, carotenoid and spad-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). Agronomy 7 (3), 61. https://doi.org/10.3390/agronomy7030061.

Simpson, C.R., Franco, J.G., King, S.R., Volder, A., 2018. Intercropping halophytes to mitigate salinity stress in watermelon. Sustain 10, 681. https://doi.org/10.3390/su10030681.

Slatni, T., Ben Slimene, I., Harzalli, Z., Taamalli, W., Smaoui, A., Abdelly, C., Elkahoui, S., 2024. Enhancing quinoa (*Chenopodium quinoa*) growth in saline environments through salt-tolerant rhizobacteria from halophyte biotope. Physiol. Plant. 176 (4), e14466. https://doi.org/10.1111/ppl.14466.

Soussi, A., Ferjani, R., Marasco, R., Guesmi, A., Cherif, H., Rolli, E., et al., 2016. Plant-associated microbiomes in arid lands: diversity, ecology and biotechnological potential. Plant Soil 405, 357–370. https://doi.org/10.1007/s11104-015-2650-y.

Stalikas, C.D., 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of separation science 30, 3268–3295.

Stewart, A.J., Bozonnet, S., Mullen, W., Jenkins, G.I., Lean, M.E., Crozier, A., 2000. Occurrence of flavonols in tomatoes and tomato-based products. J. Agric. Food Chem. 48 (7), 2663–2669.

Uertas, R., Olias, R., Eljakaoui, Z., Galvez, F.J., LI, J., De Morales, P.A., Belver, A., Rodriguez-Rosales, M.P., 2012. Overexpression of *SlSOS2* (*SlCIPK24*) confers salt tolerance to transgenic tomato. Plant Cell Environ. 35, 1467–1482. https://doi.org/10.1111/j.1365-3040.2012.02504.x.

Vallverdú-Queralt, A., Medina-Remón, A., Casals-Ribes, I., Lamuela-Raventos, R.M., 2013. Is there any difference between the phenolic content of organic and conventional tomato juices? Food Chem. 130 (1), 222–227.

Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. 46, 4113–4117.

Verma, V., et al., 2016. Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16, 86.

Wang, D., Wang, L., Zhu, F., Zhu, J., Chen, X.D., Zou, L., Saito, M., Li, L., 2008. In vitro and in vivo studies on the antioxidant activities of the aqueous extracts of Douchi (a traditional Chinese saltfermented soybean food). Food Chem. 107, 1421–1428.

Wang, S., Zhao, Z., Ge, S., Zhang, K., Tian, C., Mai, W., 2022a. The effects of *Suaeda salsa/ Zea mays* L. Intercropping on plant growth and soil chemical characteristics in saline soil. Agric. For. 12, 107. https://doi.org/10.3390/agriculture12010107.

Wang, Y., Luo, J.P., Wu, H.Q., Jin, H., 2009. Conversion of protocorm-like bodies of Dendrobium huoshanense to shoots: the role of polyamines in relation to the ratio of total cytokinins and indole-3-acetic acidindole-3-acetic acid. J. Plant Physiol. 166, 2013–2022.

Wang, Y., Li, L., Ye, T., Zhao, S., Liu, Z., Feng, Y.Q., Wu, Y., 2011. Cytokinin antagonizes ABA suppression to seed germination of Arabidopsis by downregulating ABI5 expression. Plant J. 68, 249–261. https://doi.org/10.1111/j.1365-313X.2011.04683.x.

Wang, Y., Narayanan, M., Shi, X., Chen, X., Li, Z., Natarajan, D., Ma, Y., 2022b. Plant growth-promoting bacteria in metal-contaminated soil: current perspectives on remediation mechanisms. Front. Microbiol. 13, 966226. https://doi.org/10.3389/fmicb.2022.966226.

Wang, Z., Hong, Y.C., Zhu, G.T., Li, Y.M., Niu, Q.F., Yao, J.J., Hua, K., et al., 2020. Loss of salt tolerance during tomato domestication conferred by variation in a Na^+/K^+ transporter. EMBO J. 39, e103256.

Yang, J., Zhang, J., Li, C., Zhang, Z., Ma, F., Li, M., 2019. Response of sugar metabolism in apple leaves subjected to short-term drought stress. Plant Physiol. Biochem. 141, 164–171. https://doi.org/10.1016/j.plaphy.2019.05.025.

Yang, X., Li, Y., Chen, H., Huang, J., Zhang, Y., Qi, M., Liu, Y., Li, T., 2020. Photosynthetic response mechanism of soil salinity-induced cross-tolerance to subsequent drought stress in tomato plants. Plants 9, *363*. https://doi.org/10.3390/plants9030363.

Yang, Y., Ma, C., Xu, Y., Wei, Q., Imtiaz, M., Lan, H., Gao, S., Cheng, L., Wang, M., Fei, Z., et al., 2014. A zinc finger protein regulates flowering time and abiotic stress tolerance in chrysanthemum by modulating gibberellin biosynthesis. Plant Cell 26, 2038–2054.

Yarra, R., He, S.J., Abbagani, S., Ma, B., Bulle, M., Zhang, W.K., 2012. Overexpression of a wheat Na^+/H^+ antiporter gene (*TaNHX2*) enhances tolerance to salt stress in transgenic tomato plants (*Solanum lycopersicum* L.). Plant Cell Tissue Organ Cult. 111 (1), 49–57.

Yu, Z., et al., 2019. CEPR2 phosphorylates and accelerates the degradation of PYR/PYLs in Arabidopsis. J. Exp. Bot. 70, 5457–5469.

Yu, Z., Duan, X., Luo, L., Dai, S., Ding, Z., Xia, G., 2020. How plant hormones mediate salt stress responses. Trends Plant Sci. 25 (11), 1117–1130. https://doi.org/10.1016/j.tplants.2020.06.008.

Zhang, Zhihao, Chai, Xutian, Tariq, Akash, Zeng, Fanjiang, Li, Xiangyi, Graciano, Corina, 2021. Intercropping systems modify desert plant-associated microbial communities and weaken host effects in a hyper-arid desert. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.754453.

Zhao, B., Ge, L., Liang, R., Li, W., Ruan, K., Lin, H., Jin, Y., 2009. Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. BMC Mol. Biol. 10, 29. https://doi.org/10.1186/1471-2199-10-29.

Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53, 247–273. https://doi.org/10.1146/annurev.arplant.53.091401.143329.

Zorrig, W., Rouached, A., Shahzad, Z., Abdelly, C., Davidian, J.C., Berthomieu, P., 2010. Identification of three relationships linking cadmium accumulation to cadmium tolerance and zinc and citrate accumulation in lettuce. J. Plant Physiol. 167, 1239–1247. https://doi.org/10.1016/j.jplph.2010.04.012.

Zou, J.Y., Chang, S.K.C., Gu, Y., Qian, S.Y., 2011. Antioxidant activity and phenolic compositions of lentil (Lens culinaris var. Morton) extract and its fractions. Agric. Food Chem. 59, 2268–2276.

Zuccarini, P., 2008. Ion uptake by halophytic plants to mitigate saline stress in *Solanun lycopersicon* L., and different effect of soil and water salinity. Soil Water Res 3, 62–73. https://doi.org/10.17221/25/2008-swr.