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Potential Use of Biochar as a Mitigation Strategy for Salinity-Related Issues in Tomato Plants (*Solanum lycopersicum* L.)

Eleonora Coppa, Giulia Quagliata, Rachele Venanzi, Alessandro Bruschini, Leonardo Bianchini, Rodolfo Picchio and Stefania Astolfi *

Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, via S.C. de Lellis, 01100 Viterbo, Italy; ecoppa@unitus.it (E.C.); giulia.quagliata@unitus.it (G.Q.); venanzi@unitus.it (R.V.); alessandro.bruschin@studenti.unitus.it (A.B.); l.bianchini@unitus.it (L.B.); r.picchio@unitus.it (R.P.)
* Correspondence: sastolfi@unitus.it

Abstract: The continuous growth of the population, along with climate change and the resulting surge in food demand, requires the development of alternative crop cultivation strategies that reduce the excessive use of freshwater for agricultural purposes. Biochar, which is a carbon-rich material made from organic waste through pyrolysis, has been recommended as a potential soil amendment to mitigate the negative effects of salinity. Biochar has unique properties such as high porosity, an ion exchange capacity, and the ability to retain water and nutrients. The purpose of this study was to evaluate the feasibility and effectiveness of using saline water for the cultivation of tomato plants (*Solanum lycopersicum* L.) and to investigate the potential use of biochar as a mitigation strategy for salinity-related issues in tomato cultivation. The concentration of NaCl during the experiment was 100 mM. We examined the impact of salt stress on plant growth, protein and chlorophyll content, the activation of the antioxidant response, and nutritional status. Our results indicated that salt treatments led to a significant accumulation of Na and Cl in shoots (regardless of the biochar addition) but did not result in a corresponding reduction in plant growth. However, the degree of oxidative damage caused by NaCl treatment, measured as malondialdehyde (MDA) accumulation, was reduced by biochar addition to the growth medium, most likely because of an increased guaiacol peroxidase (GPX) activity, which led to lower MDA accumulation. The strong positive effect of biochar on GPX activity could be reasonably attributed to increased Mo accumulation. In conclusion, the findings of this study represent a valuable starting point for developing crop management strategies based on biochar application to enhance plant performance under unfavorable conditions and reduce freshwater dependence in agriculture.

Keywords: biochar; salt stress; tomato; antioxidants; lipid peroxidation

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1. Introduction

Managing water resources is one of the most pressing challenges of the 21st century. This is due to the ever-increasing global population and the subsequent rise in food demand. According to the Intergovernmental Panel on Climate Change's report [1], the average global surface temperature reached 1.1 °C above 1850–1900 in 2011–2020 and is set to increase by approximately 1.4–4.8 °C by the end of this century. This will lead to a 10% decrease in rainfall due to climate change, resulting in significant water availability problems [2]. Arid and semi-arid regions may experience up to a 20% decrease in precipitation over the coming century [3,4], which will result in the depletion of good-quality irrigation water supplies [5]. Agriculture is the most affected sector due to water scarcity since it accounts for 70% of freshwater withdrawals [6]. With freshwater resources becoming scarce, alternative strategies are necessary to sustain crop cultivation. One such strategy is utilizing diluted seawater for crop cultivation since it is abundant and readily available [7,8].

Seawater is also rich in mineral nutrients that are essential for human diets and are often limited. Therefore, using seawater as irrigation water could also be an alternative strategy to enrich agricultural food production with substances that are required in the human diet [9,10].

Crop productivity does not seem to be affected by irrigating with a certain concentration of diluted seawater [10–14]; however, plants must cope with salt stress [15]. Salt stress is considered one of the most important factors that limit crop production. Salinity alters physiological processes by affecting osmotic homeostasis, causing nutritional imbalance and oxidative stress [16–18]. Therefore, the ability of plants to activate physiological responses to minimize oxidative stress under salt stress conditions is crucial for their survival. When plants are exposed to high saline concentrations, they experience oxidative stress, leading to an increased production of reactive oxygen species (ROS).

To cope with this adverse condition, plants have adopted strategies including the antioxidative system, which combines non-enzymatic (e.g., glutathione) and enzymatic (e.g., guaiacol peroxidase) antioxidants.

Biochar has been proposed as a potential soil amendment to mitigate the negative effects of salinity [19–26]. Biochar has unique properties that make it a promising candidate for improving soil conditions and plant resilience in saline environments [27,28]. These properties include its high surface area due to high porosity, an ion exchange capacity, an alkaline pH, and the capacity to retain water and nutrients or organic and inorganic pollutants. Biochar under salt stress conditions has been shown to have positive effects on plant growth and resilience by minimizing Na uptake by plants [20,22,23], increasing K availability and water retention, and improving soil physical and biological properties, photosynthesis, stomatal conductance, chlorophyll content, water use efficiency, and osmotic potential [20]. In addition, applying biochar under salt stress conditions reduces oxidative stress and improves antioxidant enzyme activity [19,20].

Biochar is a carbon-rich material derived from biomass or organic waste through different processes, including traditional charcoal production, slow/fast/microwave pyrolysis, gasification, hydrothermal and flash carbonization, and torrefaction [28–30]. Pyrolysis and gasification systems are mainly used today, with each process working with different temperatures, residence times, and product yields. Pyrolysis yields a higher biochar yield than gasification, which has a higher yield in syngas [28].

Research in Italy has shown the low economic value of woody assortments, particularly fuel wood. It is therefore crucial to investigate novel solutions for advancement in this area and/or retrieve traditional practices that are improved with new technologies. Wood charcoal is a feasible example of a traditional practice that could be exploited for innovative and effective applications linked with the food and pharmaceutical industries, as well as agricultural systems, such as biochar [31].

The current study aimed to assess the feasibility and effectiveness of utilizing saline water as an irrigation source for the cultivation of tomato plants (*Solanum lycopersicum* L.). Tomato is considered a moderately tolerant crop to salt stress [32,33], and the impact of the effects of salinity on the morphology, physiology, biochemistry, yield, fruit quality, and gene expression of tomato plants has been recently reviewed [34].

The study also aimed to evaluate the potential use of biochar as a mitigation strategy for salinity-related issues in tomato cultivation. To achieve this, the effect of salt stress alone or combined with biochar as an addition to the growth medium on plant growth, chlorophyll content, the activation of the antioxidant response, and nutritional status was evaluated.

2. Materials and Methods

2.1. Biochar Production and Characterization

Biomass pyrolysis is a process of thermo-chemical conversion [35]. The characteristics of the resulting biochar depend on the feedstock type, as well as the pyrolysis temperature. This study employs the pyrolysis process of all samples at a maximum temperature of

450 °C, using a prototype of a modern horizontal charcoal kiln, developed in our laboratory [31,35]. This novel kind of kiln is characterized by a metal structure that can be loaded on trucks or tractors and placed at the landing site. It has a loading space for fuel wood and a small opening to maintain fire, with an automatic woodchip burner and a rear chimney.

To produce biochar, a seamless carbonization cycle was followed, consisting of the following steps: (i) drying, which took 3 h from 30 °C to 150 °C; (ii) first carbonization, which took 90 min from 120 °C to 200 °C; (iii) second carbonization, which took 90 min from 200 °C to 250 °C; (iv) third carbonization, which took 120 min from 250 °C to 450 °C; and (v) final carbonization, which took 12 h from 450 °C to 50 °C. During the final carbonization step, all air accesses were blocked, and the introduction of thermal energy into the process was stopped, causing the temperature to slowly decrease from 450 °C to about 50 °C in approximately 12 h. Environmental conditions, including minimum and maximum temperatures, humidity, and wind direction, were recorded during the carbonization process.

Once the biochar was produced, it was thoroughly washed with boiling distilled water [36] to clean it. At the end of the test run, the residues (biochar) underwent visual, physical, and chemical evaluations. The wood biomass was obtained from processing P16 (ISO 17225-4) [37] pine wood chips (*Pinus nigra* Arnold) obtained by chipping whole trees which grew in pure forests on the Amiata mountain. The biochar was obtained over 3 cycles (carbonization processes), and four replicates were used for each cycle and each parameter. The heating value was determined according to EN ISO 18125 [EN ISO 18125: 2018 [38]. Solid biofuels—Determination of Heating Value]. The particle size of the biochar obtained was similar to the original wood chips' dimensions (P16 according to ISO 17225-4) [37].

The higher heating value (HHV) was determined using the calorimeter Anton Paar 6400 isoperibol oxygen bomb calorimeter (Moline, IL, USA). Twelve samples of shredded biomass were prepared using a pellet mill, the Pellet Press 2810 (Parr Instrument Company, Moline, IL, USA), to produce tablets, weighing 1 g each. Before each single set of analyses, the instrument was calibrated with benzoic acid. The bulk density was evaluated based on EN ISO 17828 [39] [EN ISO 17828: 2016. Solid biofuels—Determination of bulk density]. A standard container (PR/BDA Ray-Ran apparatus USA, Moline, IL, USA) was filled with a certain amount of shredded material of a given size and shape and then weighed. The bulk density was calculated from the net weight per standard volume and reported with the determined moisture content. The moisture content was determined according to EN ISO 18134-1 [40] [EN ISO 18134-1: 2015. Solid biofuels—Determination of moisture content—Oven dry method], using a Memmert UFP800 drying oven (Büchenbach, Germany). The samples were taken to the laboratory, where they were oven-dried at 103 ± 2 °C until a constant weight was achieved (weight variation not exceeding 0.2% during a further drying period of 60 min). The moisture content was calculated as a percentage of weight loss before and after the drying process.

The pH value was measured using a potentiometric analysis with a S500 Mettler-Toledo pH meter (COLUMBIA, MD 21045, USA), using biomass/saline solution suspensions (biomass-KCl 1 M) in a 1:2.5 proportion.

Organic matter measurement was performed via incineration in a muffle furnace, the Nabertherm L3/11 (Lilienthal, Germany), at 400 °C for 4 h following the thorough elimination of water and pre-treatment at 160 °C for 6 h. Using the direct method with the conversion factor of Van Bemmelen (1.724), the amount of organic carbon was assessed [41].

To assess the inorganic carbon content, the measurement was carried out via incineration in a mitten at 1000 °C for 2 h. Inorganic carbon was obtained by observing the weight loss between 400 °C and 1000 °C and multiplying it with a conversion constant of 0.273 to convert the mass of CO_2 to the mass of carbon. During these processes, the samples were weighed with a laboratory scale (0.0001 g) (Sartorius BCE64-1S, 37079 Goettingen, Germany) [41].

The characterization of biochar in terms of the C, H, and N contents was carried out based on the standard EN-15104 via combustion and subsequent gas-phase chromato-

graphic separation and measurement in an elemental analyzer (EMA 502, Velp Scientifica, Usmate, Italy). The Cl concentration was determined according to EN 15289, using a digestion step based on bomb combustion in oxygen and absorption in NaOH (0.05 M), followed by measurement via ion chromatography (Eco IC Analyzer, Metrohm, Italy). For the determination of the contents of major and minor ash-forming elements (excluding Cl), multi-step pressurized digestion with HNO_3 (65%)/HF (40%)/H3BO3, followed by measurement via inductively coupled plasma–optical emission spectroscopy (ICP–OES) (Agilent 5800, Santa Clara, CA, USA) or inductively coupled plasma–mass spectroscopy (ICP–MS) (Agilent 7850, Santa Clara, CA, USA), depending upon the detection limits required [42]. For the total organic carbon (TC) analyses, an aliquot was treated with acid, and the generated CO_2 was measured using infrared (IR) (Gas Analyzer Gasmet FTIR CX4000 ANSYCO GmbH, 76131 Karlsruhe, Germany). The Cl concentration of the ashes was measured using ion chromatography (Eco IC Analyzer, Metrohm, Italy) after elution for 24 h with deionized water.

The cation exchange capacity of the biochar used for this study was measured via extraction with barium chloride ($BaCl_2$ $2H_2O$) according to the method described in [43] and recommended by the Soil Science Society of America.

2.2. Plant Growth

Tomato seeds (*Solanum lycopersicum* L. cv. Marmande) were germinated in perlite in the dark, to reproduce natural germination conditions, at 20 °C for 1 week, and then uniform seedlings were put in plastic pots (2 seedlings per pot) containing perlite as a medium (control condition, C) or a mixture of perlite + biochar (10% *w*/*w*) (biochar condition, B). The pots were irrigated once daily, alternating every other day with distilled water or a complete nutrient solution (NS) [44], to maintain the soil relative water content (SRWC) at 75%. The water regime was manually controlled by weighing the pots and adding the amount of water or NS needed to reach the desired SRWC, calculated according to the field capacity. In particular, NS was applied from above every other day (three times per week, Monday, Wednesday, and Friday) and distilled water on the other days (three times per week, Tuesday, Thursday, and Saturday). During the whole experiment, the water content in individual pots was controlled through daily weighing. After 14 days from transplant in the pots, half of the plants from both the C and B conditions were treated with 100 mM of NaCl. As a result, four conditions were obtained: control ©, salt stress (S), biochar (B), and combined biochar/salt stress (BS). The salt treatment was repeated 3 times, on the 4th, 7th, and 9th day from the first application. The plants were grown in a growth chamber under a 200 µmol m^{-2} s^{-1} photosynthetic photon flux and a 16/8 h day/night regime (28/20 °C air temperature, 80% relative humidity). The experiment was arranged as a completely random design with three replications (pots), and the pots were randomly moved daily to minimize position effects. The plants were harvested 24 days after transplant and their shoot tissues were analyzed.

2.3. Determination of the Concentration of Macro- and Micronutrients in Tomato Plants

The nutrient concentration in the shoot tissues was measured after drying the samples at 121 °C for 24 h. Approximately 0.06 g (dry weight (DW)) of the shoot tissue was mineralized in 3 mL of ultrapure HNO_3 (69.5%) and 0.6 mL of HCl (37%) using a Microwave digestion system (Multiwave Go Plus, Anton Paar GmbH, Graz, Austria). Mineralized samples were filtered and diluted to 1:200 with Milli-Q water. The concentrations of nutrients were measured using inductively coupled plasma–mass spectroscopy (Agilent 7850 ICP-MS, Santa Clara, CA, USA).

2.4. Determination of Malondialdehyde Concentration

The level of lipid peroxidation was expressed as the malondialdehyde (MDA) concentration and was determined as thiobarbituric acid (TBA)-reactive metabolites, according to Quagliata et al. [45] Briefly, fresh shoot tissues (0.2 g) were homogenized in 10 mL of

0.25% TBA made in 10% trichloroacetic acid (TCA). The extract was heated at 95 °C for 30 min and then quickly cooled on ice. After centrifugation at 10,000× *g* for 10 min, the absorbance of the supernatant was measured at 532 nm. The correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was expressed as mmol g^{-1} fresh weight by using an extinction coefficient of 155 mM cm^{-1}.

2.5. Determination of Non-Protein Thiol Concentration

Water-soluble non-protein thiol compounds were determined colorimetrically with 5,5′dithio-bis-(2-nitrobenzoic acid) (DTNB), following the procedure reported by Quagliata et al. [45]. Briefly, both shoot and root tissues (1 g FW) were ground in liquid nitrogen and extracted in 3 mL of a solution composed of 80 mM of TCA, 1 mM of ethylenediaminetetraacetic acid (EDTA), 0.15% (*w*/*v*) ascorbic acid, and 10% (*w*/*v*) polyvinylpolypyrrolidone (PVP). After a centrifugation step (30 min at 4000× *g* and 4 °C), the supernatant was recovered, and the concentration of DTNB-reactive compounds was detected spectrophotometrically at 415 nm (Agilent Cary 3500 UV-Vis Spectrophotometer, Santa Clara, CA, USA).

2.6. Extraction and Determination of Guaiacol Peroxidase (GPX) Activity

Guaiacol peroxidase (E.C. 1.11.1.7) activity was measured spectrophotometrically (Agilent Cary 3500 UV-Vis Spectrophotometer) at 470 nm using guaiacol as a hydrogen donor [46]. Briefly, shoot tissues (0.5 g, fresh weight) were powdered in a pre-chilled mortar with liquid N_2, and then homogenated using an extraction buffer at pH 5.0 and added in a ratio of 1:2 (*w*/*v*). The enzyme activity was linear with time and proportional to the amount of extract used.

2.7. Other Measurements

The protein concentration in the extracts of leaf tissues was determined according to the dye-binding method of Bradford [47], using bovine serum albumin (BSA) as the standard.

The chlorophyll content was measured on the youngest fully expanded leaf of the tomato plants and expressed as SPAD units, using a non-destructive portable apparatus, the Soil Plant Analysis Development (SPAD-502 Plus, Konica Minolta, Osaka, Japan), both before the imposition of salt stress (14 days after transplant) and at harvest (24 days after transplant).

The shoot Cl concentration was determined turbidimetrically at 460 nm using the mercury (II) thiocyanate method after extraction with dilute acetic acid [48].

2.8. Statistical Analysis

Concerning the biochar characterization, all data were statistically analyzed with the statistical package STATISTICA (StatSoft, Inc. 2004, version 6, Tulsa, OK, USA) to assess the existence (or lack thereof) of variability among the three cycles (carbonization tests) analyzed. First of all, compliance with the main statistical assumptions was conducted with Levene's test (equality of variances) and the Shapiro–Wilk test (normality). Finally, a random-effects ANOVA model was used.

For physiological analysis, each reported value represents the mean ± standard deviation (SD) of measurements carried out in triplicate and obtained from three independent experiments (biological replicates). All data were statistically analyzed to compare four treatment conditions (C, S, B, BS) via a one-way analysis of variance (ANOVA) with Tukey's post hoc tests at $p < 0.05$, using the statistical software Costat (version 6.451). Using the PAST 4.0.3 software, a principal component analysis (PCA) of the nutrient concentrations (Na, Mg, K, Ca, Mn, Fe, Zn, Mo, and Cl) was applied.

3. Results

3.1. Biochar Characterization

Table 1 summarizes the characteristics of biochar derived from pine feedstock through slow pyrolysis at an average temperature of 450 °C. The results of the random-effects ANOVA test applied did not show differences among the three carbonization cycles analyzed.

Table 1. Biochar characterization. Energetic, physical, and elementary characterization of pine biochar obtained via pyrolysis at 450 °C. SD, standard deviation. Random-effects ANOVA results were applied among the three independent carbonization processes.

Parameter	Average	SD	*p*-Value
Carbonization yield	28%	3.8%	>0.05
Bulk density (kg m^{-3})	138.2	11.8	>0.05
HHV (MJ kg^{-1})	32.6	1.9	>0.05
Percentage of calorific value from pyrolysis	80%	12%	>0.05
H	1.29%	0.52%	>0.05
C	84.20%	11.18%	>0.05
N	0.84%	0.11%	>0.05
O	1.33%	0.69%	>0.05
Carbonate as CO_2	2.85%	0.54%	>0.05
Carbonate (organic) *	74.1%	11.2%	>0.05
Sulfur (total)	0.03%	0.01%	>0.05
H/C	0.21	0.08	>0.05
O/C	0.014	0.002	>0.05
C/N	201.5	81.2	>0.05
Ash	4.50%	1.21%	>0.05
pH	8.79	1.1	>0.05
Specific Surface Area (m^2 g^{-1})	274	10.4	>0.05
Volatile matter	4.1%	0.14%	>0.05
Electrical conductivity (µS cm^{-1})	341	18.2	>0.05
Salt content (g kg^{-1})	0.412	0.05	>0.05

* Carbonate in biochar can be derived from the mineral fraction of the original feedstock or from CO_2 (e.g., evolved from organic C during pyrolysis) trapped in the alkaline charred material.

The bulk density values were similar to those found in other studies [49]. The product was characterized by positive characteristics such as a good porosity value, resulting in adequate aeration of the growth medium, and potentially, in a positive impact on microbial respiration (Table 1). The surface area showed interesting values, and this is consistent with similar studies carried out on other lignocellulosic materials [50].

Volatile forms have reached important levels that may explain some microbial and plant responses observed following the addition of biochar to the soil. However, it is not clear how this interaction takes place.

The electrical conductivity values were medium–high, most likely as a result of an increased number of ions due to the increased ash fraction [51] (Table 1).

The results of the heavy-metal contaminant analysis (shown in Table 2) revealed that the recorded values were below the limit of quantitation (<LQ) (2 mg kg^{-1}) in all samples. The sixteen USEPA PAHs measured in the study were below the limit of detection (0.5 mg kg^{-1}). The results of the random-effects ANOVA test applied did not show differences among the three carbonization cycles analyzed.

Table 2. Mineral and PAH concentrations. Concentration of minerals and PAHs in pine biochar obtained via pyrolysis at 450 °C. SD, standard deviation. Random-effects ANOVA results were applied among the three independent carbonization processes.

Concentration (mg kg^{-1})	Average	SD	*p*-Value
P	590	22	>0.05
Mg	1300	95	>0.05
Ca	12,500	250	>0.05
K	5500	121	>0.05
Na	320	10	>0.05
Fe	1550	120	>0.05
Si	9900	99	>0.05
S	290	13	>0.05
Pb	30	5	>0.05
Cd	0.15	0.02	>0.05
Cu	30	2.1	>0.05
Ni	7	1.2	>0.05
Hg	0.02	0.01	>0.05
Zn	90	9.2	>0.05
Cr	21	1.6	>0.05
B	26	2.7	>0.05
Mn	350	62	>0.05
Benzo(k)Fluoranthene	<0.1	-	-
7,12-Dimethylbenz(a)anthracene	<0.1	-	-
Fluoranthene	<0.1	-	-
Naphthalene	0.4	0.01	>0.05
Phenanthrene	0.2	0.02	>0.05
SUM PAHs (EPA)	0.92	0.3	>0.05

3.2. Plant Growth Parameters

The performance of tomato plants exposed to different growth conditions was evaluated by measuring changes in the shoot fresh weight and protein concentration (Figure 1A,B) and chlorophyll content (Figure 2).

The presence of biochar in the growing medium (B condition) stimulated the shoot biomass production by 29% compared to the control (C) (Figure 1A). The treatment with 100 mM of NaCl used for the imposition of salt stress had no significant effect on the shoot biomass of plants grown on perlite (S condition). On the other hand, plants grown under the BS condition (combined biochar/salt stress) showed a significant decrease (23%) in shoot biomass production compared to their relative control (B), although no significant difference between these plants and the control (C) plants was found. Plants grown in the presence of biochar and exposed to salt stress (BS condition) contained a higher protein concentration in their shoot tissue than plants from the other three conditions (about 30%), but this difference was not statistically significant (Figure 1B).

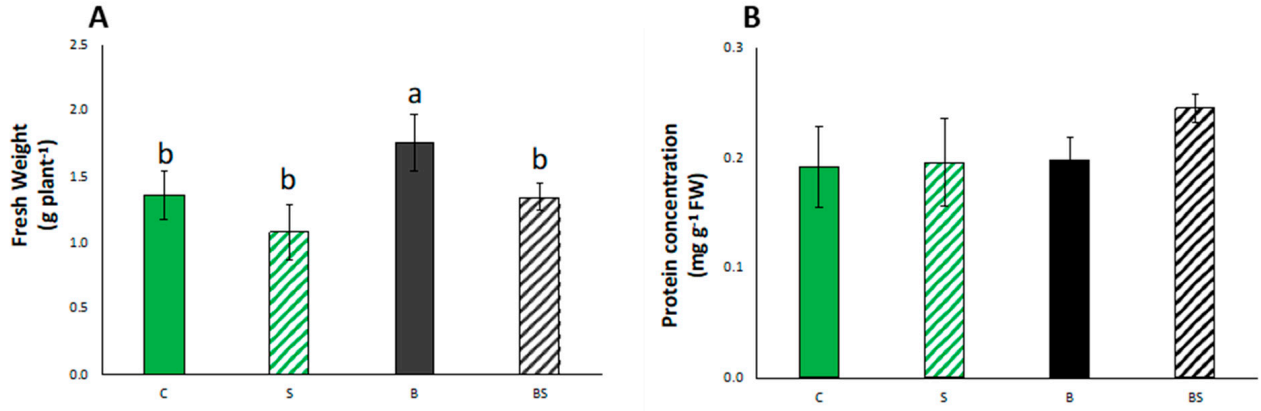

Figure 1. Growth parameters. Fresh weight (**A**) and protein concentration (**B**) of shoots from tomato plants grown in pots in four different growth conditions: control (C), salt stress (S), biochar (B), and combined biochar/salt stress (BS). Data are reported as the mean of three biological replicates ± SD (n = 3). The statistical significance was determined via a one-way ANOVA analysis with Tukey's post hoc test ($p < 0.05$). Different letters indicate significantly different values among the growth conditions.

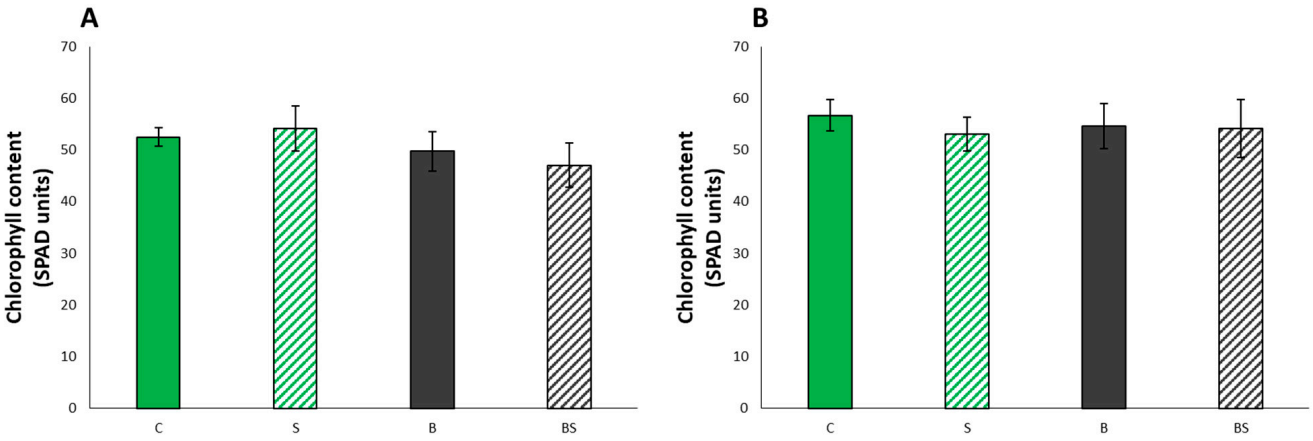

Figure 2. Relative chlorophyll content. Chlorophyll content in leaves of tomato plants, measured as SPAD readings, before (**A**) and after (**B**) salt stress (NaCl, 100 mM) imposition. Plants were grown in pots in four different growth conditions: control (C), salt stress (S), biochar (B), and combined biochar/salt stress (BS). Statistics as in Figure 1.

Concerning the relative chlorophyll content, measured as SPAD readings, plants exposed to 100 mM of NaCl did not show symptoms of salt stress injury, such as the chlorosis of leaves, as reported in Figure 2 (A and B, before and after NaCl treatments, respectively). In addition, the presence of biochar in the substrate did not result in changes in the SPAD values throughout the experimental period (Figure 2).

3.3. Lipid Peroxidation

Figure 3 shows that the application of 100 mM of NaCl did not significantly impact lipid peroxidation. However, when NaCl application was combined with the presence of biochar in the substrate (BS condition), the level of lipid peroxidation decreased. This was demonstrated by the lower MDA level in these plants, which was 32% lower compared to plants grown in the S substrate.

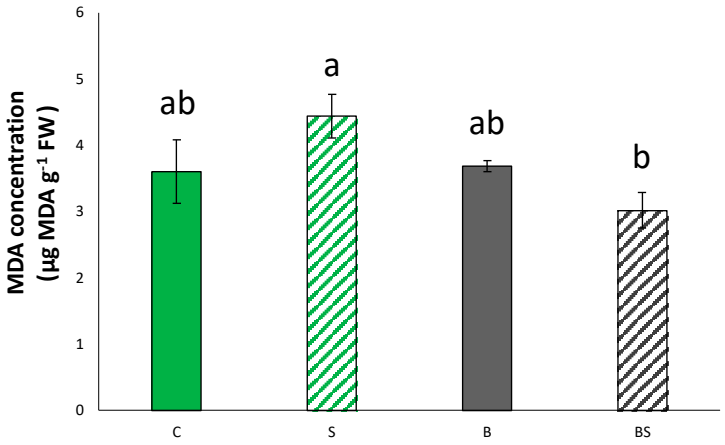

Figure 3. Degree of lipid peroxidation. Malondialdehyde (MDA) concentration in shoot tissues of tomato plants grown in pots in four different growth conditions: control (C), salt stress (S), biochar (B), and combined biochar/salt stress (BS). Statistics as in Figure 1.

3.4. Non-Enzymatic and Enzymatic Antioxidant Response

Thiols are compounds that contain a sulfhydryl group (-SH), which includes cysteine and glutathione. These compounds play a crucial role in regulating redox potential to reduce the harmful effects caused by the production of reactive oxygen species (ROS) during oxidative stress. Figure 4A shows that the presence of biochar in the substrate resulted in a significant reduction (−30%) in thiol levels in plant shoots. However, NaCl treatment did not produce a significant change in the level of these compounds, regardless of the presence or absence of biochar in the substrate.

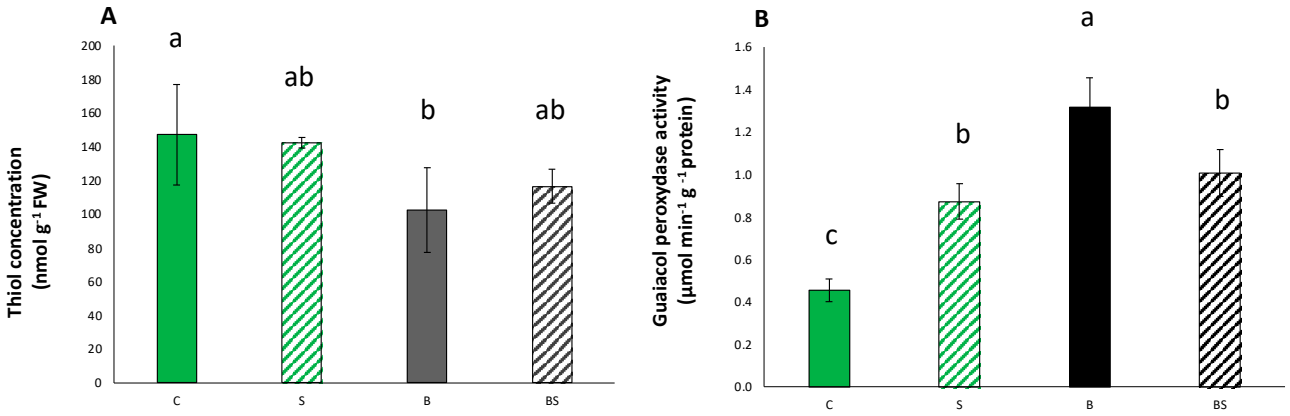

Figure 4. Non-enzymatic and enzymatic antioxidant response. Thiol concentration (**A**) and guaiacol peroxidase activity (**B**) in shoots of tomato plants grown in pots in four different growth conditions: control (C), salt stress (S), biochar (B), and combined biochar/salt stress (BS). Statistics as in Figure 1.

Figure 4B demonstrates that the presence of biochar in the growing medium led to a 132% increase in GPX activity compared to the control (C). Interestingly, NaCl treatment increased GPX activity in plant shoots by 54% compared to the control (C). However, the combination of salt and biochar treatment resulted in a significant decrease (−24%) in GPX enzyme activity compared to its relative control (B).

3.5. Shoot Elemental Composition

This study examined the impact of biochar in the growing medium and NaCl treatment on nutrient accumulation in plant shoots, including Na, Cl, Mg, K, Ca, Fe, Mn, Zn, and Mo. The results showed that biochar had no significant effect on ion accumulation in the shoots

(Figure 5). However, there were notable differences in elemental composition between plants treated and untreated with NaCl (Figure 5).

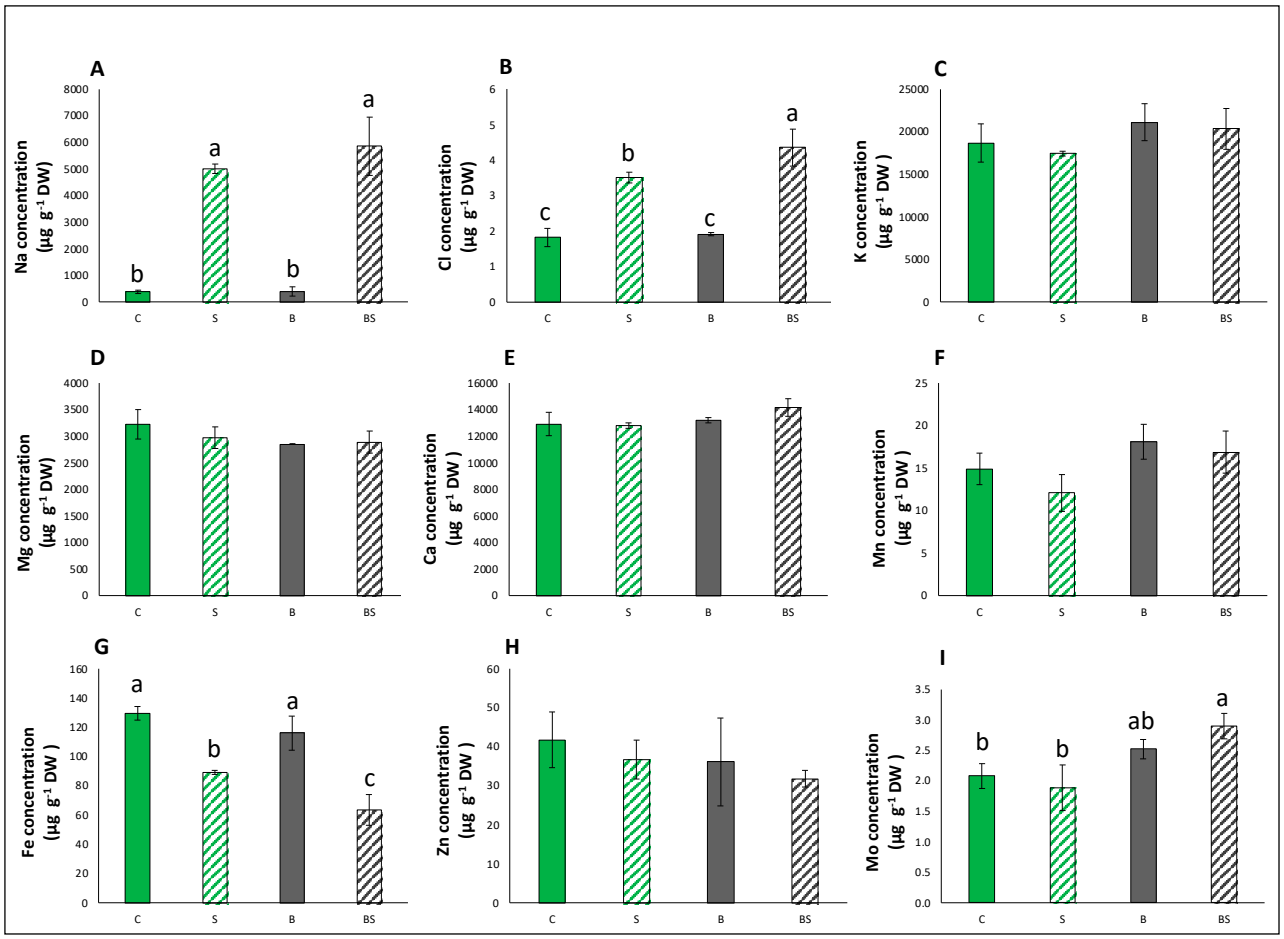

Figure 5. Shoot ionomic composition. Na (**A**), Cl (**B**), K (**C**), Mg (**D**), Ca (**E**), Mn (**F**), Fe (**G**), Zn (**H**), and Mo (**I**) concentrations detected in shoots of tomato plants grown in pots in four different growth conditions: control (C), salt stress (S), biochar (B), and combined biochar/salt stress (BS). Statistics as in Figure 1.

The salt stress caused by NaCl treatment at 100 mM significantly increased the accumulation of Na and Cl in tomato shoots (Figure 5A,B). The concentration of K remained unchanged (Figure 5C), and the Na/K ratio increased (data not shown). Interestingly, the concentration of Na and Cl was higher in plants grown with biochar than those grown without biochar (13-fold Na and 128% Cl than in those grown without biochar, 12-fold Na and 93% Cl) (Figure 5A,B).

The Fe accumulation in shoot tissues decreased significantly by 31 and 45% in plants treated with NaCl, with or without biochar in the medium (Figure 5G). However, there was a considerable increase in Mo accumulation (40%) in plants grown with biochar under salt stress conditions (Figure 5I).

There were no significant changes in the concentration of Mg (Figure 5D), Ca (Figure 5E), Mn (Figure 5F), and Zn (Figure 5H) among plants subjected to different growth conditions.

To evaluate the relationship between treatment and ionome in treated and untreated samples, a principal component analysis (PCA) was performed on the concentrations of all elements (Na, Mg, K, Ca, Fe, Mn, Zn, Mo, and Cl) measured in the shoots of tomato plants from the four different growth conditions (Figure 5).

The first two components of the PCA described 93% of the variance, and discriminated the treatments into four different clusters (Figure 6). PC1 clearly separates plants grown without biochar in the substrate (C and S conditions) from plants grown with biochar (B and BS conditions), whereas PC2 clearly separates plants not treated with NaCl at 100 mM (C and B conditions) from plants exposed to salt stress (S and BS conditions). the loading plots showed that Mo, Cl, Na, and Ca were those which contributed most to the positive side of PC1, while Fe, Zn, and Mg were the strongest contributors along the negative side of PC1. On the other hand, K and Mn drove the separation along the positive side of PC2, while the negative direction was only loaded by Na and Cl.

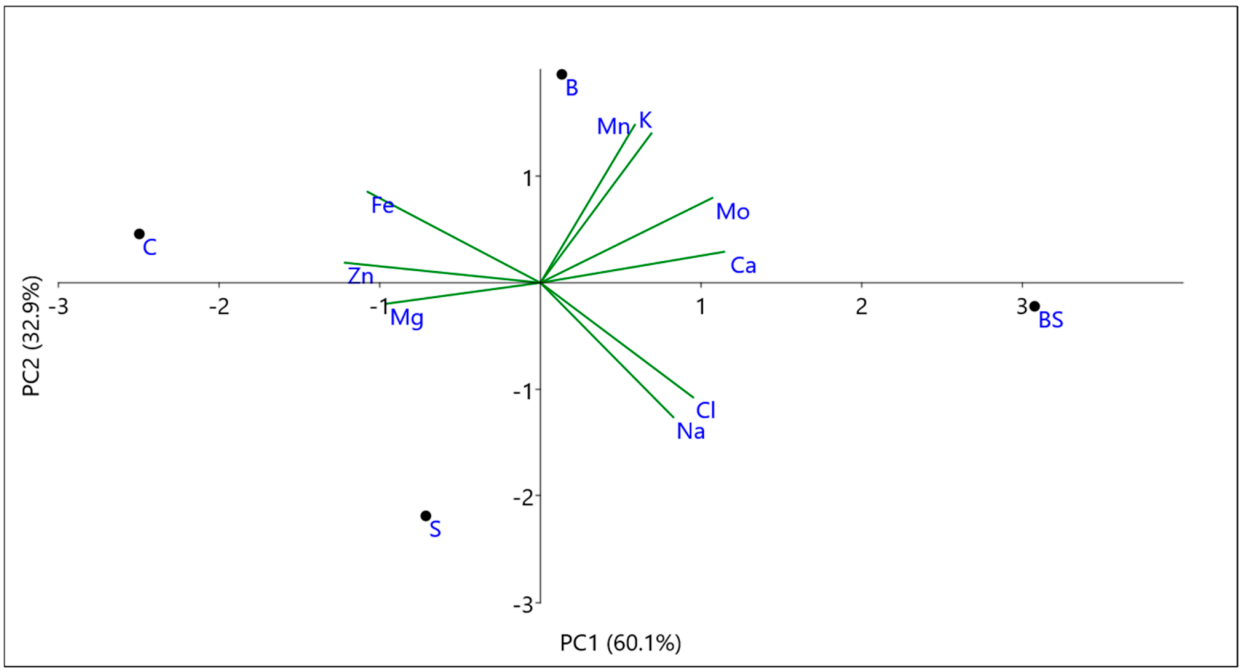

Figure 6. Principal component analysis (PCA). PCA scatter plot of shoot ionomic composition (Na, Mg, K, Ca, Mn, Fe, Zn, Mo, and Cl) of tomato plants grown in pots in four different growth conditions: control (C), salt stress (S), biochar (B), and combined biochar/salt stress (BS).

4. Discussion

Water availability is a crucial factor that affects human life, agriculture, and the environment, especially in arid, semi-arid, and tropical regions [5]. In coastal areas or places where freshwater resources are limited, using seawater for irrigation, which can be desalinized, blended, or diluted, could be an effective solution to tackle water scarcity [7].

Biochar has gained attention for its potential use as a mitigation strategy for salinity-related issues in tomato cultivation [24]. However, its effectiveness mainly depends on its chemical characteristics, which in turn depend on the feedstock, as well as the pyrolysis temperature. Here, it is reported whether and how our biochar can be utilized to alleviate the impact of salt stress in tomato production under controlled conditions.

In these experiments, a NaCl concentration of 100 mM was used, which is roughly equivalent to the NaCl concentration found in 1:6 diluted seawater [52].

The effectiveness of biochar produced from pine feedstock for agricultural purposes was thoroughly analyzed. This material is known for its high variability, as stated by other authors [28,52–55]. Studies have identified feedstock and temperature as the most important parameters for biochar production from woody and herbaceous biomasses [56]. Temperature was found to be the main factor influencing yield, which decreased as the temperature increased [49]. The biochar used in this study was produced at a low pyrolysis temperature of 450 °C, which resulted in a theoretically suitable material for this research. The surface area values found for the biochar were consistent with those found in similar

studies carried out on similar lignocellulosic materials [50]. The electrical conductivity values were medium–high and likely due to the increase in the ion amount, resulting from the increase in the ash fraction [51], closely related to the pyrolysis temperature (Table 1). The relationship between the ash content and pyrolysis temperature for biochar is influenced by multiple factors, such as the type of feedstock and pyrolysis temperature. Generally, increasing the pyrolysis temperature leads to a reduced ash content in the resulting biochar [57]. Polycyclic aromatic hydrocarbons (PAHs) are toxic organic compounds that form during biomass pyrolysis. The initial limit value for PAHs in biochar was set by the IBI (International Biochar Initiative) at 20 mg kg^{-1} [50], and later increased from 20 mg kg^{-1} to 300 mg kg^{-1} [58]. Recent studies have shown that PAH concentrations in biochar produced at 400–750 °C usually range from 0.4 to 2000 mg kg^{-1} [59]. Further studies have demonstrated that lowering the pyrolysis temperature (to less than 500 °C) prevents lignin from being converted into a hydrophobic polycyclic aromatic hydrocarbon (PAH) [60]. Therefore, the total concentration of PAHs in our biochar produced from pine wood at 450 °C was 0.92 mg kg^{-1}, with naphthalene being the dominant PAH. Naphthalene is the only PAH with two aromatic rings and is the least toxic one [61].

The use of biochar in agriculture is related to its ability to retain and release cations, which is measured based on its cation exchange capacity (CEC). Studies have shown that the CEC of biochar decreases as the pyrolysis temperature increases [62,63]. Moreover, the CEC values depend on the starting feedstock [51], with biochar derived from broadleaf species possessing a higher CEC than that from conifers (black pine) [54]. The biochar produced in this study had low CEC values (approximately 13 cmolc kg^{-1}), but still had a suitable surface area.

By evaluating the potential of biochar to mitigate salinity-related issues in tomato cultivation, we found a first interesting finding. Biochar addition to the growth medium had a beneficial effect on the shoot growth of plants not exposed to salt stress (Figure 1). Previous studies have suggested that biochar can enhance plant performance through various mechanisms, such as improving the retention of nutrients and water in the substrate and releasing nutrients [20,64–66].

As discussed earlier, the biochar used in the experiments had a CEC of approximately 13 cmolc kg^{-1}, which was moderately low. Therefore, its ability to retain nutrients is rather limited. Furthermore, it does not play a significant role in releasing nutrients, except for molybdenum (Figure 5I). The experiment did not directly investigate the impact of biochar on nitrogen (N) and phosphorus (P) concentrations. However, since both nutrients are involved in protein synthesis, we can reasonably assume that there was no effect of biochar on the accumulation of these elements in plant tissues, as no significant differences were found among treatments for protein concentrations (Figure 1B). The presence of NaCl reduced the growth of plants grown in the presence of biochar (BS condition), but the shoot biomass of these plants was still not significantly different from that of the control plants (C condition). Furthermore, salt stress did not affect the chlorophyll content of the plants, regardless of the presence of biochar in the growth medium (Figure 2B). Leaf chlorophyll content is often used as an indicator of salt stress-induced effects [24,67,68]. Previous studies have suggested that chlorosis resulting from salt treatments can be attributed to a limited nitrogen uptake [69] and/or osmotic stress. However, our data indicated that the salt stress caused by NaCl in the medium had little to no effect on the shoot biomass and chlorophyll level, most likely due to the acknowledged moderate tolerance of tomato crops to salt stress [32,33]. However, both the Na and Cl concentrations in the shoot tissues of the tomato plants strongly increased following NaCl treatment (Figure 5A,B). In particular, plants grown without biochar had a Na level that was 12 times higher than the control, while plants grown with biochar had a Na level that was 13 times higher than the control (as shown in Figure 5A). Similarly, the Cl level increased by 93% and 128% in plants grown without and with biochar, respectively (as shown in Figure 4B). It is worth noting that most crops are more susceptible to Na toxicity than Cl toxicity [70,71]. Excessive amounts of Cl can impact cell division and photosynthesis efficiency [55,72]; however, this study did not

find any effect on plant growth or chlorophyll content despite the high Cl accumulation. This suggests that the salt stress did not have any significant impact on the photosynthetic activity of the tomato plants. A high Na concentration is known to trigger oxidative stress and impair osmotic homeostasis, which can cause a nutrient imbalance [16].

The harmful effects of salt stress on plants, such as the production of free radicals and reactive oxygen species (ROS), have been well-documented. These can cause damage to macromolecules and cellular integrity [73]. One of the earliest symptoms of oxidative stress is lipid peroxidation, which can be measured by assessing the degree of oxidative damage through the concentration of MDA [74]. In our experiment, we found that the MDA concentration increased by 23% in tomato plants grown without biochar (S condition) when treated with NaCl, compared to the C plants (Figure 3). However, the tomato plants grown with combined biochar/salt (BS condition) showed a lower accumulation of MDA (−32%) than those from the S condition (Figure 3). Previous studies have also shown that biochar addition to the growth medium can inhibit the increase in MDA accumulation caused by saline treatment in bean [19] and tomato [24] plants. Therefore, our results suggest that, although the presence of biochar in the medium did not reduce the accumulation of Na and Cl, it can somehow mitigate salt toxicity and the resulting oxidative damage. This effect could be attributed to biochar's high carbon content, which, acting as a source of stable organic carbon, may serve as an electron buffer reducing the impact of ROS on plant cells [75]. Furthermore, it has been demonstrated that biochar can allow plants to alleviate salt stress effects by promoting the biosynthesis and activity of some antioxidative enzymes, such as ascorbate peroxidase [19,76]. However, further studies are required to understand the mechanisms of action of applied biochar on the plant physiological and biochemical responses.

Plants have a complex antioxidant system that combines enzymatic and non-enzymatic components to mitigate oxidative stress [77–79].

The concentration of thiol compounds, indicating the second mechanism and directly reflecting the glutathione concentration [80], remained unaffected by the NaCl treatments, independent of biochar addition (Figure 4A).

We measured the guaiacol peroxidase (GPX) activity, an indicator of antioxidant enzyme activity [81], and found a significant negative correlation between GPX activity and the level of thiols in the shoot tissues (R^2 = 0.872) (Table 3). The GPX activity increased significantly in the NaCl-treated plants, especially in those grown with biochar, suggesting a biochar-mediated GPX induction. Moreover, we found that the highest GPX activity was in the shoots of plants grown with biochar and not exposed to salt stress (Figure 4B). It is well known that molybdenum (Mo), playing a role in the activation of antioxidative enzymes, such as superoxide dismutase, ascorbate peroxidase, and glutathione reductase, indirectly contributes to a plant's antioxidative defense mechanisms [82]. Previous studies have shown that exogenous molybdenum treatments can stimulate GPX activity [83–85]. Therefore, the higher GPX activity in plants grown with biochar and NaCl might be due to the higher Mo accumulation in those plants (Figure 5I). The beneficial effects of Mo in reducing oxidative stress by lowering MDA levels in plants exposed to abiotic stress could be explained by its role in the modulation of endogenous nitric oxide (NO) accumulation, which in turn exerts positive effects on the upregulation of antioxidant defense induced by Mo [86]. Thus, it is reasonable to suggest that the endogenous Mo accumulation observed in the present work could regulate ROS homeostasis through a NO-dependent pathway, although further detailed investigations are needed.

Table 3. Correlation between MDA concentration and Mo concentration, and between GPX activity and thiol concentration (in brackets, the slopes) ($p \leq 0.05$).

	MDA	**GPX**
Mo	R^2 0.764 (−1.117)	-
Thiols	-	R^2 0.872 (−63.952)

Although the increase in Mo concentration in the biochar-treated plants was not significant compared to the control plants, we found a significant negative correlation between Mo accumulation and MDA production in the shoot tissues (R^2 = 0.764) (Table 3). Consequently, a biochar-enriched medium might stimulate GPX activity, which reduces oxidative damage, thus resulting in lower MDA accumulation (under the BS condition) (Figure 3) and a lower production of thiols (under the B condition) (Figure 4A).

Many studies have shown that salt stress can cause an imbalance of nutrients in plants [16,87–91]. We conducted experiments and found that, when plants were treated with NaCl, the ionomic composition of their shoot tissues changed (as shown in Figures 5 and 6). Through a principal component analysis (PCA), we were able to identify four different clusters. The first principal component (PC1) distinguished between plants grown with and without biochar in the substrate (C and S conditions versus B and BS conditions). On the other hand, the second principal component (PC2) separated plants that were not exposed to a NaCl concentration of 100 mM (C and B conditions) from those that were exposed to salt stress (S and BS conditions) (as illustrated in Figure 6).

Previous studies have shown that salt stress can have a negative impact on Fe [92–96]. Our own research found a strong negative correlation between the accumulation of Na and Fe (R^2 = 0.940), as well as between Cl and Fe (R^2 = 0.966) (see Table 4). This correlation is likely due to salt's effect on either Fe(III)-chelate reductase activity or the plant's ability to acidify the rhizosphere soil [97]. Interestingly, biochar application did not significantly affect nutrient accumulation, except for Mo. Indeed, the tomato plants exposed to salt stress and grown with biochar showed a significantly higher concentration of Mo in their shoot tissues (see Figure 5I).

Table 4. Na and Cl effect on Fe accumulation. Correlation between Na and Fe concentration, and between Cl concentration and Fe concentration (in brackets, the slopes) ($p \leq 0.05$).

	Na	**Cl**
Fe	R^2 0.904 (−95.185)	R^2 0.966 (−0.042)

To summarize, the tested biochar met the contaminant burden guidelines established by the International Biochar Initiative. Irrespective of the biochar addition, the salt treatments resulted in a substantial accumulation of sodium (Na) and chloride (Cl) in the shoots, but this did not lead to a corresponding reduction in plant growth. However, the addition of biochar to the growth medium reduced the oxidative damage caused by the NaCl treatment, which was measured as MDA accumulation. This was likely due to an increase in GPX activity, which resulted in lower MDA accumulation. The reason for the strong positive effect of biochar on GPX activity could be possibly attributed to increased Mo accumulation.

Although further research is necessary, the results suggest that the application of this biochar could be a useful strategy for improving plant performance under unfavorable conditions, such as saline soils, and reducing freshwater dependence in agriculture. The findings could provide valuable insights for farmers, researchers, and policymakers seeking innovative approaches to mitigate the negative effects of salinity and ensure food security in water-scarce regions.

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