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Effects of Two Biochar Types on Mitigating Drought and Salt Stress in Tomato Seedlings

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Abstract: Biochar's underlying biochemical and physiological mechanisms in reducing irrigation and salinity stress are elusive. This paper investigates the effects of two types of biochar (wood biochar and poultry biochar) on the growth and physiology of tomato seedlings exposed to the combined effects of drought and salinity stress. Two types of biochar, wood biochar (WB) and poultry biochar (PB), were added to the soil separately, with three salinity gradients of 0, 100, and 200 mmol/L and two water supply conditions of full irrigation (FI) and deficit irrigation (DI). Results showed that biochar addition effectively improved the root water potential and osmotic potential of tomato plant under drought and salinity stress. Biochar application also mitigated leaf relative water content by 9.86% and 24.37% under drought and salinity stress, respectively. Furthermore, biochar application decreased abscisic acid concentrations in xylem sap under drought and salinity stress. Biochar altered the soil structure and increased field water holding capacity, indirectly increasing the soil water supply. While water use efficiency did not increase significantly after biochar application, a synergistic increase in seedling growth and water consumption occurred. In conclusion, biochar addition shows promise for promoting seedling growth to help mitigate the adverse impacts of drought and salinity stress on plant growth and physiology.

Keywords: biochar; water relationship; photosynthesis; ABA; water use efficiency (WUE)

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1. Introduction

According to the FAO (2009), there will be a 34% increase in the world's population by 2050, resulting in 2.3 billion more people to feed and necessitating a 70% increase in staple crop production. However, the use of chemical fertilizers and environmental stresses like drought and salinity pose significant challenges to food production and agricultural sustainability. The land area affected by extreme drought has increased by 1.74% per decade from 1950 to 2008, with the drought frequency projected to increase sevenfold. The global area of salinized land is about 831 million ha, of which 770 billion ha are affected by secondary salinization [1]. Salinity-affected planting land exists in more than 70 countries, including 19.5% of irrigated and 2.1% of dry land, with a global average of 6.0% [2].

Climate change has increased the likelihood of extreme arid climates emerging [3], which is expected to impact food production due to varying degrees of drought for prolonged periods [4]. Water is a driving force of plant growth [5]. Drought stress can affect the physiological characteristics of plant leaves, lowering leaf photosynthetic and transpiration rates and stomatal conductance, and can disturb seed germination, crop heading, pollination, filling, and maturity, reducing crop yields [6–8]. Several studies have reported decreased tomato yields under deficit irrigation [9–11].

Salt stress also adversely affects crop growth and production, exposing plants to osmotic stress and ion toxicity [12,13]. The direct damage of salt stress to plants includes ionic stress and disrupted ionic homeostasis. Plant Na^+ accumulation under salt stress affects metabolic processes in the environment with low Na^+ and high K^+ and Ca^{2+} concentrations [14]. Osmotic stress in plants is secondary damage from salt stress [15]. Under salt stress, the absorptive soil water sharply declined due to the decreased soil water potential caused by the increased salt concentration [16]. Hence, salt stress reduces crop growth by decreasing the photosynthetic rate [17] and root water and nutrient uptake from rhizosphere soil.

Strong evaporation in arid and semi-arid regions can bring salt to the soil surface, forming saline-alkali soils. Therefore, crops grown in these areas often face the dual stress of drought and salinity. Thus, mitigating and compensating for their adverse effects on crop growth is necessary for agricultural sustainability and food security. A promising agronomic measure is biochar addition to soil, which has been well-received due to its excellent performance in improving harsh soil environmental conditions. Biochar is a natural material obtained by pyrolyzing organic waste such as plant straw and animal excrement under limited oxygen conditions at 300–1000 °C. Due to its low density, large surface area, and excellent adsorption capacity [18], biochar can dramatically change soil properties (bulk density, pH, cation exchange capacity, soil structure, and water retention capacity) and improve the physicochemical environment for plants and microorganisms [19–24].

However, there is limited research on the role of biochar in tomato plants under combined drought and salinity stress. Therefore, this study evaluated the effect of biochar addition on tomato seedling growth and clarified the regulation of biochar addition on the physiological processing of tomato seedlings under combined drought and salinity stress.

2. Materials and Methods

2.1. Soil and Biochar

The experimental soil was sandy soil collected from farmland at the Taastrup campus, University of Copenhagen. The bulk density was 1.38 g/cm^3 and field water holding capacity (WHC) was 23.77 mass%. Two biochar types were used—wood biochar (WB) and poultry manure biochar (PB)—made from chemically untreated wood and poultry manure, respectively, pyrolyzed at 550 °C, ground, and passed through a 2 mm sieve.

The fine biochar powder was mixed thoroughly with soil before filling 2 L planting pots. The biochar weight accounted for 5% of the soil weight. The bulky density of the WB-soil mixture was 1.32 g/cm^3, and the field WHC was 27.75 mass%. The bulk density of the PB-soil mixture was 1.28 g/cm^3, and the field WHC was 26.20 mass%. Table 1 details the physical and chemical properties of the biochars. Figure 1 shows the cultivation environment for the tomato plants.

Table 1. Basic information of the two biochar types—wood biochar (WB) and poultry manure biochar (PB).

	WB	**PB**
EC (μS/cm)	–	2650
pH (in $CaCl_2$)	8.6	10.1
C (%)	–	67.6
N (%)	–	2.9
P (%)	–	1
K (%)	–	3
Surface area (m^2/g)	369	117
Total ash (%)	1.25	22.2
Volatile matter	4	6.2
C stability (%)	36	44.3
Bulk density (g/cm^3)	0.150	0.362

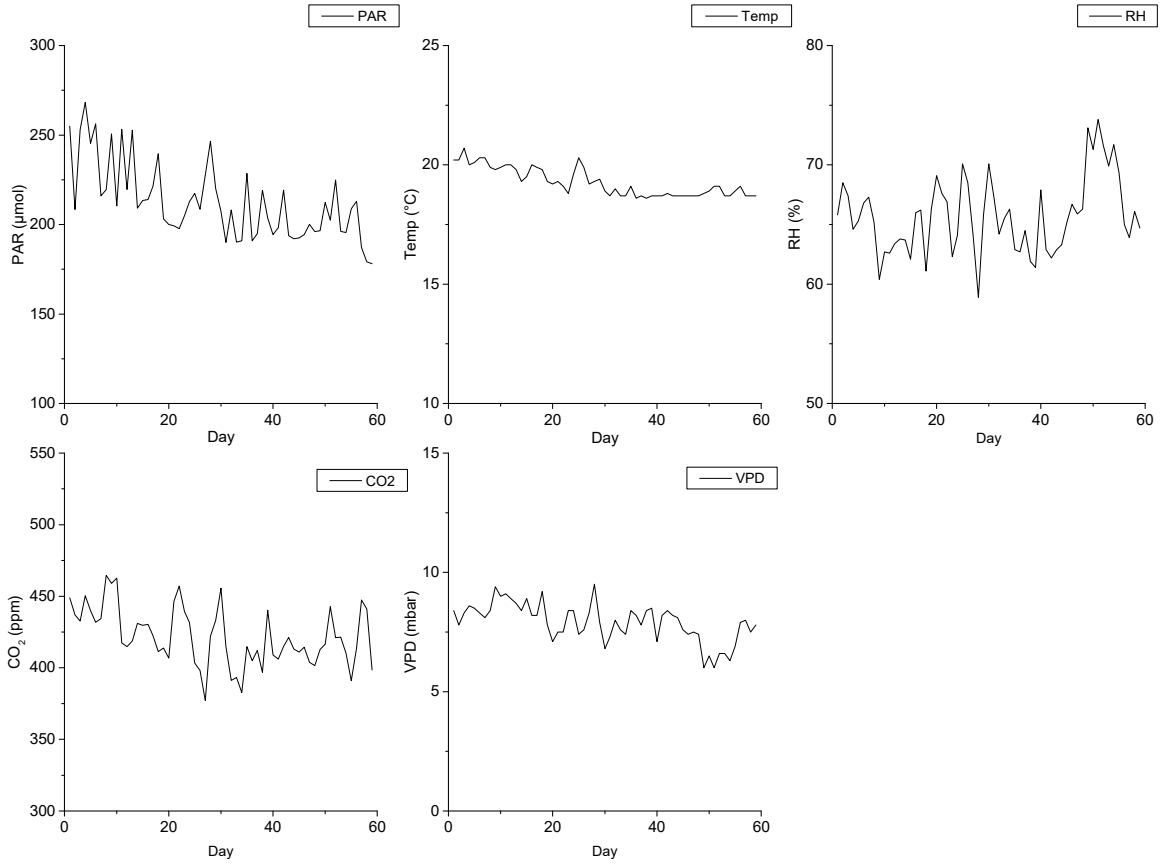

Figure 1. Cultivation environment for tomato plants. PAR, Temp, RH, CO_2, and VPD represent photosynthetically active radiation, air temperature, relative humidity, CO_2 concentration, and vapor pressure deficit, respectively. The data were recorded from 26 October 2021 to 23 December 2021.

2.2. Experimental Design

The experiment had three factors: biochar type, water supply volume, and degree of salt stress. For biochar type, pots containing wood biochar-soil mixture (WB) or poultry biochar-soil mixture (PB) were the treated groups, while those without biochar served as controls (CK). For water supply, half the plants were kept at 90% pot WHC (full irrigation, FI) throughout the experiment, whereas the rest of the pots were irrigated daily with 70% of the FI water supply (deficit irrigation, DI). There were three salt-stress treatments: 0, 0.1, and 0.2 mol/L NaCl (S0, S1, and S2). In total, there were 18 treatments, with four repetitions for each treatment.

2.3. Experimental Setup

Tomato (*Solanum lycopersicum*) seeds (cv. Ailsa Craig) were sown in peat on 26 October 2021. After 21 days, at the fourth leaf stage, the seedlings were transplanted into pots and supplied with 0.5 g N and 0.5 g K as NH_4NO_3 and KH_2PO_4 in a nutrient solution. The first harvest occurred on 3 December 2021, with four plants in each biochar treatment harvested to obtain the initial plant biomass. To impose salt stress, totally 5.45 g NaCl per pot was added to the respective treatment pots, three times for S2 treatment and two times for S1 during 3 to 6 December 2021. All pots were irrigated to 90% pot WHC until 10 December 2021, from which drought stress was imposed. After weighing the pots, the FI treatments were irrigated to 90% pot WHC, while the DI treatments received 70% of the FI amount. The second harvest occurred on 20 December. The experiment was conducted in a greenhouse at Taastrup campus, the University of Copenhagen, Denmark.

2.4. Measurement of Parameters

2.4.1. Soil Water Content and Plant Water Consumption

Each pot was weighted daily with a balance (SB24001DR, Mettler Teledo) at 15:00 from the first to the second harvest. The daily water supply volume (Vs) was calculated as:

$$Vs = p \times ((1 + 90\% \times FC) \times Ms - Mw) \quad (1)$$

where p is water-satisfying degree (100% for FI treatment and 70% for DI treatment), FC is water-holding capacity (mass%), Ms is dry mass of the soil in the pot (g), and Mw is real-time mass of the pot before being irrigated every day.

The cumulative plant water consumption (Vc) is the sum of irrigation volume and changes in soil water content during the treatment period:

$$Vc = \sum_{i=1}^{n} Vs_i + \left(Mw_f - Mw_s\right) \quad (2)$$

where Vs_i is water supply volume at i days after the first harvest day, n is days from the first to second harvest, Mw_f and Mw_s are plant mass values at the first and second harvests, respectively.

2.4.2. Plant Water Relations

At the second harvest, a fresh circle of a fully developed young leaf was collected with a hole puncher and weighed immediately. The leaf circle was soaked in water for 2 h and weighed in a water-saturated condition. Leaf relative water content (LRWC, %) was calculated as follows:

$$\text{LRWC} = \frac{M_{lc} - M_{ld}}{M_{ls} - M_{ld}} \times 100\% \quad (3)$$

where M_{lc} is fresh leaf mass (g), M_{ld} is dry leaf mass (g), M_{ls} is leaf mass after water saturation (g).

Another fully developed young leaf was excised and wrapped in a transparent plastic bag. The sampled leaf was placed into a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) and pressurized to measure leaf water potential (LWP, MPa). The leaf was divided into two parts, immediately frozen in liquid nitrogen after wrapping in tin foil, and stored at −80 °C for ABA concentration and osmotic potential (OP) measurements.

Leaf osmotic potential (OP, MPa) was measured using the gas-phase equilibrium method with a psychrometer (C-52 sample chambers, Wescor Inc., Logan, UT, USA) connected to a microvoltmeter (HR-33T, Wescor, Logan, UT, USA) at 22 °C. Firstly, marking a line about the relationship between NaCl concentration and the osmotic potential was made by filter papers soaked in 0.1, 0.2, 0.3, 0.4, and 0.5 mmol/L NaCl solution and then leaf juice was squeezed from the leaf samples to measure OP.

For root water potential (RWP, MPa), tomato branches were removed from the plants (after removing leaves), leaving the main stem. Tomato plants (with pot and soil) were

pressurized in a pressure chamber, with RWP read from the pressure gauge once bubbles appeared in the stem cross-section.

2.4.3. Gas Exchange Measurements

Before the second harvest, photosynthetic rate (An), stomatal conductance (gs), transpiration rate (Tr), and intercellular carbon dioxide concentration (Ci) were determined twice on the youngest fully developed leaf from 9:00 to 13:00 with a portable photosynthetic system (LiCor-6400XT, Li-Cor, Lincoln, NE, USA). The chamber temperature was set to 20 °C, photon flux density to 1200 μmol/m^2/s, and reference CO_2 concentration (e[CO_2]) to 400 ppm.

2.4.4. Leaf Area, Biomass, Ratio of Root and Shoot (Rrs), and Specific Leaf Area (SLA)

Leaves were clipped from plants and flattened in the leaf area meter before measuring leaf area.

Harvested plants were divided into stem, leaf, and root. Fresh biomass was weighed, and dry mass was measured after oven-drying the samples at 60 °C to a constant weight.

The ratio of root and shoot (Rrs) reflects the correlation between underground and aboveground plant parts, calculated as follows:

$$\mathrm{Rrs} = \frac{\mathrm{DL} + \mathrm{DS}}{\mathrm{DR}} \tag{4}$$

where DL is leaf dry mass (g), DS is stem dry mass (g), and DR is root dry mass (g).

Specific leaf area (SLA) was calculated as follows:

$$\mathrm{SLA} = \frac{\mathrm{DL}}{\mathrm{A_l}} \tag{5}$$

where A_l is leaf area (cm^2).

2.4.5. ABA Concentration

Leaf and xylem sap ABA concentrations were determined by enzyme-linked immunosorbent assay following the protocol of Asch (2000).

Leaves stored at −80 °C were ground with liquid nitrogen in a precooled mortar and pestle to avoid thermal decomposition. For each treatment, a 0.03 ± 0.003 g sample was added to a 2 mL test tube along with 1 mL distilled water. The tubes were homogenized by shaking for 16 h at 4 °C and then centrifuged at 10,000 r/min for 5 min at 4 °C. A 0.7 mL supernatant was extracted to measure leaf ABA concentration (L_{ABA}, ng/g FW).

Xylem sap (~1.5 mL) was collected into 2 mL tubes using disposable plastic straws, immediately frozen in liquid nitrogen, and stored at −80 °C. The ABA concentration in the xylem sap ($X_{ABA,}$ pmol/mL) was measured in 1.0 mL supernatant after defrosting.

2.4.6. iWUE and WUE

Instantaneous water use efficiency (iWUE, mmol CO_2/mol H_2O) was calculated as the ratio of An (μmol/m^2/s) to Tr (mmol/m^2/s), as follows:

$$\mathrm{iWUE} = \frac{\mathrm{An}}{\mathrm{Tr}} \tag{6}$$

Plant WUE was calculated as the ratio between water consumption and dry biomass accumulation during the treatment period, as follows:

$$\mathrm{WUE} = \frac{\mathrm{DM}}{\mathrm{Wc}} \tag{7}$$

where DM and Wc are total mass accumulation (g) and water consumption (mL) from the first to second harvest.

2.5. Data Analysis

The data were homogeneous and normally distributed and thus subjected to three-way analysis of variance (ANOVA) for the independent variables: biochar (B), water supply (W), salt stress (S), and their interactions at $p \leq 0.05$ using SPSS (IBM SPSS Statistics 23, Chicago, IL, USA). Plotting the results was performed in OriginPro 9.1.

3. Results

3.1. Morphological Parameters

Biochar did not significantly affect tomato plant height (Table 2). Drought stress and salt stress had significant main effects on plant height. Average plant height decreased by 5.04 cm under drought stress (Figure 2). At 100 and 200 mmol/L salt stress, tomato plant height decreased by 1.92 and 8.88 mm, respectively, compared with no salt stress.

Table 2. Analysis of variance (ANOVA) for plant height (H), dry mass accumulation (DM), dry root mass (DR), dry leaf mass (DL), ratio of root and shoot (Rrs), and specific leaf area (SLA) as affected by biochar addition (B), drought stress (W), and salt stress (S).

	B	**W**	**S**	**B × W**	**B × S**	**W × S**	**B × W × S**
H/cm	ns	**	**	ns	ns	ns	ns
DM (g)	**	**	**	ns	ns	ns	ns
DR (g)	**	**	**	**	**	ns	ns
DL (g)	**	**	**	ns	ns	ns	ns
Rrs	**	ns	ns	ns	**	ns	ns
SLA ($g \cdot cm^{-2}$)	**	*	ns	ns	ns	ns	ns

Note: * and ** indicate significance levels at $p < 0.05$ and $p < 0.01$; ns indicates there is no significantly effect at $p < 0.05$.

All major factors (B, W, S) significantly affected DM, DR, and DL. The average dry matter accumulation decreased by 2.25% with WB, but increased by 30.98% with PB. The average dry matter accumulation decreased by 27.7% under drought stress and 4.3–35.5% under salt stress.

Leaf dry mass increased by 5.3% and 22.5% with WB and PB, respectively, and decreased by 8.4% under drought stress and 3.5% and 19.8% at 100 and 200 mmol/L NaCl, respectively.

The interaction effects of B × W and B × S significantly affected DR, increasing by 20.3% and 51.5% with WB and PB, respectively. Under drought stress, DR increased in the CK treatment but decreased in the biochar treatments. Under severe salt stress, DR increased and then decreased under CK and PB, but gradually decreased with WB.

Biochar addition significantly increased Rrs and SLA. Drought stress significantly increased SLA. There was also a significant B × S interactive effect on Rrs. As the degree of salt stress increased, Rrs increased and then decreased under CK and PB, but gradually decreased with WB.

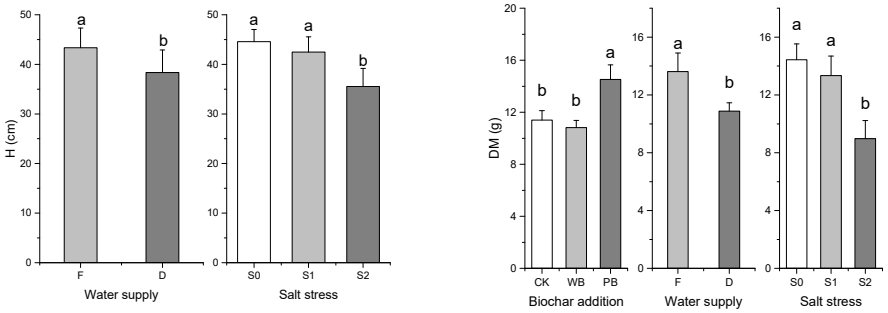

Figure 2. *Cont.*

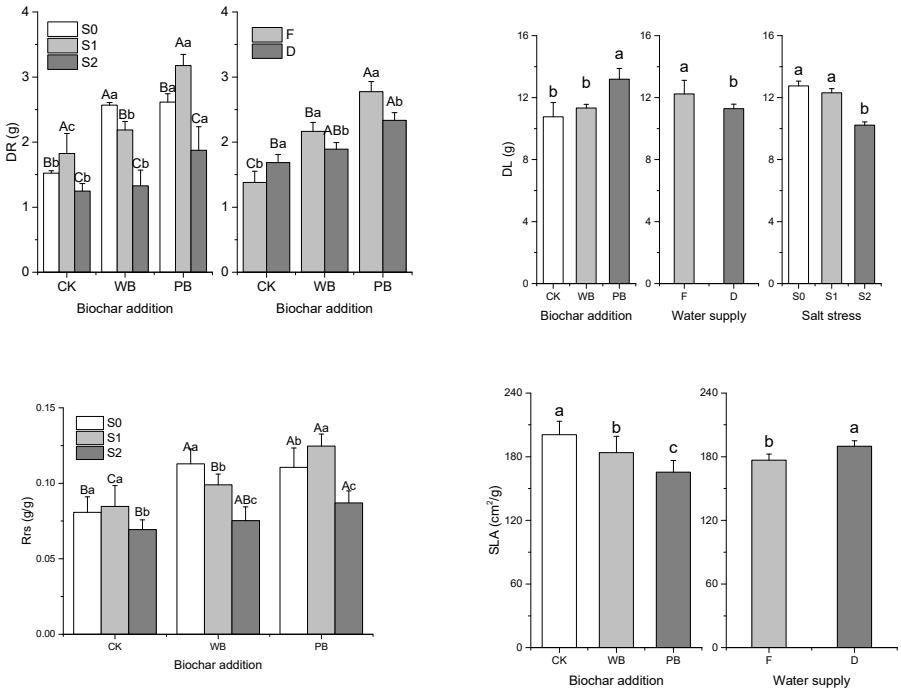

Figure 2. Plant height (H), dry mass accumulation (DM), dry root mass (DR), dry leaf mass (DL), ratio of root and shoot (Rrs), and specific leaf area (SLA) as affected by biochar addition (B), drought stress (W), and salt stress (S). The treatments are biochar type (no biochar control group, CK; wood biochar treatment, WB; poultry biochar, PB), water supply (F and D), and salt condition (S0, S1, and S2). Different uppercase letters indicate significant differences at the 0.05 level among CK, WB, and PB treatments. Different lowercase letters indicate significant differences at the 0.05 level within groups.

3.2. Gas Exchange

The B × S interaction significantly affected the leaf gas exchange parameters (An, Tr, and gs) of tomato plants (Table 3). For all treatment without salt stress (S0), adding biochar increased An. For 100 mmol/L NaCl (S1), WB did not significantly increase An, while PB increased An under FI but decreased An under DI (Figure 3). For 200 mmol/L NaCl (S1), WB contributed to the maximum An compared with CK and PB. Biochar increased Tr under no salt stress (WBS0 and PBS0) and 200 mmol/L treatment (S2). Taking S2 as an example, gs decreased by 21.6–58.6% for CK, WB, and PB, respectively. For S0 and S2, the biochar treatments had higher gs than CK. For S1, WB did not significantly improve gs, while PB did. The Ci response to B, W, and S was insignificant.

Table 3. Analysis of variance (ANOVA) for photosynthetic rate (An), stomatal conductance (gs), transpiration rate (Tr), and intercellular carbon dioxide concentration (Ci) as affected by biochar addition (B), drought stress (W), and salt stress (S).

	B	**W**	**S**	**B × W**	**B × S**	**W × S**	**B × W × S**
An	*	ns	ns	ns	*	ns	ns
Tr	ns	**	ns	ns	**	ns	ns
gs	ns	*	ns	ns	*	ns	ns
Ci	ns	ns	ns	ns	ns	ns	ns

Note: * and ** indicate significance levels at $p < 0.05$ and $p < 0.01$; ns indicates there is no significantly effect at $p < 0.05$.

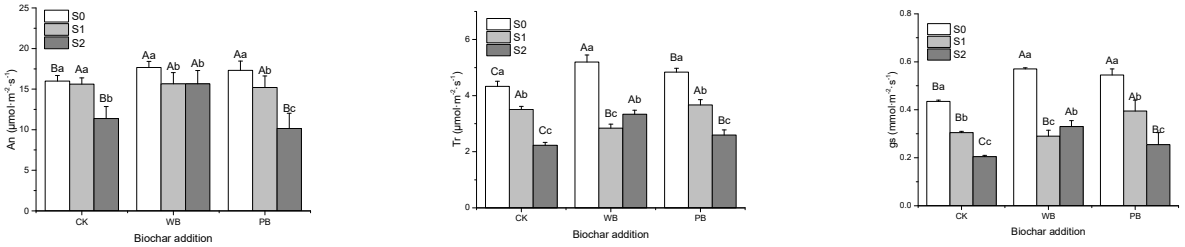

Figure 3. Photosynthetic rate (An), stomatal conductance (gs), and transpiration rate (Tr) as affected by biochar addition (B), drought stress (W), and salt stress (S). The treatments are biochar type (CK, WB, and PB), water supply (F and D), and salt condition (S0, S1, and S2). Different uppercase letters indicate significant differences at the 0.05 level among CK, WB, and PB treatments. Different lowercase letters indicate significant differences at the 0.05 level within groups.

3.3. Water Relations

Table 4 shows that B, W, and S affected plant water relations to some degree. B, W, and S significantly affected LRWC individually and in pairs. Under no salt stress (S0), biochar addition did not significantly improve LRWC and even decreased slightly under drought stress. For 100 mmol/L NaCl (S1), WB and PB increased LRWC by 19.5% and 9.6% without drought stress and 6.2% and 9.7% under drought stress, respectively. For 200 mmol/L NaCl (S2), WB and PB significantly increased LRWC, except for WB under FI, where LRWC gradually decreased. Drought stress also significantly decreased LRWC.

Table 4. Effect and output of three-way ANOVA for leaf relative water content (LRWC), leaf water potential (LWP), osmotic potential (OP), and root water potential (RWP) as affected by biochar addition (B), drought stress (W), and salt stress (S).

Parameters	Biochar Treatment	F			D			ANOVA						
		0	100 mmol/L	200 mmol/L	0	100 mmol/L	200 mmol/L	B	W	S	B × W	B × S	W × S	B × W × S
LRWC (%)	CK	88.08 ± 1.02	75.46 ± 0.87	70.43 ± 1.13	83.35 ± 1.78	72.89 ± 3.40	61.65 ± 2.14	**	**	**	*	**	**	ns
	WB	88.97 ± 0.87	90.18 ± 3.25	82.84 ± 0.20	82.38 ± 1.27	77.39 ± 3.24	64.81 ± 1.73							
	PB	89.63 ± 3.25	82.67 ± 1.40	73.39 ± 8.60	81.80 ± 3.13	79.96 ± 9.67	63.79 ± 8.08							
RWP (–MPa)	CK	0.23 ± 0.13	0.25 ± 0.06	0.42 ± 0.08	0.28 ± 0.03	0.35 ± 0.08	0.52 ± 0.14	**	**	**	ns	ns	ns	ns
	WB	0.23 ± 0.13	0.22 ± 0.15	0.37 ± 0.09	0.25 ± 0.08	0.23 ± 0.03	0.43 ± 0.04							
	PB	0.10 ± 0.06	0.06 ± 0.02	0.19 ± 0.08	0.21 ± 0.09	0.33 ± 0.07	0.47 ± 0.03							
LWP (–MPa)	CK	0.33 ± 0.16	0.48 ± 0.05	0.50 ± 0.06	0.57 ± 0.01	0.59 ± 0.04	0.63 ± 0.34	ns	**	ns	ns	ns	ns	*
	WB	0.47 ± 0.06	0.33 ± 0.04	0.31 ± 0.03	0.59 ± 0.03	0.58 ± 0.09	0.69 ± 0.22							
	PB	0.47 ± 0.03	0.44 ± 0.07	0.49 ± 0.06	0.64 ± 0.07	0.50 ± 0.06	0.45 ± 0.21							
OP (–MPa)	CK	0.64 ± 0.17	0.88 ± 0.06	1.06 ± 0.01	0.83 ± 0.06	1.05 ± 0.28	1.10 ± 0.16	*	ns	**	ns	ns	ns	ns
	WB	0.57 ± 0.22	0.71 ± 0.11	0.92 ± 0.26	0.74 ± 0.07	0.70 ± 0.32	1.07 ± 0.21							
	PB	0.51 ± 0.28	0.69 ± 0.37	0.82 ± 0.12	0.80 ± 0.20	0.55 ± 0.34	1.09 ± 0.23							

Note: * and ** indicate significance levels at $p < 0.05$ and $p < 0.01$; ns indicates there is no significantly effect at $p < 0.05$.

Individually, B, W, and S significantly affected RWP. The RWP of plants increased with biochar addition and decreased significantly under drought stress. RWP under 100 mmol/L NaCl treatment (S1) did not change significantly compared with no salt stress (S0); RWP under 200 mmol/L NaCl treatment (S2) decreased significantly.

The B × W × S interaction significantly affected LWP. With no biochar addition, LWP decreased gradually under salt stress, and drought stress (DI) decreased LWP relative to FI. Under FI, WB increased LWP at 200 mmol/L NaCl (S2); under DI, WB did not significantly affect LWP at 100 mmol/L NaCl (S1) but increased LWP by 16.9% at 200 mmol/L NaCl (S2). Under FI, PB increased and then decreased LWP under salt stress; under DI, LWP increased by 0.14 and 0.19 MPa at 100 and 200 mmol/L NaCl, respectively. Drought stress also decreased LWP by 22.0–80.6%.

Biochar and salt stress individually affected plant osmotic potential. Biochar addition significantly increased leaf osmotic potential and decreased leaf osmotic potential, particularly at the higher salt concentration.

3.4. Leaf and Xylem Sap ABA Concentrations

Overall, biochar addition reduced leaf ABA concentration (Table 5). Salt stress affected the regulation effect of biochar on leaf ABA concentration. For example, WB under FI decreased leaf ABA concentration by 41.2% under no salt stress (S0) and 22.1% at 100 mmol/L NaCl (S1), and increased leaf ABA concentration by 84% at 200 mmol/L (S2). Without biochar addition, leaf ABA contents under salt stress ranged from 688.7 (DI) to 868.8 (FI) mmol/L, decreasing to 148.7–205.3 mmol/L with WB and 42.5–305.1 mmol/L with PB.

Table 5. Effect and output of three-way ANOVA for leaf ABA concentration (L_{ABA}) and xylem sap ABA concentration (X_{ABA}) as affected by biochar addition (B), drought stress (W), and salt stress (S).

Parameters	Biochar Treatment	F			D			ANOVA						
		0	100	200	0	100	200	B	W	S	B × W	B × S	W × S	B × W × S
L_{ABA} (ng/g FW)	CK	2263 ± 355	1971 ± 597	1394 ± 185	2298 ± 769	1627 ± 746	1609 ± 705	***	ns	ns	ns	*	ns	ns
	WB	1330 ± 058	1536 ± 256	1511 ± 139	1324 ± 496	1621 ± 438	1394 ± 097							
	PB	1411 ± 268	1106 ± 086	1341 ± 102	1037 ± 479	997 ± 176	1039 ± 245							
X_{ABA} (pmol/mL)	CK	355 ± 05	411 ± 40	547 ± 43	653 ± 32	578 ± 33	1181 ± 216	***	*	ns	***	**	ns	*
	WB	268 ± 14	348 ± 37	528 ± 26	555 ± 26	567 ± 43	1841 ± 145							
	PB	119 ± 25	284 ± 41	373 ± 36	451 ± 16	376 ± 44	695 ± 023							

Note: *, ** and *** indicate significance levels at $p < 0.05$, $p < 0.01$ and $p < 0.001$; ns indicates there is no significantly effect at $p < 0.05$.

The B × W, B × S, and B × W × S interactions significantly affected xylem sap ABA content. The W and S treatments with biochar addition significantly affected xylem sap ABA content. Under FI, PB reduced xylem sap ABA content more than WB. Under DI, PB decreased xylem sap ABA contents by 30.9–41.2% compared to CK, while WB decreased xylem sap ABA content under no salt stress (S0), had no effect at 100 mmol/L NaCl (S1), and increased it by 660 mmol/L at 200 mmol/L NaCl (S2), relative to CK.

3.5. WUE and iWUE

Salt stress decreased the water consumption of tomato plants (Table 6). Biochar addition significantly increased water consumption under no salt stress (S0), had no effect at 100 mmol/L NaCl (S1), and at 200 mmol/L NaCl (S2), did not change much with WB but increased significantly with PB (Figure 4). The B × S interaction significantly affected WUE. Under no salt stress (S0), PB increased WUE, while WB did not. At 100 mmol/L NaCl (S1), WB and PB increased WUE significantly, while at 200 mmol/L NaCl (S2), PB decreased WUE, but WB did not. The B × W interaction significantly affected iWUE. For CK, DI significantly decreased iWUE, while for WB and PB, DI significantly increased iWUE.

Table 6. Analysis of variance (ANOVA) for water consumption, instantaneous water use efficiency (iWUE), and plant water use efficiency (WUE) as affected by biochar addition (B), drought stress (W), and salt stress (S).

	B	W	S	B × W	B × S	W × S	B × W × S
Water consumption	***	***	***	ns	*	ns	ns
WUE	*	ns	***	ns	**	ns	ns
iWUE	ns	ns	*	*	ns	ns	ns

Note: *, ** and *** indicate significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$; ns indicates there is no significantly effect at $p < 0.05$.

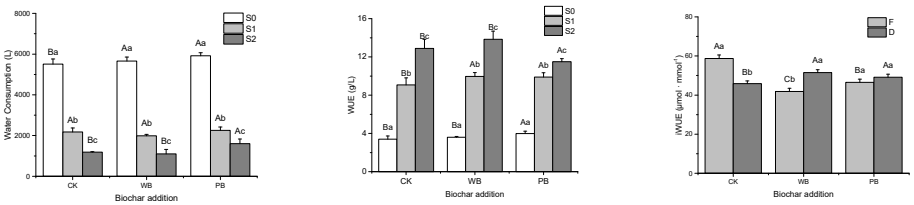

Figure 4. Water consumption, instantaneous water use efficiency (iWUE), and plant water use efficiency (WUE) as affected by biochar addition (B), drought stress (W), and salt stress (S). The treatments included biochar type (CK, WB, and PB), water supply (F and D), and salt conditions (S0, S1, and S2). Different uppercase letters indicate significant differences at the 0.05 level among CK, WB, and PB treatments. Different lowercase letters indicate significant differences at the 0.05 level within groups.

4. Discussion

The results revealed that the effect of biochar application on crop growth is reflected more in changes in biomass accumulation than in plant height. The root dry weight and root–shoot ratio of tomato seedlings significantly increased with biochar addition (Figure 2), indicating stimulated root development at the seedling stage and consistent with the findings of Abiven et al. [25], Backer et al. [26], Xiang et al. [27], and Zou et al. [28]. Bruun et al. [29] and Sun et al. [30] also reported that biochar addition optimized root morphology, including root length, root surface area, and root density, and improved root cell viability. The root system is a key pathway regulating aboveground plant growth in some biochar-improved soils, such that PB compensated for the reductions in total dry weight and leaf dry weight under DI. In addition, the improved soil water status increased the water consumption of tomato plants. The biochar addition increased the field soil WHC from 24.4% to 26.0% (WB) and 27.1% (PB), improving the soil structure, especially for sandy soils [31]. Soils with added biochar had 50% higher soil water permeability than those without biochar [32]. Biochar application mainly enhances soil water retention and WHC for coarse-textured soils and promotes water infiltration and drainage (i.e., saturated hydraulic conductivity) for fine-textured soils [33,34]. Root systems, especially those with fine roots, are significantly enhanced in biochar-applied soils [35], strengthening the ability of plants to fix soil particles. Thus, biochar is also significant for soil and water conservation in arid and semi-arid regions [36]. However, WB failed to have the same compensatory effect as PB in term of DR mass, indicating a significant effect of biochar type. Additionally, differences in preparation time may contribute to different compensatory effects. High temperature during pyrolysis reduces the concentration of functional groups (e.g., -OH, -COOH, -CH, and -C=O) [37], such that fresh biochar usually has a lower CEC (compared to old biochar) and is not immediately effective [38]. WB is freshly prepared wood biochar, which may delay when it begins to function in the soil. Biochar application changed the water regime and relations within tomato plants. Insufficient water supply decreases the soil water potential, reducing plant water availability. In such cases, plants tend to actively reduce their water content and increase the solute concentration to lower cellular water potential, thereby improving their ability to obtain soil water [39]. Consistent with Ghosh et al. [40] and Yang et al. [41], we showed that drought reduced plant LRWC, root water

potential, LWP, and osmotic potential to varying degrees. Crop LRWCs were not sensitive to biochar application when drought stress acted alone, probably because biochar is a physical measure that did not alter the available soil water under controlled VPD and temperature conditions. However, drought is often accompanied by continuous high-temperature climates, leading to severe soil surface evaporation. Biochar retains water and reduces soil evaporation. The increased salt concentration in soil could sever the soil solute potential and impose osmotic stress on plants. The experimental results showed that salt stress decreased LWP and osmotic potential relative to the control group. Biochar can adsorb sodium ions, increase the free activity of soil water, and reduce the salt concentration, reducing the soil water potential. The results also showed that biochar significantly increased the root water potential and osmotic potential of tomato plants. Biochar application under drought and salinity stress improved LWRC by 9.86–100% and 24.37–100%, respectively, indicating that biochar helped plants to maintain firm leaves under abiotic stress. Studies have shown that ionic and osmotic stresses brought by drought and salinization lead to cellular stress responses, stimulate the production of reactive oxygen species (ROS), and interfere with normal cellular functions [42]. Abscisic acid concentrations (ABA) is known to regulate ROS production, which is an important chemical signal for plants to sense environmental stress conditions and regulate crop growth [43]. Under stressful conditions, ABA promotes stomatal closure by rapidly altering ion flux in protective cells, minimizing evaporative water loss [44,45]. ABA positively influences crop adaptation to salt stress by reducing cytoplasmic salinity and increasing sodium ion content in vacuoles. In this study, ABA responses to stress varied with plant parts. Leaf ABA concentrations did not significantly differ from CK under drought and salinity stress but increased in xylem sap. Reportedly, ABA increases in xylem sap much earlier than leaf ABA, and is generally believed to be produced by roots and transported to the leaves through xylem [46]. Guo et al. [47] reported a better correlation between X_{ABA} and gs than between L_{ABA} and gs, such that X_{ABA} may be a better indicator of crop stress status than L_{ABA}.

Consistent with the conclusions presented in [48], biochar reduced leaf ABA concentration, likely improving the soil stress environment. Studies have shown that biochar maintains ion balance and alleviates ion toxicity caused by Na^+/K^+ imbalance by absorbing Na^+ and releasing K^+ [13,49,50]. The increased soil WHC after biochar addition also dilutes salinity, mitigating osmotic stress [13]. However, the ability of biochar to adsorb ions is limited and restricted by the degree of stress. Our findings suggest that improving photosynthesis with biochar addition depends on the degree of salinity stress. In addition, biochar can decrease ROS production in plants. According to Natasha [51], biochar application to soil decreased the ROS level by 33% compared to no biochar application. Biochar significantly decreases superoxide dismutase, catalase, and peroxidase activities and malondialdehyde and proline contents, reducing oxidation and osmotic stress and promoting plant growth [52–56].

5. Conclusions

Biochar addition to soil could offset some adverse effects of deficit irrigation and salt stress by improving soil texture and water status and slowing ion absorption, which can alleviate osmotic and ion stresses faced by crops and help optimize their internal water status. Biochar addition did not improve WUE in tomato plants but accelerated the transpiration rate and increased plant water consumption, which is beneficial for tomato seedling growth under drought and salt stress.

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