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The combination of biochar and rhizobacteria improved leaf pigments and growth of oilseed rape under salinity

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Abstract

Objective: Soil salinity is a major environmental constraint that significantly influences plant growth and productivity. Application of carbon-rich materials and rhizobacteria may reduce the negative impacts of environmental stresses such as soil salinity on plants. Thus, this research aimed to investigate the possible roles of solid and enriched biochars with *Pseudomonas putida* RS-198 and *Azotobacter chroococcum* RS-106 on the physiological performance and grain yield of oilseed rape under salinity.

Methods: A factorial experiment with randomized complete block design in three replicates was laid out in a greenhouse at the University of Tabriz, Iran, to find out the effects of solid biochar (30 g biochar per 1 kg soil) and enriched biochars with *Pseudomonas putida* (100 ml bacteria in 1 kg^{-1} of biochar), *Azotobacter chroococcum* (100 ml bacteria in 1 kg^{-1} of biochar), and *P. putida* + *A. chroococcum* (50 ml *P. putida* + 50 ml *A. chroococcum* in 1 kg^{-1} of biochar) on nutrient uptake, osmotic adjustment, photosynthetic activity, and yield components of oilseed rape plants under salt stress (0, 6, and 12 dS m^{-1} NaCl; as non-saline, and moderate and high salinities, respectively).

Results: The Na^+ uptake and osmolytes were enhanced, but the K^+ uptake, Ca^{2+} and Mg^{2+} contents, leaf water content, photosynthetic activity, plant growth, grain yield, and yield components were decreased with increasing salt stress. The biochar-related treatments reduced the negative impacts of salt stress by decreasing Na^+ uptake and increasing nutrients and water contents, soluble sugars, leaf pigments, plant biomass, seeds per plant, and grain yield, particularly under high salinity. In addition, the combination of biochar and *P. putida* + *A. chroococcum* was the superior treatment in enhancing pods per plant of oilseed rape.

Conclusion: The integrated application of biochar and rhizobacteria could be a new method worthy of consideration for improving plant growth and productivity in saline soils.

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Introduction

Crop production relies on fertile soils and a stable environment. Therefore, any undesirable factor that disrupts the sustainability of the environment can restrict agricultural production (Ghassemi-Golezani and Mousavi 2022). Numerous environmental challenges, including salt stress, may limit plant growth and survival (Tanveer and Shah 2017; Ghassemi-Golezani and Abdoli 2021). The occurrence of elevated levels of toxic ions (especially Na^+ and Cl^-) in saline soils has multifaceted effects on plant physiology, biochemistry, and morphology, threatening the sustainability and productivity of agricultural systems (Arif *et al.* 2020; Abdoli and Ghassemi-Golezani 2025). Salt stress is specified by detrimental impacts on plant growth, productivity, and quality, which are strongly associated with decline in osmotic potential of soil in the rhizosphere, ionic toxicity, nutritional imbalance, oxidative stress, or a combination of these factors (Farouk *et al.* 2020; Ayuso-Calles *et al.* 2021). Salt stress is initially realized by the root system and impairs root growth (Ghassemi-Golezani and Abdoli 2023) by reducing water availability, ionic toxicity, and nutrient imbalances in the cells. Excessive toxic ions also appear as reactive oxygen species (ROS)-mediated oxidative stress (Abdoli and Ghassemi-Golezani 2021). Salt stress lowers the water potential of the soil in the vicinity of roots, causing an osmotic stress, which elevates the energy required for water and nutrient uptake. Many plants can maintain an osmotic equilibrium and water uptake by synthesis of compatible solutes such as proline, glycine betaine, soluble proteins, and sugars (Abdoli *et al.* 2020) to overcome osmotic stress caused by salinity. Increasing ionic and oxidative stresses in plants under salinity can reduce chlorophyll and other leaf pigments (Abdoli and Ghassemi-Golezani 2021). Salinity has been shown to reduce Rubisco activity, photosynthesis rate, gas exchanges, and metabolism of stressed plants (Ghassemi-Golezani and Farhadi 2021). Salinity negatively affects chlorophyll synthesis and accelerates its degradation through enhancing ethylene production and chlorophyllase activity (Jamil *et al.* 2007). Disturbances of photosynthesis under salinity may also be related to the restricted electron transport through PSII and/or with structural injuries to PSII (Kan *et al.* 2017). Stomatal closure under saline conditions reduces the ratio of CO_2/O_2 and disrupts electron transport, leading to an unbalanced ATP/NADPH ratio (Lokhande *et al.* 2011a, b) that limits plant growth (Ghassemi-Golezani and Abdoli 2024).

Several economic and environmentally friendly ameliorative approaches have been established to combat the toxic impacts of salinity on plants. A constructive strategy to reduce the destructive

effects of salt stress on plants might be amending the soil with biochar (Solhi-Khajehmarjan *et al.* 2025). Biochar is a solid carbon-rich material, which is mainly manufactured from organic waste residuals under high temperature and low oxygen conditions (Busch and Glaser 2015). The recent progress in carbon-based technology in mitigating salt toxicity and improving plant tolerance indicates great ameliorative effects of biochar on salt-affected plants through improving soil properties (Amini *et al.* 2016), increasing relative photosynthetic electron transport rate, photosynthetic pigments, maximum quantum yield of photosystem II, and decreasing sodium accumulation and ROS generation (Ghassemi-Golezani *et al.* 2021). Addition of biochar to the soil noticeably enhances soil pH, respiration, microbial metabolism, nutrient availability (Wu *et al.* 2020), water holding (Adhikari *et al.* 2023), and cation exchange capacities (Šimanský *et al.* 2018). Adding biochar to the soil can also improve root growth, that in turn modifies soil structure and promotes plant growth. Increasing plant nutrient absorption capacity is a key aspect of these improvements (Ghassemi-Golezani and Abdoli 2023).

Another efficient approach to improve the growth and productivity of stressed plants is inoculation with plant growth-promoting rhizobacteria (PGPR) (Arkhipova *et al*. 2020). The plant's roots may act as a niche for the multiplication of specific species of rhizosphere bacteria in the soil, inculcating the suitable growth of plants, especially under adverse conditions. Numerous studies have illustrated the beneficial effects of rhizobacteria on plant growth under high saline conditions with detailed emphasis on nutrient acquisition, root growth, and potential roles in conferring salt tolerance by releasing acids, proteins, hormones, antibiotics, and other chemicals (Gupta and Pandey 2019), revealing that rhizosphere engineering with PGPR has a wide-ranging benefits in improving plant tolerance against environmental stresses as well as developing eco-friendly sustainable agriculture. Utilization of rhizobacteria enhances nutrient accessibility, antioxidant potential, photosynthetic activities, and osmo-protectants, leading to an improvement in plant growth (Ansari *et al.* 2019; Abdel Latef *et al.* 2020). According to Zhang *et al*. (2008), inoculation with PGPR downregulates *HKT1* expression in roots, but upregulates it in the shoots, resulting in lower Na^+ uptake and translocation under salt conditions. Banaei-Asl *et al.* (2015) found that increased salt tolerance of oilseed rape by bacterial inoculation is associated with proteins for energy metabolism and division of root cells. Inoculation with PGPR containing ACC deaminase significantly increases the growth of salt-affected plants (Bal *et al.* 2013). Mayak *et al.* (2004) reported that a decrement in ethylene content by ACC-producing *Achromobacter piechaudii* under salinity led to a 66% increment in the growth of tomato seedlings under stress. Nia *et al.* (2012) and Ramadoss *et al.* (2013) also showed that inoculation with saline-adapted rhizobacteria resulted in increased salinity tolerance and higher growth and grain

productivity of wheat plants. The ameliorative effects of rhizosphere bacteria and carbon-rich materials in mitigating the toxic impacts of salinity might be considered as potential tools and approaches for improving the production of major crops such as oilseed rape *(Brassica napus* L.) under saline conditions.

Oilseed rape, as an important oilseed crop (Song *et al.* 2020), is moderately salt-tolerant (Ashraf and McNeilly 2004). Cultivation of this plant has increased tremendously for its various critical economic uses such as human food, animal fodder, and fuel. Understanding the mechanism of biochar inoculation with rhizobacteria on plant growth and tolerance can contribute toward the long-term goal of improving plant–bacteria–biochar triple interactions for saline soils. As a strategic crop, the changes in plant growth and reproductive characteristics of oilseed rape in response to biochar inoculated with rhizobacteria have not been documented so far. Hence, this research was conducted to scrutiny the possible roles of biochar inoculation with *Pseudomonas putida* RS-198 and *Azotobacter chroococcum* RS-106 on nutrient uptake, photosynthetic pigments, osmolytes, plant growth, and yield related traits of oilseed rape under various salinity levels.

Materials and Methods

Soil and biochar characteristics

The peach (*Prunus persica* L. Batsch) residues were used to produce biochar by pyrolysis under anoxic conditions at about 560 °C (Qian *et al.* 2013). The method of Carter and Gregorich (2008) was applied to analyze the soil and biochar samples. An elemental analyzer (Elementar group, Hanau, Germany) was used to assay the carbon, hydrogen, nitrogen, and oxygen contents of soil and biochar. Other nutrients were measured by a flame photometer (Corning flame photometer, 410). The pH and CEC of soil and biochar were analyzed by a pH meter (Model: HI 99121, Hanna Instrument, USA) and the ammonium acetate method (Chapman 1965), respectively. The soil and biochar properties are listed in Table 1.

Experimental conditions and treatments

A greenhouse experiment as factorial based on randomized complete block design with three replicates was conducted at the University of Tabriz, to evaluate the possible roles of the combination of biochar and rhizobacteria on physiological and yield components of salt-exposed oilseed rape plants. The bacterial strains (10^8 cfu mL^{-1}) were obtained from the Soil and Water Research Institute, Karaj, Iran. In the first step, salt-tolerant strains of *Pseudomonas putida* RS-198 *and Azotobacter chroococcum* RS-106 were mixed with biochar (100 mL bacteria in 1 kg^{-1} of biochar) individually

Table 1. Some physical and chemical properties of the experimental soil and biochar.

Soil		Biochar	
Texture	Silty loam	pH	7.8
pH	6.3	C (%)	52.6
EC (dS m^{-1})	1.95	N (%)	0.61
Organic carbon (g kg^{-1})	9.8	H (%)	1.9
K (mg kg^{-1})	120.1	O (%)	26.2
Ca (mg kg^{-1})	73.6 mg kg^{-1}	Na (mg kg^{-1})	3.04
Mg (mg kg^{-1})	82.8 mg kg^{-1}	K (mg kg^{-1})	4380
Total N (%)	1.1%	Ca (mg kg^{-1})	2600
P (mg kg^{-1})	42.7 mg kg^{-1}	Mg (mg kg^{-1})	102
CEC (cmol kg^{-1})	19.8 cmol kg^{-1}	P (mg kg^{-1})	5180
		S (mg kg^{-1})	4840
		CEC (cmol kg^{-1})	24.6

CEC: Cation exchange capacity, EC: Electrical conductivity.

and/or in combined form. Treatments were salinity levels (0, 6, and 12 dS m^{-1} NaCl; as non-saline, moderate, and high salinities, respectively) and soil amendments (NB: non-biochar, B: biochar, BP: combination of biochar and *P. putida* RS-198, BA: combination of biochar and *A. chroococcum* RS-106, and BPA: combination of biochar and *P. putida* RS-198 + *A. chroococcum* RS-106). In this experiment, 50 plastic pots (20 × 20 cm), each filled with 3 kg untreated and treated soil (30 g biochar per 1 kg soil) were used. All of the pots were placed in a glass greenhouse with day and night temperatures of 28 and 23 ℃, respectively, 146 W m^{-2} light intensity, 33-40% relative humidity, and about 13 h photoperiod. 45 pots were considered for sowing, and 5 pots were kept unsown for measuring the water loss of the pots. 10 seeds of *Brassica napus* (cv. Delgan) were then sown at a depth of 1 cm from the soil surface of each pot and instantly irrigated with tap water and saline solutions up to field capacity (FC) according to the treatments. Specific amounts of sodium chloride were dissolved in tap water (pH of 7.2 and EC of 0.6 dS m^{-1}), and then saline solutions were uniformly added to the pots until the electrical conductivity of leaked water from the pots reached the intended EC (6 and 12 dS m^{-1} NaCl). The established seedlings were thinned to keep four plants per pot.

Ions analysis

At early flowering, two plants from each pot were harvested, and the nutrient content of the plants was measured. A flame photometer (Corning flame photometer, 410) was used to measure the ion

contents in plant tissues. Initially, the dried plant samples were burned in an electric furnace at 560 ℃ for 7 h. Subsequently, the resulting ashes were digested in 10 mL of 1 N hydrochloric acid at 25 ℃ for 24 h, and the sodium, potassium, calcium, and magnesium contents in the digested samples were then measured as mg g^{-1} dry weight. Subsequently, the Na^+ and K^+ uptakes by the roots were calculated as the ratio of plant Na^+ and K^+ contents per root dry weight.

Osmolytes

Oilseed rape leaf and root samples were homogenized in 3% sulfosalicylic acid, and then 1 mL of ninhydrin and 1 mL of glacial acetic acid were added to 1.5 mL of the extracted sample. Proline content was determined with a calibration curve of pure proline and expressed as mg g^{-1} fresh weight (FW) (Bates *et al.* 1973). Glycine betaine content of roots and leaves was estimated according to Grieve and Grattan (1983). A sample of 500 mg of leaves and roots was ground in liquid nitrogen (-80 °C) and mixed with a toluene-water mixture (0.05% toluene) in a plastic tube, and then shaken for 24 h at 25 °C. Afterwards, 0.5 ml of each sample was added to 1 ml of 2 N hydrochloric acid and 0.1 ml of potassium tri-iodide solution and it was again shaken in an ice-cold water container for 90 min. The upper layer was removed, and the absorbance was recorded at 365 nm. The phenol sulfuric acid test (Kochert 1978) was undertaken to determine soluble carbohydrate content as mg g^{-1} FW.

Leaf and root water contents

Leaves and roots of a plant from each pot were cut and separately weighed (FW). These samples were then dried at 75 °C for 48 h and reweighed (DW). The root and leaf water contents (RWC and LWC, respectively) were calculated as:

$$WC = ((FW - DW)/FW)) \times 100$$

Leaf pigments and chlorophyll fluorescence

Fresh leaf samples were cut and homogenized in 10 ml of 80% acetone for 24 h. Each sample was then centrifuged at 10,000 g for 5 min. The absorbance of supernatant was read at 663, 645, and 470 nm, to quantify chlorophylls a, b, and carotenoid content, respectively (Arnon 1949). A fresh leaf sample (1 g) was homogenized in the 3 ml extraction mixture (methanol 2.37 ml: distilled water 0.6 ml: HCl 0.03 ml). After centrifugation at 12,000 g and 4 °C for 20 min, the absorbance was read at 550 nm for anthocyanin measurement. The maximum quantum yield of photosystem II was recorded by a portable fluorometer (OS-30, OPTISCIENCES, USA).

Plant biomass, yield, and yield components

At maturity, two remaining plants from each pot were removed, and the shoots were separated by cutting from the crown. Subsequently, the shoot samples were weighed after drying at 75 ℃ for 48 h. The seeds of the plants from each pot were separated, and the number of pods (siliques) per plant, seeds per plant, 1000-seed weight, and grain yield per plant were calculated. Eventually, the plant biomass was determined.

Statistical analysis

According to the experimental design, a two-way analysis of variance was carried out by the MSTAT-C software. Comparison of means was carried out by Duncan multiple range test at $p \leq 0.05$ and presented as mean ± standard error. All figures were drawn using Excel 2019.

Results

Cations contents

Significant interaction of salt toxicity and biochar-based rhizobacteria was observed for Na^+, K^+, Ca^{2+}, and Mg^{2+} content of the oilseed rape tissues ($p \leq 0.01$). In general, rising salinity increased Na^+ content and decreased K^+, Ca^{2+}, and Mg^{2+} content. Under non-saline conditions, the biochar-related treatments had no significant effects on Na^+, Ca^{2+}, and Mg^{2+} content. Whereas, the biochar with bacteria treatments, particularly the BP and BPA treatments, remarkably reduced the Na^+ content and enhanced the K^+, Ca^{2+}, and Mg^{2+} content under saline conditions (Table 2).

The Na^+ and K^+ uptake

The interaction of salinity × biochar-related treatments for Na^+ and K^+ uptakes and K^+/Na^+ ratio was also significant ($p \leq 0.01$). The Na^+ uptake was augmented, but the K^+ uptake and K^+/Na^+ ratio were diminished as a result of salinity increment (Figure 1a-c). The biochar-related treatments reduced Na^+ uptake of the salt-stressed plants, especially under 12 dS m^{-1} NaCl. This reduction in Na^+ uptake was about 56% and 59% by plants grown under the BP and BPA treatments, respectively (Figure 1a). However, the superiority of biochar-related treatments in enhancing K^+ uptake and K^+/Na^+ uptake ratio was more evident under moderate salinity (6 dS m^{-1} NaCl). No significant differences in K^+ uptake of the enriched-biochar treated plants under moderate and high salinities and in the K^+/Na^+ ratio under high salinity were observed (Figure 1b-c).

Table 2. Changes in Na^+, K^+, Ca^{2+}, and Mg^{2+} content (mg g^{-1} DW) of oilseed rape plant tissues affected by biochar and biochar-based rhizobacteria under different levels of NaCl salinity.

Salinity	Soil treatment	Na^+	K^+	Ca^{2+}	Mg^{2+}
Non-saline	NB	6.06 ± 0.30h	35.23 ± 0.92b	13.86 ± 0.68a	11.36 ± 0.68a
	B	6.15 ± 0.22h	37.42 ± 0.48a	14.40 ± 0.83a	11.58 ± 0.73a
	BP	6.01 ± 0.37h	37.33 ± 0.99a	14.13 ± 0.28a	11.75 ± 0.13a
	BA	5.97 ± 0.44h	36.83 ± 0.21ab	13.94 ± 0.18a	11.55 ± 0.26a
	BPA	6.04 ± 0.22h	38.10 ± 0.44a	14.09 ± 0.16a	11.61 ± 0.16a
6 dS m^{-1}	NB	16.83 ± 0.52d	19.50 ± 0.74f	8.10 ± 0.14cd	7.06 ± 0.12cd
	B	14.26 ± 0.19e	25.33 ± 0.13e	9.35 ± 0.61c	7.36 ± 0.38c
	BP	9.90 ± 0.70g	29.27 ± 0.16d	11.11 ± 0.40b	9.32 ± 0.13b
	BA	12.78 ± 0.39f	28.87 ± 0.78d	9.42 ± 0.30c	9.26 ± 0.16b
	BPA	8.67 ± 0.52g	31.82 ± 0.19c	10.81 ± 0.91b	9.41 ± 0.37b
12 dS m^{-1}	NB	32.60 ± 0.44a	10.52 ± 0.47j	4.50 ± 0.59f	4.05 ± 0.20f
	B	26.68 ± 0.10b	13.30 ± 0.40i	6.36 ± 0.33e	5.03 ± 0.41ef
	BP	20.80 ± 0.82c	15.68 ± 0.60gh	8.06 ± 0.72cd	6.09 ± 0.41de
	BA	25.49 ± 0.39b	14.85 ± 0.72hi	7.79 ± 0.37d	5.10 ± 0.48ef
	BPA	20.05 ± 0.71c	17.24 ± 0.72g	8.03 ± 0.19cd	6.84 ± 0.29cd
Source of variation	**df**	**Mean squares**			
Replication	2	0.003^{ns}	0.168^{ns}	3.095^{*}	1.154^{*}
Salinity (S)	2	1412.38^{**}	1934.87^{**}	193.65^{**}	141.59^{**}
Soil treatment (ST)	4	69.903^{**}	68.686^{**}	7.535^{**}	4.995^{**}
S × ST	8	19.898^{**}	11.275^{**}	2.076^{**}	1.291^{**}
Error	28	0.700	1.123	0.615	0.377

Data represent the average of three replicates ± standard error; ns, *, **: Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test; B: Biochar, BA: Biochar-based *Azotobacter chroococcum* RS-106, BP: Biochar-based *Pseudomonas putida* RS-198, BPA: Biochar-based *Pseudomonas putida* RS-198 + *Azotobacter chroococcum* RS-106, NB: Non-biochar.

Osmolytes and water content of roots and leaves

The interaction of salinity × biochar-inoculated bacteria was significant for osmolytes and water content of the oilseed rape roots and leaves. The soluble sugars, glycine betaine, and proline content were increased, but the water content of roots and leaves was decreased with the increment of salinity (Figure 2). The biochar-related treatments had no significant effect on root and leaf osmolytes and water content under non-saline conditions. In contrast, the combination of biochar and bacteria considerably reduced proline and glycine betaine content and enhanced soluble sugars and water contents of roots and leaves under saline conditions. The combination of biochar and rhizobacteria

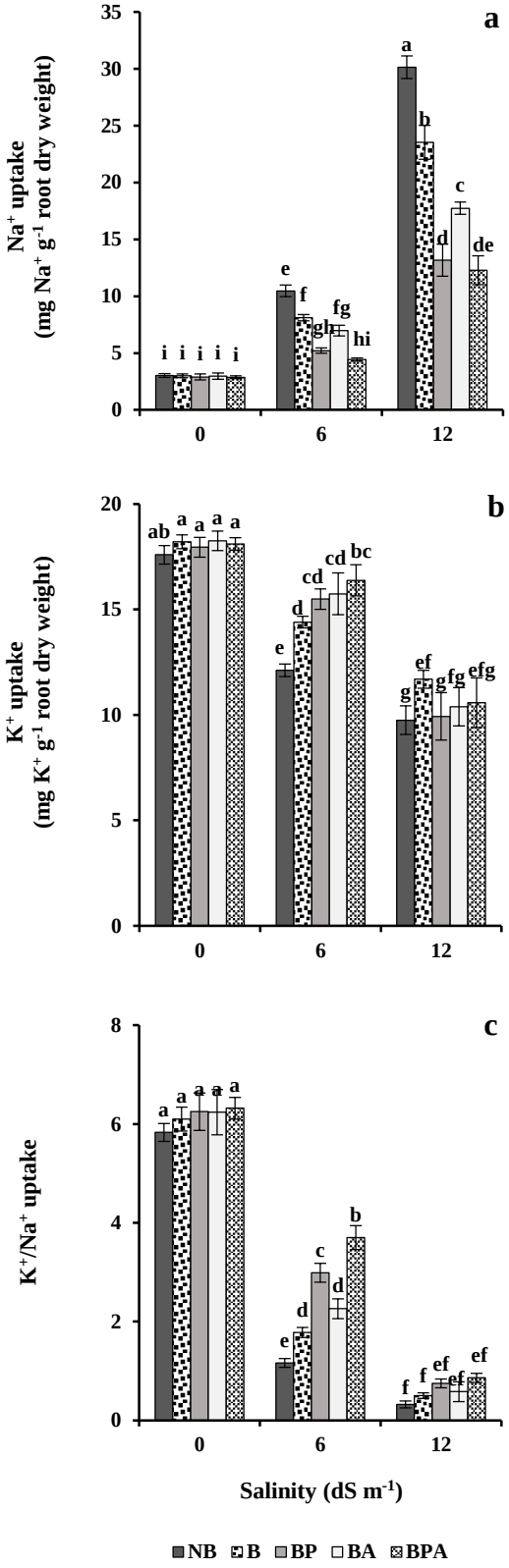

Figure 1. Changes in Na^+ (a) and K^+ (b) uptakes, and K^+/Na^+ ratio (c) of oilseed rape plants in response to biochar-based rhizobacteria under different levels of salinity; Data represent the average of three replicates ± standard error; Different letters indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test; B: Biochar, BA: Biochar-based *Azotobacter chroococcum* RS-106, BP: Biochar-based *Pseudomonas putida* RS-198, BPA: Biochar-based *Pseudomonas putida* RS-198 + *Azotobacter chroococcum* RS-106, NB: Non-biochar.

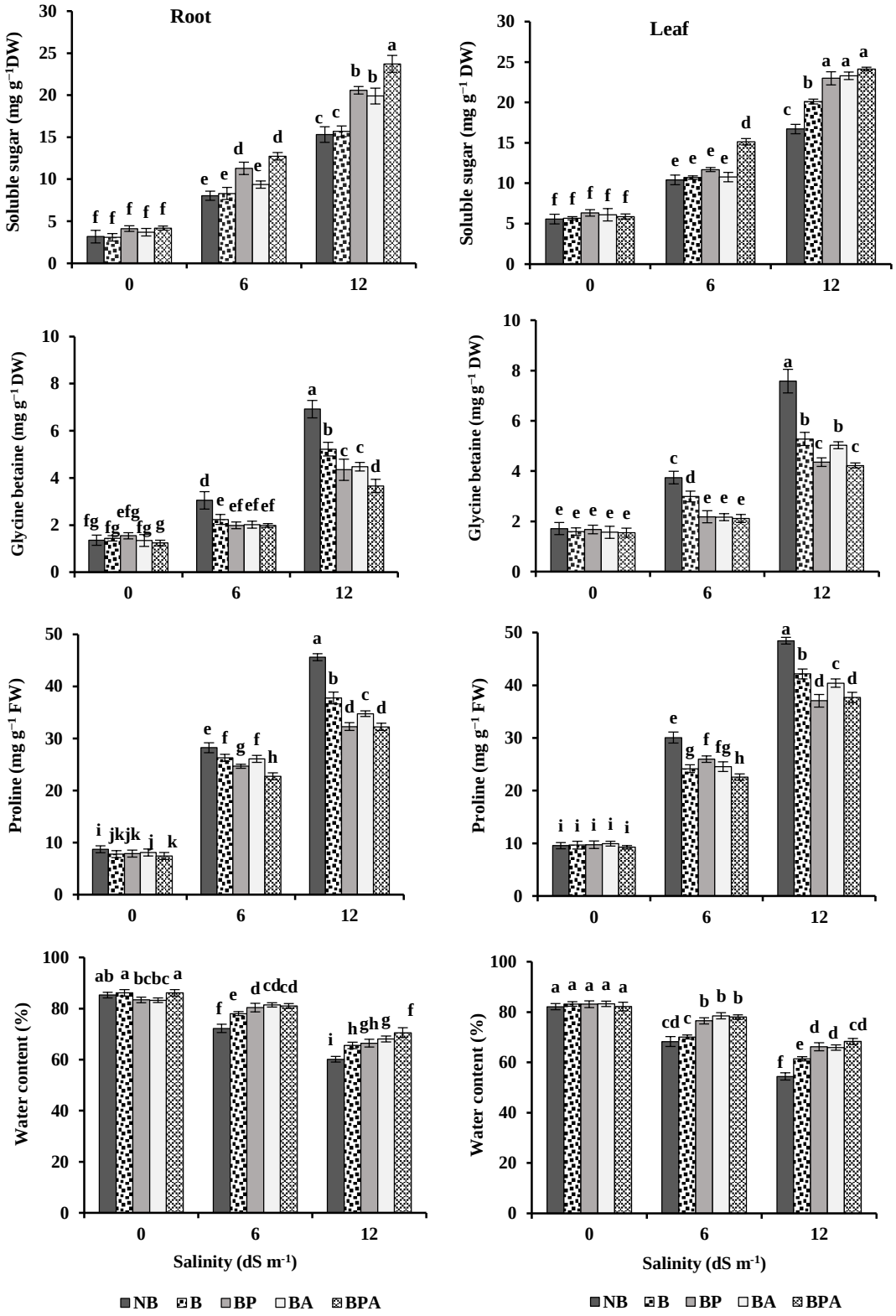

Figure 2. Changes in osmolytes and water content of oilseed rape roots and leaves in response to biochar-based rhizobacteria under different levels of salinity; Data represent the average of three replicates ± standard error;
Different letters indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test; B: Biochar, BA: Biochar-based *Azotobacter chroococcum* RS-106, BP: Biochar-based *Pseudomonas putida* RS-198, BPA: Biochar-based *Pseudomonas putida* RS-198 + *Azotobacter chroococcum* RS-106, NB: Non-biochar.

increased the soluble sugars of roots and leaves, especially under high salinity. Maximum soluble sugars and minimum glycine betaine and proline contents were recorded for the combination of biochar and both bacteria under saline conditions. The bacteria-related treatments resulted in statistically similar root and leaf glycine betaine under moderate salinity, and water content of the roots and leaves under moderate and high salinities, except for BPA under high salinity, which was significantly higher than other bacteria-related treatments. In general, soluble sugars, glycine betaine, and proline content of the oilseed rape leaves were greater than those of in the roots, but root water content was greater than leaf water content (Figure 2).

Leaf pigments and fluorescence

The interaction of salinity × biochar-related treatments was significant for chlorophylls a and b and a/b ratio, carotenoids, anthocyanin, and the maximum quantum yield of photosystem II. Increasing salt stress led to a decrease in chlorophylls a and b, carotenoids, and anthocyanin contents and efficiency of photosystem II, and an increase in chlorophyll a/ chlorophyll b ratio (Table 3). The biochar-related treatments did not influence these traits, except anthocyanin and efficiency of photosystem II, under non-saline conditions. The BP and BPA similarly increased chlorophyll a and anthocyanin contents under moderate salinity and chlorophyll b, chlorophyll a/chlorophyll b, carotenoids, and maximum quantum yield of photosystem II under moderate and high salinities, except for carotenoids under the BP treatment and moderate salinity. Plants grown in biochar inoculated with bacteria-related treatments showed statistically similar chlorophyll b, chlorophyll a/ chlorophyll b ratio, and carotenoids under high salinity (Table 3).

Plant growth and yield components

Significant interaction of salt stress and biochar treatments was found for plant biomass, seed number per plant, and grain yield of oilseed rape (Table 4). An increment of salt stress led to a decrease in plant growth and yield components. However, biochar + bacteria treatments, especially biochar-based *P. putida* RS-198 + *A. chroococcum* RS-106, considerably enhanced these characteristics under all salinity levels. The plant biomass, seeds per plant, and grain yield under non-saline conditions were similarly affected by different bacteria-related treatments. Moreover, the BP and BPA treatments had similar effects on plant biomass under high salinity. No significant differences between BP and BA for seed number and grain yield under high salinity were recorded. The ombination of biochar and both bacteria in comparison with non-biochar enhanced plant biomass, seeds per plant, and grain yield of oilseed rape by 75%, 94%, and 98% under high salinity, respectively (Table 4).

Table 3. Changes in leaf area (cm^2 $plant^{-1}$), leaf pigments (mg g^{-1} DW), and maximum quantum yield of photosystem II of oilseed rape affected by biochar and biochar-based rhizobacteria under different levels of NaCl salinity.

Salinity	Soil treatments	Chl a	Chl b	Chl a/Chl b ratio	Carotenoids	Anthocyanin	Maximum quantum yield of photosystem II
Non-saline	NB	2.03 ± 0.04a	1.26 ± 0.02a	1.62 ± 0.06e	0.65 ± 0.08ab	0.065 ± 0.006d	0.78 ± 0.03bc
	B	2.04 ± 0.06a	1.27 ± 0.02a	1.61 ± 0.06e	0.68 ± 0.03a	0.070 ± 0.004b	0.75 ± 0.03cd
	BP	2.08 ± 0.02a	1.27 ± 0.01a	1.64 ± 0.02de	0.66 ± 0.03ab	0.068 ± 0.005c	0.82 ± 0.01ab
	BA	2.06 ± 0.04a	1.26 ± 0.02a	1.64 ± 0.06de	0.65 ± 0.02ab	0.072 ± 0.007a	0.83 ± 0.01ab
	BPA	2.06 ± 0.02a	1.26 ± 0.02a	1.63 ± 0.03de	0.66 ± 0.04ab	0.071 ± 0.001ab	0.84 ± 0.01a
6 dS m^{-1}	NB	1.39 ± 0.05c	0.83 ± 0.04c	1.68 ± 0.14cde	0.34 ± 0.02ef	0.038 ± 0.004i	0.68 ± 0.03ef
	B	1.76 ± 0.05b	1.03 ± 0.07b	1.71 ± 0.09cde	0.43 ± 0.03de	0.048 ± 0.004f	0.70 ± 0.01de
	BP	1.97 ± 0.09a	1.09 ± 0.03b	1.81 ± 0.06cde	0.53 ± 0.02cd	0.066 ± 0.003d	0.82 ± 0.02ab
	BA	1.80 ± 0.08b	1.11 ± 0.02b	1.62 ± 0.07de	0.55 ± 0.01bc	0.060 ± 0.004e	0.74 ± 0.02cd
	BPA	2.05 ± 0.03a	1.05 ± 0.03b	1.95 ± 0.02bc	0.59 ± 0.04abc	0.065 ± 0.003d	0.82 ± 0.01ab
12 dS m^{-1}	NB	0.90 ± 0.07e	0.53 ± 0.06f	1.74 ± 0.13cde	0.19 ± 0.03g	0.025 ± 0.003j	0.51 ± 0.04h
	B	1.14 ± 0.09d	0.60 ± 0.02ef	1.90 ± 0.12bcd	0.21 ± 0.02g	0.038 ± 0.002i	0.58 ± 0.04g
	BP	1.53 ± 0.06c	0.73 ± 0.03d	2.09 ± 0.07ab	0.29 ± 0.01fg	0.045 ± 0.002g	0.68 ± 0.02ef
	BA	1.41 ± 0.04c	0.66 ± 0.03de	2.16 ± 0.07ab	0.30 ± 0.03fg	0.040 ± 0.001h	0.64 ± 0.01f
	BPA	1.71 ± 0.05b	0.74 ± 0.05cd	2.33 ± 0.11a	0.40 ± 0.05ef	0.048 ± 0.004f	0.72 ± 0.01de
Source of variation	**df**	**Mean squares**					
Replication	2	0.055^{**}	0.004^{ns}	0.023^{ns}	0.004^{ns}	0.00002^{**}	0.013^{**}
Salinity (S)	2	1.958^{**}	1.428^{**}	0.681^{**}	0.549^{**}	0.00302^{**}	0.127^{**}
Soil treatments (ST)	4	0.343^{**}	0.037^{**}	0.111^{**}	0.033^{**}	0.00112^{**}	0.031^{**}
S × ST	8	0.082^{**}	0.012^{**}	0.049^{*}	0.010^{*}	0.00001^{**}	0.003^{**}
Error	28	0.007	0.003	0.021	0.004	0.00001	0.001

Data represent the average of three replicates ± standard error; ns, *, **: Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test; Chl a: Chlorophyll a, Chl b: Chlorophyll b; B: Biochar, BA: Biochar-based *Azotobacter chroococcum* RS-106, BP: Biochar-based *Pseudomonas putida* RS-198, BPA: Biochar-based *Pseudomonas putida* RS-198 + *Azotobacter chroococcum* RS-106, NB: Non-biochar.

The pod number per plant was diminished by salinity. The plants grown under non-saline conditions had the highest pods per plant, but the lowest pods were similarly achieved by plants grown under moderate and high salinities (Figure 3a). the addition of biochar-related treatments to the soil

enhanced pods per plant, with no significant difference between B and NB treatments. The BP, BA, and BPA treatments similarly led to a significant increase in the pods per plant (Figure 3b). The 1000-seed weight was declined by rising salinity levels of the soil. This decrement was about 6% and 21% under 6 and 12 dS m^{-1} NaCl, respectively (Figure 4).

Table 4. Changes in plant biomass (g $plant^{-1}$), seeds per plant, and grain yield (g $plant^{-1}$) of oilseed rape affected by biochar and biochar-based rhizobacteria under different levels of NaCl salinity.

Salinity	Soil treatments	Plant biomass	Seeds per plant	Grain yield
Non-saline	NB	9.97 ± 0.12b	610.7 ± 29.1b	2.38 ± 0.05b
	B	9.89 ± 0.05b	606.7 ± 14.4bc	2.41 ± 0.03b
	BP	10.47 ± 0.25a	647.1 ± 24.1ab	2.59 ± 0.09a
	BA	10.46 ± 0.25a	661.5 ± 29.2a	2.57 ± 0.08a
	BPA	10.55 ± 0.20a	654.1 ± 14.5a	2.61 ± 0.06a
6 dS m^{-1}	NB	5.71 ± 0.25h	349.9 ± 30.9h	1.30 ± 0.08g
	B	7.01 ± 0.24f	443.2 ± 13.8g	1.65 ± 0.07ef
	BP	8.25 ± 0.32d	569.4 ± 22.5cd	2.12 ± 0.07c
	BA	7.37 ± 0.26e	484.4 ± 17.1f	1.80 ± 0.08d
	BPA	8.84 ± 0.23c	634.4 ± 22.5ab	2.38 ± 0.04b
12 dS m^{-1}	NB	3.80 ± 0.20j	281.3 ± 22.7i	0.87 ± 0.07i
	B	4.67 ± 0.22i	351.2 ± 31.8h	1.09 ± 0.10h
	BP	6.47 ± 0.20g	516.6 ± 31.2ef	1.64 ± 0.08ef
	BA	5.94 ± 0.17h	496.9 ± 12.0f	1.57 ± 0.07f
	BPA	6.65 ± 0.14g	545.9 ± 35.3de	1.72 ± 0.07de
Source of variation	**df**		**Mean squares**	
Replication	2	1.584^{**}	20563.69^{**}	0.183^{**}
Salinity (S)	2	86.08^{**}	154885.3^{**}	4.869^{**}
Soil treatments (ST)	4	7.214^{**}	59508.03^{**}	0.778^{**}
S × ST	8	0.983^{**}	9547.152^{**}	0.101^{**}
Error	28	0.038	497.515	0.004

Data represent the average of three replicates ± standard error; ns, *, **: Not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test; B: Biochar, BA: Biochar-based *Azotobacter chroococcum* RS-106, BP: Biochar-based *Pseudomonas putida* RS-198, BPA: Biochar-based *Pseudomonas putida* RS-198 + *Azotobacter chroococcum* RS-106, NB: Non-biochar.

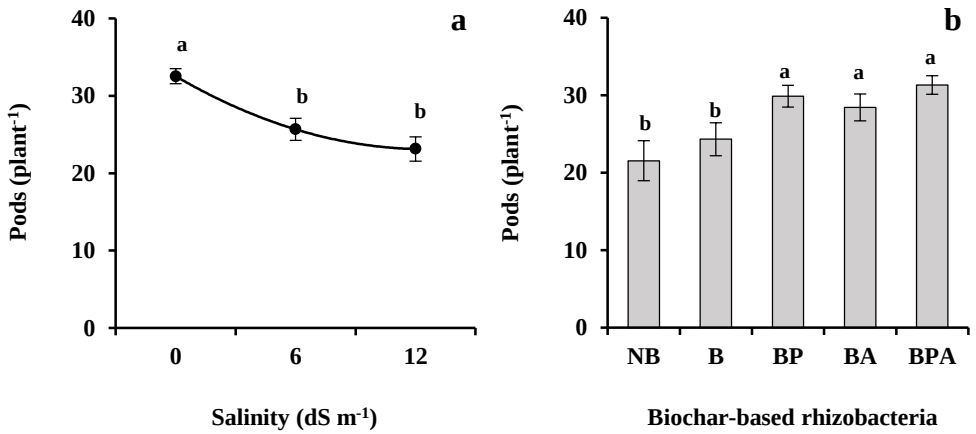

Figure 3. Changes in pods per plant of oilseed rape in response to salinity (a) and biochar-based rhizobacteria (b); Data represents the average of three replicates ± standard error; Different letters indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test; B: Biochar, BA: Biochar-based *Azotobacter chroococcum* RS-106, BP: Biochar-based *Pseudomonas putida* RS-198, BPA: Biochar-based *Pseudomonas putida* RS-198 + *Azotobacter chroococcum* RS-106, NB: Non-biochar.

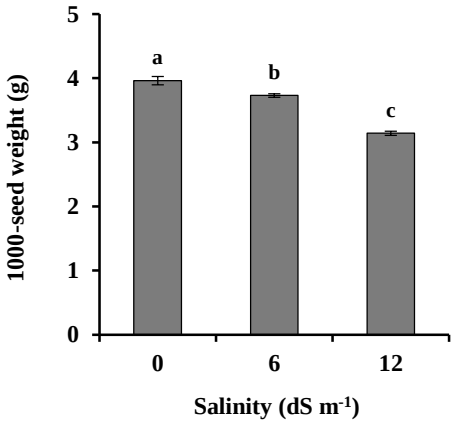

Figure 4. Changes in 1000-seed weight of oilseed rape in response to different levels of salinity; Data represent the average of three replicates ± standard error; Different letters indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test.

Discussion

Utilizing biochar (Abd El-Mageed *et al.* 2020) and beneficial plant growth-promoting rhizobacteria (Arkhipova *et al.* 2020) can be effective approaches to help plants withstand salt stress. The results revealed that salinity negatively affects the photosynthetic pigments, water status, growth, and productivity of oilseed rape plants, but application of biochar and especially biochar-based rhizobacteria helped to minimize the damaging effect of salinity on physiological performance, plant growth, and grain yield. Salt stress enhanced Na^+ uptake by root cells (Figure 1), while reducing K^+, Ca^{2+}, and Mg^{2+} concentrations in plant tissues (Table 2). This can be due to the changes in the soil quality as a consequence of the salinity increment. Declining soil pH and cation exchange capacity (Ghassemi-Golezani and Rahimzadeh 2024), as well as augmenting Na^+ concentration in soil

solution, have pivotal roles in reducing the bioavailability of essential nutrients for plants under saline conditions. Moreover, the disturbed ionic relations may result from the changes in gene transcription coding different types of transporters and/or channels involved in regulating transmembrane fluxes of Na^+ and K^+ in salt-subjected plants. Impaired Na^+ and K^+ homeostasis in plant tissues may be attributed to differential expression of *HKT* transporters such as *HKT1* and *HKT2* (mostly for K^+ exclusion) in roots under salinity (Rizk *et al.* 2021). Most likely, the improvement of soil properties by the addition of biochar is related to high sodium sorption and cation exchange capacities of biochar, leading to reduced exchangeable sodium percentage in soil (Luo *et al.* 2017). The superiority of biochar in diminishing Na^+ uptake was enhanced by the combination with *P. putida* RS-198 and *A. chroococcum* RS-106 (Figure 1), which might be due to rhizobacteria-mediated improvement in soil quality, solubilities of nutrients, and root growth (Kapadia *et al.* 2021; Ghassemi-Golezani and Abdoli 2023). Reducing the exchangeable sodium percentage in the soil by these treatments increased the bioavailability and absorption capacity of essential nutrients in the rhizosphere, leading to increased uptake and accumulation of K^+, Ca^{2+}, and Mg^{2+} in plant tissues (Figure 1; Table 2). Moreover, rhizobacteria can restrict Na^+ influx to the root cells by releasing some chemical compounds. Kasotia *et al.* (2016) found that *Pseudomonas sp.* AK-1 was able to make Na^+ inaccessible to plants by binding exopolysaccharides to free sodium in saline soils.

Increasing osmo-regulators such as soluble sugars, glycine betaine, and proline (Figure 2) is an effective mechanism to protect plants from osmotic stress caused by salinity. High levels of sugars can maintain membrane integrity and cell turgor and prevent water loss (Aziz *et al.* 2018). Some gene expressions and enzyme activations could be effective in proline accumulation in response to salinity (Guerzoni *et al.* 2014; Wang *et al.* 2015). It was also reported that proline, as an osmo-protectant, was effective for protein stabilizer, inhibitor of lipid peroxidation, and scavenger of ROS (Trovato *et al.* 2008). This can maintain photosynthetic pigments, enzyme activities, and membrane integrity (Szabados and Savouré 2010; Surender Reddy *et al.* 2015). Glycine betaine removes excess ROS, maintains Rubisco activity and oxygen-evolving complex of PSII (Ohnishi and Murata 2006), and activates genes related to stress (Chen and Murata 2008; Khan *et al.* 2012). Despite a proline and glycine betaine decrement, soluble sugar accumulation markedly increased in oilseed rape plants grown under different biochar-related treatments, indicating the critical roles of soluble sugars in osmo-adjusting of plants grown under these treatments in saline conditions, especially under high salinity (Figure 2). Interestingly, the root and leaf water contents of plants grown under different biochar + bacteria treatments were not accompanied by an increase of glycine betaine and proline accumulations (Figure 2). The results revealed an increase in water absorption and maintenance of

water dynamics under salt stress by plants grown under biochar inoculated with bacteria individually and in combination. The increased ability of the roots to uptake water is facilitated by the activation of root growth and modification of its architecture, which was previously proven in response to biochar (Ghassemi-Golezani and Abdoli 2023). Arkhipova *et al.* (2020) found an accumulation of abscisic acid in response to bacteria, accompanied by an increase in root growth, which can enhance the water and nutrient uptake.

Photosynthesis is the main process in the formation of plant productivity. The content of the photosynthetic pigments is an indirect index of photosynthetic activity and the most important biochemical indicator of photosynthesis rate (Ghassemi-Golezani and Farhadi 2021). Decreasing mineral nutrients' uptake by plants (Figure 1) and contents in plant tissues (Table 2) under salinity also resulted in a decline in chlorophylls a and b, carotenoids, and anthocyanins, which led to a decrease in maximum quantum yield of PSII (Table 3). Salt-induced oxidative stress can potentially reduce photosynthetic pigments and activity (Rasool *et al.* 2013). This stress has a negative impact on PSII electron transfer (Ghassemi-Golezani *et al.* 2021) and their photoinhibition, resulting in the demolition of pigments. Block of electron transfer from the main receptor (plastoquinone, QA) to the secondary receptor (plastoquinone, QB) at the acceptor side of PSII by salt stress can reduce photochemical efficiency of photosystem II (Shu *et al.* 2012). It is possible that increasing ethylene synthesis caused by salinity (Hussain *et al.* 2020) can also elevate degradation of leaf pigment (Figure 3), thereby decreasing photosynthesis and plant growth. The beneficial effects of biochar amendment on photosynthetic activities of plants are likely linked to the improved soil water holding capacity (Adhikari *et al.* 2023), nutrients availability and retention (Liu *et al.* 2018), and soil pH and cation exchange capacity (Ghassemi-Golezani and Rahimzadeh 2024), and limited bioavailability of toxic Na^+ ions (Figure 1; Table 2). Soil amendment by biochar enriched with sulfur and effective microorganisms enhanced photosynthetic activities by improving dehydration tolerance and water use efficiency of *Capsicum annuum* plants under salt stress (Abd El-Mageed *et al.* 2020). The ameliorative effects of biochar and biochar + bacteria treatments on salt tolerance of plants could also be attributed to the anthocyanin- and flavonoid-rich plant extracts (Table 3). Anthocyanins and flavonoids can be considered as two major groups of plant phenolics, which protect against oxidative stress caused by salinity. Radyukina *et al.* (2012) found that flavonoids and particularly anthocyanins are involved in the plant defense system, stimulating salt resistance in three medicinal plants.

Salt stress led to growth reduction, which negatively affected pods per plant (Figure 3a), 1000-seed weight (Figure 4), seeds per plant, and grain yield (Table 4). This stress reduced crop growth and productivity in favor of enhancing the osmo-protectants synthesis (Figure 2) and limiting the

energy loss, which increase the chance of plant survival. Rengasamy (2010) has found that the intense decline in water absorption during the flowering and seed filling period resulted in a great adverse impact on the grain yield of wheat plants under saline conditions. Salinity may also influence the plant phenology by diminishing reproductive duration or delaying the onset of flowering (Pushpavalli *et al.* 2016), and consequently reducing the yield components and grain yield (Figures 3 and 4; Table 4). Moreover, the transcriptional changes in pivotal components of the photoperiodic flowering pathway due to salinity can remarkably modify flowering time (Kim *et al.* 2013). The biochar and biochar inoculated with bacteria increased the growth and yield of oilseed rape plants, particularly under salt stress (Table 4). The simultaneous application of biochar and rhizobacteria improved nutrients and water uptake, leaf pigments, and photochemical efficiency of photosystem II, causing an increase in synthesis and transmission of more photo-assimilates during seed filling, thus elevating plant biomass and, more importantly, yield-related characteristics under salt stress (Figures 3 and 4; Table 4). These useful effects of biochar-related treatments in mitigating salt toxicity are most likely associated with improving the soil properties and its structure.

Conclusion

Salinity impedes plant growth and productivity due to the excessive accumulation of Na^+ and the reduction of essential nutrients and leaf pigments. The adverse effects of salinity on plants were alleviated by the application of biochar + bacteria treatments. The improved plant biomass and yield and components of salt-affected oilseed rape plants in response to biochar-related treatments, particularly by the combination of biochar and *P. putida* RS-198 + *A. chroococcum* RS-106, are highly attributed to improved water and nutritional relations, photosynthetic pigments, and maximum quantum yield of photosystem II. Amendment of soil with biochar and biochar + bacteria treatments noticeably reduced the sodium uptake by plants and enriched the plant cells with potassium, calcium, and magnesium under saline conditions. Ultimately, these beneficial impacts of biochar-related treatments resulted in the enhanced grain yield via increasing pods and seeds per plant. Overall, the potential provided by the integrated application of biochar and rhizobacteria is a valuable resource for promoting salt tolerance and productivity of plants. Future work may reveal other beneficial effects of biochar and rhizobacteria on plant performance under various environmental conditions.

Conflict of Interest

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

Data Availability

Data will be available by the corresponding author on a reasonable request.

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References

Abdel Latef AAH, Abu Alhmad MF, Kordrostami M, Abo-Baker Abd-Elmoniem Abo-Baker ABAE, Zakir, A. 2020. Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions. J Plant Growth Regul. 39: 1293-1306. https://doi.org/10.1007/s00344-020-10065-9

Abd El-Mageed TA, Rady MM, Taha RS, Abd El Azeam S, Simpson CR, Semida WM. 2020. Effects of integrated use of residual sulfur-enhanced biochar with effective microorganisms on soil properties, plant growth and short-term productivity of *Capsicum annuum* under salt stress. Sci Hortic. 261: 108930. https://doi.org/10.1016/j.scienta.2019.108930

Abdoli S, Ghassemi-Golezani K. 2021. Salicylic acid: an effective growth regulator for mitigating salt toxicity in plants. J Plant Physiol Breed. 11(1): 1-15. https://doi.org/10.22034/JPPB.2021.13681

Abdoli S, Ghassemi-Golezani K. 2025. Foliar treatments of salicylic acid and iron nanoparticles enhanced antioxidant potential and essential oil production of ajowan under salt stress. Plant Biosyst. 159(1): 92-102. https://doi.org/10.1080/11263504.2024.2446790

Abdoli S, Ghassemi-Golezani K, Alizadeh-Salteh S. 2020. Responses of ajowan (*Trachyspermum ammi* L.) to exogenous salicylic acid and iron-oxide nanoparticles under salt stress. Environ Sci Pollut Res Int. 27(29): 36939-36953. https://doi.org/10.1007/s11356-020-09453-1

Adhikari S, Mahmud MP, Nguyen MD, Timms W. 2023. Evaluating fundamental biochar properties in relation to water holding capacity. Chemosphere. 328: 138620. https://doi.org/10.1016/j.chemosphere.2023.138620

Amini S, Ghadiri H, Chen C, Marschner P. 2016. Salt-affected soils, reclamation, carbon dynamics, and biochar: a review. J Soils Sediments. 16: 939-953. https://doi.org/10.1007/s11368-015-1293-1

Ansari M, Shekari F, Mohammadi MH, Juhos K, Végvári G, Biró B. 2019. Salt-tolerant plant growth-promoting bacteria enhanced salinity tolerance of salt-tolerant alfalfa (*Medicago sativa* L.)

cultivars at high salinity. Acta Physiol Plant. 41: 195. https://doi.org/10.1007/s11738-019-2988-5

Arif Y, Singh P, Siddiqui H, Bajguz A, Hayat S. 2020. Salinity induced physiological and biochemical changes in plants: an omic approach towards salt stress tolerance. Plant Physiol Biochem. 156: 64-77. https://doi.org/10.1016/j.plaphy.2020.08.042

Arkhipova T, Martynenko E, Sharipova G, Kuzmina L, Ivanov I, Garipova M, Kudoyarova G. 2020. Effects of plant growth promoting rhizobacteria on the content of abscisic acid and salt resistance of wheat plants. Plants. 9(11): 1429. https://doi.org/10.3390/plants9111429

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24(1): 115. https://doi.org/10.1104/pp.24.1.1

Ashraf M, McNeilly T. 2004. Salinity tolerance in Brassica oilseeds. Crit Rev Plant Sci. 23(2): 157-174. https://doi.org/10.1080/07352680490433286

Ayuso-Calles M, Flores-Félix JD, Rivas R. 2021. Overview of the role of rhizobacteria in plant salt stress tolerance. Agronomy. 11: 1759. https://doi.org/10.3390/agronomy11091759

Aziz A, Akram NA, Ashraf M. 2018. Influence of natural and synthetic vitamin C (ascorbic acid) on primary and secondary metabolites and associated metabolism in quinoa (*Chenopodium quinoa* Willd.) plants under water deficit regimes. Plant Physiol. Biochem. 123: 192-203. https://doi.org/10.1016/j.plaphy.2017.12.004

Bal HB, Nayak L, Das S, Adhya TK. 2013. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil. 366: 93-105. https://doi.org/10.1007/s11104-012-1402-5

Banaei-Asl F, Bandehagh A, Dorani Uliaei E, Farajzadeh D, Sakata K, Mustafa G, Komatsu S. 2015. Proteomic analysis of canola root inoculated with bacteria under salt stress. J Proteomics. 124: 88-111. https://doi.org/10.1016/j.jprot.2015.04.009

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205-207. https://doi.org/10.1007/BF00018060

Busch D, Glaser B. 2015. Stability of co-composted hydrochar and biochar under field conditions in a temperate soil. Soil Use Manag. 31(2): 251-258. https://doi.org/10.1111/sum.12180

Carter MR, Gregorich EG. 2008. Soil sampling and methods of analysis. Second edition. Manitoba, Canada: Canadian Society of Soil Science. 1262 pages. https://doi.org/10.1201/9781420005271

Chapman HD. 1965. Cation exchange capacity. In: Black CA (ed.). Methods of soil analysis. Madison, Wisconsin: American Society of Agronomy. pp. 891-901.

Chen THH, Murata N. 2008. Glycine betaine: an effective protectant against abiotic stress in plants. Trends Plant Sci. 13(9): 499-505. https://doi.org/10.1016/j.tplants.2008.06.007

Farouk S, Elhindi KM, Alotaibi MA. 2020. Silicon supplementation mitigates salinity stress on *Ocimum basilicum* L. via improving water balance, ion homeostasis, and antioxidant defense system. Ecotoxicol Environ Saf. 206: 111396. https://doi.org/10.1016/j.ecoenv.2020.111396

Ghassemi-Golezani K, Abdoli S. 2021. Improving ATPase and PPase activities, nutrient uptake and growth of salt stressed ajowan plants by salicylic acid and iron-oxide nanoparticles. Plant Cell Rep. 403): 559-573. https://doi.org/10.1007/s00299-020-02652-7

Ghassemi-Golezani K, Abdoli S. 2023. Alleviation of salt stress in rapeseed (*Brassica napus* L.) plants by biochar-based rhizobacteria: new insights into the mechanisms regulating nutrient uptake, antioxidant activity, root growth and productivity. Arch Agron Soil Sci. 69(9): 1548-1565. https://doi.org/10.1080/03650340.2022.2103547

Ghassemi-Golezani K, Abdoli S. 2024. Salicylic acid and iron-oxide nanoparticles improved the growth and productivity of ajowan under salt stress. Adv Hortic Sci. 38(2): 129-139. https://doi.org/10.36253/ahsc-15671

Ghassemi-Golezani K, Farhadi N. 2021. The efficacy of salicylic acid levels on photosynthetic activity, growth, and essential oil content and composition of pennyroyal plants under salt stress. J Plant Growth Regul. 41: 1953-1965. https://doi.org/10.1007/s00344-021-10515-y

Ghassemi-Golezani K, Mousavi SA. 2022. Improving physiological performance and grain yield of maize by salicylic acid treatment under drought stress. J Plant Physiol Breed. 12(2): 1-10. https://doi.org/10.22034/JPPB.2022.16041

Ghassemi-Golezani K, Rahimzadeh S. 2024. Biochar-based nanoparticles mitigated arsenic toxicity and improved physiological performance of basil via enhancing cation exchange capacity and ferric chelate reductase activity. Chemosphere. 362: 142623. https://doi.org/10.1016/j.chemosphere.2024.142623

Ghassemi-Golezani K, Farhangi-Abriz S, Abdoli S. 2021. How can biochar-based metal oxide nanocomposites counter salt toxicity in plants? Environ Geochem Health. 43(5): 2007-2023. https://doi.org/10.1007/s10653-020-00780-3

Grieve CM, Grattan SR. 1983. Rapid assay for determination of water-soluble quaternary ammonium compounds. Plant Soil. 70: 303-307. https://doi.org/10.1007/BF02374789

Guerzoni JTS, Belintani NG, Moreira RMP, Hoshino AA, Domingues DS, Filho JCB, Vieira LGE. 2014. Stress-induced D1-pyrroline-5-carboxylate synthetase (P5CS) gene confers tolerance to

salt stress in transgenic sugarcane. Acta Physiol Plant. 36(9): 2309-2319. https://doi.org/10.1007/s11738-014-1579-8

Gupta S, Pandey S. 2019. ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. Front Microbiol. 10: 1506. https://doi.org/10.3389/fmicb.2019.01506

Hussain S, Huang J, Zhu C, Zhu L, Cao X, Hussain S, Ashraf M, Khaskheli MA, Kong Y, Jin Q, *et al.* 2020. Pyridoxal 5′-phosphate enhances the growth and morpho-physiological characteristics of rice cultivars by mitigating the ethylene accumulation under salinity stress. Plant Physiol Biochem. 154: 782-795. https://doi.org/10.1016/j.plaphy.2020.05.035

Jamil M, Lee KJ, Kim JM, Kim HS, Rha ES. 2007. Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. Sci Agric. 64(2): 111-118. https://doi.org/10.1590/S0103-90162007000200002

Kan X, Ren J, Chen T, Cui M, Li C, Zhou R, Zhang Y, Liu H, Deng D, Yin Z. 2017. Effects of salinity on photosynthesis in maize probed by prompt fluorescence, delayed fluorescence and P700 signals. Environ Exp Bot. 140: 56-64. https://doi.org/10.1016/j.envexpbot.2017.05.019

Kapadia C, Sayyed RZ, El Enshasy HA, Vaidya H, Sharma D, Patel N, Malek RA, Syed A, Elgorban AM, Ahmad K, *et al.* 2021. Halotolerant microbial consortia for sustainable mitigation of salinity stress, growth promotion, and mineral uptake in tomato plants and soil nutrient enrichment. Sustainability. 13(15): 8369. https://doi.org/10.3390/su13158369

Kasotia A, Varma A, Tuteja N, Choudhary DK. 2016. Amelioration of soybean plant from saline-induced condition by exopolysaccharide producing *Pseudomonas*-mediated expression of high affinity K^+ -transporter (*HKT1*) gene. Curr Sci. 111(12): 1961-1967. https://doi.org/10.18520/cs/v111/i12/1961-1967

Khan MIR, Iqbal N, Masood A, Khan NA. 2012. Variation in salt tolerance of wheat cultivars: Role of glycine betaine and ethylene. Pedosphere. 22(6): 746-754. https://doi.org/10.1016/S1002-0160(12)60060-5

Kim WY, Ali Z, Park HJ, Park SJ, Cha JY, Perez-Hormaeche J, Quintero FJ, Shin G, Kim MR, Qiang Z, *et al.* 2013. Release of SOS2 kinase from sequestration with GIGANTEA determines salt tolerance in *Arabidopsis*. Nat Commun. 4: 1352. https://doi.org/10.1038/ncomms2357

Kochert A, 1978. Carbohydrate determination by phenol-sulfuric acid method. In: Hellebust JA, Craige JS (eds.). Handbook of physiology and biochemical methods. London: Cambridge University Press. pp. 95-97.

Liu Q, Zhang Y, Liu B, Amonette JE, Lin Z, Liu G, Ambus P, Xie Z. 2018. How does biochar influence soil N cycle? A meta-analysis. Plant Soil. 426: 211-225. https://doi.org/10.1007/s11104-018-3619-4

Lokhande VH, Nikam TD, Patade VY, Ahire ML, Suprasanna P. 2011a. Effects of optimal and supra-optimal salinity stress on antioxidative defense, osmolytes and in vitro growth responses in *Sesuvium portulacastrum* L. Plant Cell Tiss Organ Cult. 104: 41-49. https://doi.org/10.1007/s11240-010-9802-9

Lokhande VH, Srivastava AK, Srivastava S, Nikam TD, Suprasanna P. 2011b. Regulated alterations in redox and energetic status are the key mediators of salinity tolerance in the halophyte *Sesuvium portulacastrum* L. Plant Growth Regul. 65: 287-298. https://doi.org/10.1007/s10725-011-9600-3

Luo X, Liu G, Xia Y, Chen L, Jiang Z, Zheng H, Wang Z. 2017. Use of biochar-compost to improve properties and productivity of the degraded coastal soil in the Yellow River Delta, China. J Soils Sediments, 17: 780-789. https://doi.org/10.1007/s11368-016-1361-1

Mayak S, Tirosh T, and Glick BR, 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiology and Biochemistry 42: 565–572. https://doi.org/10.1016/j.plaphy.2004.05.009

Nia SH, Zarea MJ, Rejali F, Varma A. 2012. Yield and yield components of wheat as affected by salinity and inoculation with *Azospirillum* strains from saline or non-saline soil. J Saudi Soc Agric Sci. 11(2): 113-121. https://doi.org/10.1016/j.jssas.2012.02.001

Ohnishi N, Murata N. 2006. Glycine betaine counteracts the inhibitory effects of salt stress on the degradation and synthesis of D1 protein during photoinhibition in *Synechococcus* sp. PCC 7942. Plant Physiol. 141(2): 758-765. https://doi.org/10.1104/pp.106.076976

Pushpavalli R, Quealy J, Colmer TD, Turner NC, Siddique KHM, Rao MV, Vadez V. 2016. Salt stress delayed flowering and reduced reproductive success of chickpea (*Cicer arietinum* L.), a response associated with Na^+ accumulation in leaves. J Agron Crop Sci. 202(2): 125-138. https://doi.org/10.1111/jac.12128

Qian L, Chen B, Hu D. 2013. Effective alleviation of aluminum phytotoxicity by manure derived biochar. Environ Sci Technol. 47(6): 2737-2745. https://doi.org/10.1021/es3047872

Radyukina NL, Toaima VIM, Zaripova NR. 2012. The involvement of low-molecular antioxidants in cross-adaptation of medicine plants to successive action of UV-B radiation and salinity. Russ J Plant Physiol. 59: 71-78. https://doi.org/10.1134/s1021443712010165

Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K., 2013. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. SpringerPlus. 2: 6. https://doi.org/10.1186/2193-1801-2-6

Rasool S, Ahmad A, Siddiqi TO, Ahmad P. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiol Plant. 35: 1039-1050. https://doi.org/10.1007/s11738-012-1142-4

Rengasamy P. 2010. Soil processes affecting crop production in salt-affected soils. Funct Plant Biol. 37(7): 613-620. https://doi.org/10.1071/FP09249

Rizk MS, Mekawy AM, Assaha DV, Chuamnakthong S, Shalaby NE, Ueda A. 2021. Regulation of Na^+ and K^+ transport and oxidative stress mitigation reveal differential salt tolerance of two Egyptian maize (*Zea mays* L.) hybrids at the seedling stage. J Plant Growth Regul. 40: 1629-1639. https://doi.org/10.1007/s00344-020-10216-y

Shu S, Guo SR, Sun J, Yuan LY. 2012. Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. Physiol Plant. 146(3): 285-296. https://doi.org/10.1111/j.1399-3054.2012.01623.x

Šimanský V, Horák J, Igaz D, Balashov E, Jonczak J. 2018. Biochar and biochar with N fertilizer as a potential tool for improving soil sorption of nutrients. J Soils Sediments. 18: 1432-1440. https://doi.org/10.1007/s11368-017-1886-y

Solhi-Khajehmarjan R, Ghassemi-Golezani K, Alizadeh Salteh S. 2025. The efficacy of solid and enriched biochars with magnesium and iron nanoparticles on growth and essential oil composition of German chamomile under salt stress. J Plant Physiol Breed. 15(1): 1-15. https://doi.org/10.22034/jppb.2025.64958.1354

Song JM, Guan Z, Hu J, Guo C, Yang Z, Wang S, Liu D, Wang B, Lu S, Zhou R, *et al.* 2020. Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. Nat Plants. 6(1): 34-45. https://doi.org/10.1038/s41477-019-0577-7

Surender Reddy P, Jogeswar G, Rasineni GK, Maheswari M, Reddy AR, Varshney RK, Kavi Kishor PB. 2015. Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. Plant Physiol Biochem. 94: 104-113. https://doi.org/10.1016/j.plaphy.2015.05.014

Szabados L, Savouré A. 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15(2): 89-97. https://doi.org/10.1016/j.tplants.2009.11.009

Tanveer M, Shah AN. 2017. An insight into salt stress tolerance mechanisms of *Chenopodium album*. Environ Sci Pollut Res Int. 24(19): 16531-16535. https://doi.org/10.1007/s11356-017-9337-2

Trovato M, Mattioli R, Costantino P. 2008. Multiple roles of proline in plant stress tolerance and development. Rend Fis Acc Lincei. 19: 325-346. https://doi.org/10.1007/s12210-008-0022-8

Wang H, Tang X, Wang H, Shao HB. 2015. Proline accumulation and metabolism-related genes expression profiles in *Kosteletzkya virginica* seedlings under salt stress. Front Plant Sci. 6: 792. https://doi.org/10.3389/fpls.2015.00792

Wu S, Zhang Y, Tan Q, Sun X, Wei W, Hu C. 2020. Biochar is superior to lime in improving acidic soil properties and fruit quality of *Satsuma mandarin*. Sci Total Environ. 714: 136722. https://doi.org/10.1016/j.scitotenv.2020.136722

Zhang H, Kim MS, Sun Y, Dowd SE, Shi H, Paré PW. 2008. Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter *HKT1*. Mol Plant Microbe Interact. 21(6): 737-744. https://doi.org/10.1094/MPMI-21-6-0737