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Integrated application of plant growth promoting rhizobacteria and biochar improves salt tolerance in eggplant seedlings

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Abstract: A pot study was conducted to determine the effects of the combination of plant growth promoting rhizobacteria (PGPR) and biochar on the growth, physiological, and biochemical characteristics of eggplant seedlings under salinity stress. The greenhouse experiment included two salinity levels of NaCl [S0 (0 mM NaCl) and S1 (100 mM NaCl)], three biochar levels [B0 (non-biochar), B1 (5%) and B2 (10%)] and two PGPR [R0 (non-PGPR), R1 (combination of *Bacillus megaterium* TV-6D, *Paenibacillus polymyxa* KIN-37, and *Pantoea agglomerans* RK92). Results showed that plant growth, relative leaf water content (LRWC), and chlorophyll content of eggplant seedlings decreased significantly, while malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, sucrose and ABA content, and electrolyte leakage (EL) increased significantly with increase in salinity levels. Biochar and PGPR applications mitigated the negative influence of salinity stress on plant growth and physiological and biochemical characteristics of eggplant seedlings, enhancing chlorophyll content, plant nutrient element uptake, and antioxidant enzyme activity. The enhanced salinity tolerance due to biochar and PGPR applications could be associated with a significant reduction in Na and Cl uptake, MDA, H_2O_2 and EL and an increase in LRWC, chlorophyll content, antioxidant activity and plant nutrient element uptake. Therefore, it can be concluded that combining biochar and PGPR could be used to minimize the detrimental impacts of salinity stress conditions in eggplant seedlings.

Key words: Salinity stress, PGPR, biochar, eggplant, physiology, biochemistry

1. Introduction

Global threats such as climate change, land degradation, environmental pollution and water shortages cause economic, social, and environmental damage (Siddiqui et al., 2016). These stress factors in the world significantly limited productivity of agricultural production. In combating stress conditions, traditional breeding methods, biotechnological approaches, the use of molecular markers and transgenic technologies and the development of resistant species, cultivars or genotypes are among the most effective solutions in plant production. However, these methods can be time-consuming, expensive and quite complex (Zaidi et al., 2015).

The term "Plant Growth Promoting Rhizobacteria" was first used in 1978 (Kloepper, 1978). These rhizobacteria increase plant and root growth by colonizing the rhizosphere. Although the rhizosphere is a closed food pool containing all necessary macro- and micronutrients for its plants, it is the region where microbial activity is at its highest (Vejan et al., 2016). The effects of PGPRs on plants are examined concerning direct and indirect effects. Direct mechanisms include biological nitrogen fixation (Fukami et al., 2018), production of plant hormones such as auxin, gibberellin and cytokine, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Ali and Kim, 2018), increasing the solubility of inorganic phosphorus and mineralization of organic phosphorus compounds (Khosravi et al., 2018; Singh and Gera, 2018), increasing potassium (Meena et al., 2014) and iron uptake through siderophore production (Patel et al., 2018), and inhibition of ethylene synthesis, reducing environmental stress (Ali and Kim, 2018). Many studies have reported that PGPR applications mitigated the deleterious impacts of salinity stress in several vegetable crops (Yildirim et al., 2006; Yildirim et al., 2008; Ilangumaran and Smith, 2017).

The porous material produced by thermal decomposition of biomass by pyrolysis, in a low or oxygen-free environment, is called biochar (Lehmann et al., 2012). Most of the biochar produced by pyrolysis is carbon and the residence time in the soil is estimated to be between hundreds and thousands of years (Lehmann et al., 2006). The effects of biochar have direct effects on providing nutrients and indirect effects as soil regulators for plants. The positive effect of biochar on crop yield

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is mainly attributed to the nutrients present in biochar and indirectly to an increase in productivity. Direct and indirect fertility functions are reported as fertilizer and soil regulator properties of biochar, respectively (Glaser et al., 2002). It may contain valuable nutrients such as K, Ca, and Mg (Novak et al., 2009). Biochar application may also substantially modify soil field capacity (Glaser et al., 2002).

Biochar has a highly porous internal structure. Thus, it increases the water and nutrient holding capacity of the soil, improving the soil structure and can be used as a soil improver with its low volume weight. The pore distribution of the soils where biochar is applied changes and the soil's resistance to agricultural tools decreases (Obia et al., 2016).

Eggplants are rich in vitamins, minerals, protein, antioxidants, fiber etc. (Naeem and Ugur, 2019). Eggplant may be stated as moderately sensitive to salinity by the classification scheme indicated by Maas and Hoffman (1977).

Studies show the positive effects of PGPR and biochar applications separately on many vegetable species grown under salt stress. However, no studies have shown the combined effect of PGPR and biochar application on eggplant. Therefore, this study was conducted to determine whether the effects of the combination of PGPR and biochar have a positive effect on eggplant seedlings under salinity stress and to determine the physiological and biochemical characteristics.

2. Materials and methods

2.1. Material and greenhouse conditions

The experiment was carried out at a greenhouse belonging to the Agriculture Faculty of Atatürk University. The temperature and humidity of greenhouse were 29.8 ± 3.5 ° C and 66 ± 5.9%, respectively. "Topan" eggplant (*Solanum melongena* L.) was used. The experiment was done in a completely randomized design with three replications including 5 pots in each repeat. The experiment design included 12 treatments, two salt stress treatments (S0 and S1), together with three biochar doses (B0, B1, and B2) and two rhizobacteria treatments (R0 and R1) and their combinations. Biochar was mixed with soil at the rate of 0% (B0), 5% (B1), and 10% (B2) by soil weight. Eggplant seeds were sown at 1 cm depth on 45-cell seedling trays filled with peat and when they reached three true leaf periods, they were transplanted to annealed soil.

2.2. Experiment soil and properties

The physical and chemical properties of the experiment soil are shown in Table 1. The texture of soils was determined with the Bouyoucos scale (g L^{-1}) using a standard hydrometer (Bouyoucos, 1951; Gee and Bauder, 1986). Soil pH was determined potentiometrically according to the method described by McLean (1982). The Loeppert and Suarez (1996) volumetric method was used to determine the amount of calcium carbonate ($CaCO_3$) in the soil. The Smith–Weldon (1941) age-burning method was used to determine the organic matter content of the soil. The Rhoades (1982) saturated paste extract method was used to determine electrical conductivity of soil (EC). Bremner and Keeney's steam distillation procedure was used to determine the contents of exchangeable ammonium (NO_3) and nitrate (NH_4) in the soil (1965). The total nitrogen (N) content of the soil was determined with the micro-Kjeldahl method and a Vapodest Rapid Distillation System (Bremmer and Mulvaney, 1982). The amount of phosphorus (P) in the soil was determined by ICP-OES Inductively Couple Plasma spectrophotometer (Lindsay and Norvell, 1978). The exchangeable cations in the soil, sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) was determined on the basis of the atomic absorption method expressed by Rhoades (1982). The concentration of plant-absorbable microelements in the soil, ferritin (Fe), manganese (Mn), zinc (Zn), copper (Cu) was determined with the spectrophotometric method described by Lindsay and Norvell (1978). The amount

Table 1. Physical and chemical properties of the starting soils (mean ± standard deviation, n = 5).

Properties	Unit	Analysis results
Sand	%	33.40 ± 3.03
Silt	%	29.70 ± 2.98
Clay	%	36.90 ± 3.23
pH	*1:2.5 w/s*	7.30 ± 0.26
EC	*micromhos* cm^{-1}	115.00 ± 10.17
$CaCO_3$	%	2.80 ± 0.19
Organic matter	%	0.96 ± 0.09
NH_4 -N	*mg* kg^{-1} *dw*	1.70 ± 0.25
NO_3-N	*mg* kg^{-1} *dw*	0.89 ± 0.07
P	*mg* kg^{-1} *dw*	3.20 ± 0.42
K	*cmolc* kg^{-1} *dw*	2.55 ± 0.24
Ca	*cmolc* kg^{-1} *dw*	14.40 ± 1.16
Mg	*cmolc* kg^{-1} *dw*	1.28 ± 0.15
Na	*cmolc* kg^{-1} *dw*	0.20 ± 0.04
B	*mg* kg^{-1} *dw*	0.07 ±0.00
Cu	*mg* kg^{-1} *dw*	0.80 ± 0.06
Fe	*mg* kg^{-1} *dw*	6.54 ± 0.74
Zn	*mg* kg^{-1} *dw*	0.17 ± 0.02
Mn	*mg/*kg^{-1} *dw*	0.30 ± 0.05

Each data in the table is given as the mean of three replicates ± standard error.

of B in soil was determined with the spectrophotometric method from the supernatant extracted with 0.01M Mannitol + 0.01M CaCl2 solution (AOAC, 2005).

2.3. Salt application

Before each irrigation, soil moisture was measured from control pots without salt application and the amount of water was calculated by averaging it. The soil was then irrigated twice a week until the soil moisture reached 90% of the field capacity. Salt treatments were started approximately 1 week after the seedlings were planted in the pots. The saline treatments were applied with a nutrient solution containing 0 (S0) and 100 (S1) mM NaCl. However, the first two irrigations were performed with 25 mM and 50 mM NaCl solutions, followed by the other irrigations that were performed with 100 mM NaCl solution. At the end of the study, the electrical conductivities in soils of all treatments were determined with a portable EC meter as dS m^{-1} (Figure 1).

2.4. Biochar production and application

The biochar used in the study was produced by the Synpet Company using the thermal conversion process (TDP). The biochar sample for analysis was oven dried at 68 °C for 48 h and ground. For total N determination, the Kjeldahl method (Bremner, 1996) was used. Macroelements (P, K, Na, Ca, and Mg) and microelements (Fe, Mn, Zn, Cu, Pb, Ni, and Cd) were determined by atomic absorption spectrophotometry (Perkin Elmer 3690) (AOAC 1990). Investigated physical and chemical properties of biochar are given in Table 2.

Two biochar doses as low (B1) and high (B2) biochar addition rate were applied to 5% and %10 by weight of 3 kg soil, respectively. For control treatment (B0), only soil was filled in the pots. The doses were determined based on the literature review that suggests that the application of biochar was effective in mitigating the salinity stress (Farhangi-Abriz and Torabian, 2018; Elshaikh et al., 2018; Torabian et al., 2018; Nikpour-Rashidabad et al., 2019; Hussien Ibrahim et.al., 2020; Zhang et al., 2020).

2.5. Rhizobacterial application

The study was planned with two application treatments with rhizobacteria formulation (R1) and no treatment with rhizobacteria formulation (R0). Bacterial strains selected in the study were cultured by the Department of Plant Protection, Faculty of Agriculture at Atatürk University. Approximately 460 bacterial strains were isolated from the plant rhizosphere-phyllosphere in the East Anatolia region of Turkey (Kotan et al., 2005). The selection of rhizobacterial isolates was based on their auxin (IAA)–producing, 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing, N_2- fixing, and P-solubilizing potential. Rhizobacteria applications were created from two treatments not inoculated (control) with bacteria and inoculated with a bacterial formulation consisting of a combination of *Bacillus megaterium* TV-6D, *Paenibacillus polymyxa* KIN-37, and *Pantoea agglomerans* RK92. The bacterial isolates were incubated in nutrient agar (NA) at 27 °C for 24 h. Subsequently, a single colony was transferred to 500-mL flasks containing nutrient broth (NB) and grown aerobically in flasks on a rotating shaker (95 rpm) for 48 h at 27 °C (Merck KGaA, Germany). The obtained bacterial suspension was diluted in sterile distilled water (sdH_2O) to an ultimate concentration of 10^8 CFU mL L^{-1}. Before application, the rhizobacteria formulation was diluted 1/30 in chlorine-free tap water, and 10 g granulated sugar was added per liter. The roots of the eggplant seedlings were dipped in this culture

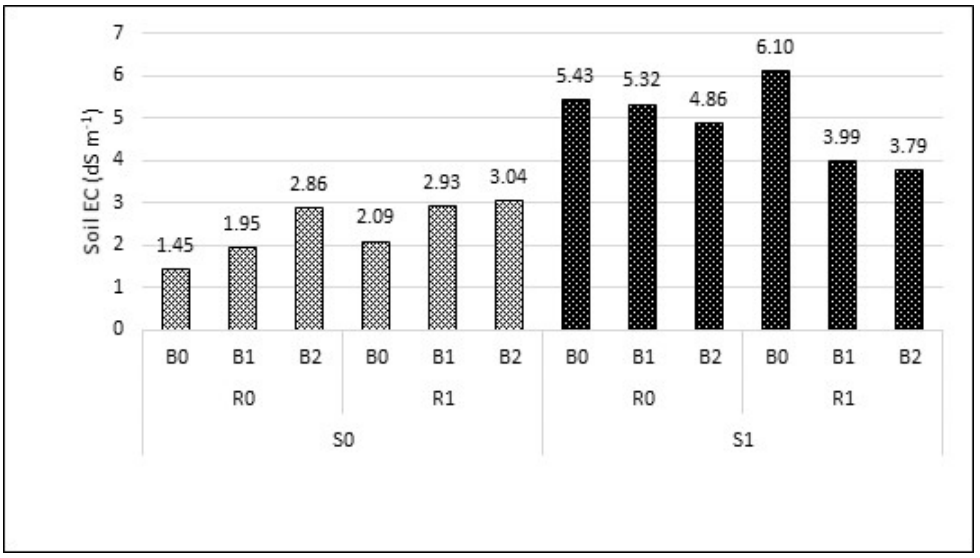

Figure 1. Soil EC results at the end of the trial of applications.

Table 2. Chemical characteristics of biochar produced from urban wastes (mean ± standard deviation, n = 5).

Properties	Unit	Analysis results
pH	1:2.5 w/s	7.8 ± 0.23
EC	dS m^{-1}	0.38 ± 0.01
Total (humic + fulvic)	%	4.9 ± 0.2
Organic nitrogen	%	1.6 ± 0.05
C	%	21.54 ± 2.04
H	%	1.26 ± 0.03
N	%	1.38 ± 0.04
O	%	2.1 ± 0.06
Pb	mg kg^{-1}	162 ± 15.88
Cd	mg kg^{-1}	10 ± 0.5
Cu	mg kg^{-1}	393 ± 27.51
Ni	mg kg^{-1}	310 ± 21.08
Zn	mg kg^{-1}	1187 ± 74.78
Cr	mg kg^{-1}	449 ± 28.74
Mn	mg kg^{-1}	549 ± 39.53
K	mg kg^{-1}	10290 ± 216.09
P	mg kg^{-1}	22980 ± 689.4
Mg	mg kg^{-1}	7372 ± 221.16
Ca	mg kg^{-1}	57500 ± 920
Fe	mg kg^{-1}	25680 ± 539.28

Each data in the table is given as the mean of three replicates ± standard error.

formulation for 30 min before transplanting. Control seedlings dipped in fresh nutrient broth were diluted 10 times. After transplanting the seedlings, 100 mL of the remaining solution was inoculated directly into soil for each pot. The cultures were maintained in Luria broth (LB) with 15% glycerol at –80 °C at the Department of Plant Protection, Faculty of Agriculture at Atatürk University.

2.6. Harvest and plant growth properties

Plant height was determined by measuring the height from the root crown to the growth tip of the plant through a ruler. The area of the seedling green leaves was quantified as cm^2 with a leaf area meter (LI-3100, LI-COR). Plants were harvested after the completion of measurements and analyses on fresh plant and root samples following a 50-day development period, and their fresh weights were determined. Afterward, the dry weights of the seedlings and roots, which were dried at 70 °C for 48 h, were determined in a precision balance (±0.001 g) and ground for mineral analysis. Leaf relative water content (LRWC) was determined by the method of Smart and Bingham (1974). Leaf discs (5 discs for each repeat) cut at a diameter of 10 mm were immediately weighed for fresh weight (FW) determination. The leaf discs were then suspended in ultrapure water at 25 °C for 24 h, and then the turgor weights (TW) were determined. Finally, to record their dry weight (DW), leaf discs were weighed after being kept at 70 °C for 48 h in an oven. LRWC (%) was calculated using the equation of Arora et al. (1998).

$$LRWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100.$$

To determine electrolyte leakage (EL%) in leaf tissues, fresh leaf discs (5 discs for each repeat) were taken at a diameter of 10 mm. Leaf discs were placed in 50-mL capped glass test tubes containing 30 mL of ultrapure water. Following incubation at 25 °C for 24 h, the EC (EC1) of the solution in the tubes was measured by an electrical conductivity meter. Tubes were autoclaved for 20 min at 95 °C and refrigerated to 25 °C, and then the EC (EC2) was measured again. Ion leakage or membrane permeability in leaf tissues was calculated by the following equation (Sairam and Srivastava, 2002).

$$EL(\%) = (EC1/EC2) \times 100$$

To determine the leaf chlorophyll concentration, plant samples with diameters of 10 mm were cut from the middle leaves and put into 2-mL Eppendorf tubes. The samples used were frozen at –80 °C as they were easier to grind in a matrix mill. They were ground to a fine powder by shaking for 3 min at 50 Hz in a vibratory ball mill. After the samples were shaken with 0.2 mL of 80% cold acetone for 3 min at 50 Hz, they were centrifuged at 10,000 rpm at 5 °C and brought to a final volume (2 mL) with 80% cold acetone. Finally, the absorbance values were measured at 663 and 645 nm by a microplate spectrophotometer and chlorophyll a (mg cm^{-2}), chlorophyll b (mg cm^{-2}), and total chlorophyll content (mg cm^{-2}) were estimated according to Arnon's (1949) equations:

$$Chlorophyll\ a = (ml\ solvent)\ [(0.0127x\ Abs\ 663) - (0.00269\ x\ Abs\ 645)]/Leaf\ area\ (cm^2)$$

$$Chlorophyll\ b = (ml\ solvent)\ [(0.0229\ x\ Abs\ 645) - (0.00468\ x\ Abs\ 663)]/Leaf\ area\ (cm^2)$$

$$Total\ chlorophyll = (ml\ solvent)\ [(0.0202\ x\ Abs\ 645) + (0.00802\ x\ Abs\ 663)]/Leaf\ area\ (cm^2)$$

For hormone, MDA, H_2O_2, sucrose, proline, and antioxidant enzyme activity determination of the plants, nearly 20 g of fresh leaf was taken from each repeat, and then the plants were chilled in liquid nitrogen, and stored at –80 °C for starting the analysis. Dried eggplant seedling samples were used for plant mineral element analysis.

2.7. MDA and H_2O_2 contents

Lipid peroxidation was determined by measuring the malondialdehyde content, which is an output of lipid peroxidation by following the method signified by Heath and Packer (1968). To assess the MDA content from the absorbance, the molar attenuation coefficient (ε) was 155

mM^{-1} cm^{-1}. Plant samples (0.5 g) were crushed with 10 mL of 0.1% (w/v) trichloroacetic acid (TCA). Afterward, 4 mL of 0.5% (w/v) thiobarbituric acid containing 20% (w/v) TCA was added to 1 mL supernatant. The mixture was heated at 98 °C for 10 min and cooled and then briefly centrifuged at 10,000× *g* at 4 °C for approximately 15 min. The activity-specific and nonspecific absorbance of the supernatant was determined at 532 and 600 nm, respectively and the MDA content MDA in mmol kg^{-1} FW was calculated.

The amount of hydrogen peroxide (H_2O_2) in leaf tissues was determined using the method of Ohkawa et al. (1979). Leaf tissues of approximately 0.5 g were homogenized in a mortar with 10 mL of 5% trichloroacetic acid (TCA). After this mixture was centrifuged at +4 °C at 10,000 rpm for 10 min, 0.5 mL of the supernatant was taken and 0.1 M 0.5 mL Tris buffer (pH 7.6) and 1 M 1.5 mL potassium iodide (KI) were added. Absorbances were measured at 390 nm wavelength in a spectrophotometer. The H_2O_2 concentration (mmol kg^{-1} fresh weight) of the supernatant was determined from its absorbance value at 390 nm a using calibration curve constructed in the range of 10 to 200 nmol.

2.8. Antioxidant enzyme activities

For the extraction of antioxidant enzymes, 0.1 g of the fresh leaf was ground adding liquid nitrogen in a mortar to powder form. The ground samples were homogenized with 1.8 mL cold homogenate buffer [50 mM KH_2PO_4 pH: 7.0 containing, 1% PVP (w/v) and 1 mM EDTA]. The mixture was transferred to a centrifuge tube and centrifuged at 15,000 rpm and +4 °C for 15 min. The supernatant obtained as a result of centrifugation was used as a source for activity measurements of antioxidant enzymes.

Superoxide dismutase (SOD) activity was determined by spectrophotometry (Agarwal and Pandey, 2004; Yordanova et al., 2004). One unit of enzyme activity was determined as the amount of the enzyme needed for the inhibition of 50% nitro blue tetrazolium (NBT) reduction rate by monitoring absorbance at 560 nm with a spectrophotometer. Peroxidase (POD) activity determination is based on the observation of the absorbance increase at 470 nm caused by the colored compound, which is the product of the reaction in which guaiacol and H_2O_2 are substrates (Angelini et al., 1990). The method applied by Havir and Mchale (1987) was used to determine the catalase (CAT) activity. According to this method, CAT activity is based on the principle of measuring the absorbance decrease at 240 nm during the conversion of H_2O_2 in the measurement environment to O_2 and H_2O (Havir and Mchale, 1987).

2.9. Proline and sucrose content

Proline contents were determined according to Bates et al. (1973). Proline extension in leaf samples was obtained by adding 2 mL of 40% methanol to 0.1 g of fresh leaf sample. The 1 mL homogenate was mixed with 1 mL orthophosphoric acid (6 M): glacial acetic acid (2:3, v/v) mixture and 25 mg of ninhydrin. After holding at 100 °C for 1 h, the tubes were chilled and mixed thoroughly with a vortex by adding 5 mL of toluene. The absorbance of the supernatant was spectrophotometrically determined at 528 nm. The proline concentration was determined as mmol kg^{-1} fresh weight using a standard curve.

The sucrose content in the plants was determined using by HPLC method. Free-sugars were analyzed according to the decided method by Van Huylenbroeck and Debergh (1996) and Karkacier et al. (2003). Fresh leaf samples (0.1 g) were crushed in a mortar by using 1 mL 80% ethanol containing melezitose as the internal standard (Sigma, USA). The samples were placed in a water bath at 90 °C for 30 min to heat the homogenate. Then the homogenate was centrifuged at 3000 rpm and +4 °C for 15 min. Next, the supernatant was evaporated until the solvent is completely removed, and dried samples were dissolved in 2 mL of distilled water. The solution was first proceeded through a Sep Pak Accell QMA cartridge column balanced with 0.1 mol dm^{-3} NaOH, and then a Sep Pak plus C18 cartridge column. The sugar content of the samples was detected by using a Shodex SC1011 column at 80 °C by HPLC liquid chromatography. Solutions were eluted at a flow rate of 1.2 mL min^{-1} with 0.1 mol dm^{-3} NaOH and then sugar contents were appointed by a pulsed amperometric detector with palladium electrodes. Sucrose was quantified by their holding times and by integrating peak areas towards the internal standard.

2.10. Hormone analysis

Extraction and purification procedures for hormone analysis were conducted according to the method reported by Kuraishi et al. (1991) and Battal and Tileklioğlu (2001). Methanol 80% at –40 °C was added to the fresh tissue sample (Davies, 1995). The samples were then homogenized for 10 min by ultra-Turrax, and the obtained solution was incubated in dark conditions for 24 h. The solutions were filtered through 11 µm pore size filter papers, and the filtered supernatants were filtered again through 0.45 µm pore size filter papers (Cutting, 1991). Next, the supernatants were dried at 35 °C by using evaporator pumps. Dried supernatants were dissolved in 0.1 M KH_2PO_4 (pH 8.0). Fatty acids were separated by centrifuging the extracts at 5000 rpm for 1 h at 4 °C (Palni et al., 1983). Polyvinylpyrrolidone (PVPP) was added to the extracts to separate colored and phenolic materials. Next, the extracts were filtered through 11 µm pore size filter papers to remove the PVPP. Supernatants were passed through a Sep-Pak C18 (Waters) cartridge for further specific separation. Hormones absorbed by this cartridge were transferred to vials through 80% methanol.

Indoleacetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA), salicylic acid (SA), cytokinin (CK), zeatin (ZT), and jasmonic acid (JA) were analyzed by high-performance liquid chromatography (HPLC) through a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC) and by a UV detector at 265 nm. The flow speed and column temperature were set to 1.2 mL min^{-1} and 25 °C, respectively. Thirteen percent acetonitrile (pH: 4.98) was used as the mobile phase.

2.11. Mineral element analysis

The contents of the plant and root samples P, K, Ca, Mg, S, Mn, Fe, Zn, B, Cl, and Na were subjected to combustion with nitric acid-hydrogen peroxide (2: 3) acid in 3 different steps (1st step: 5 min at 145 °C and 75% microwave power; 2nd step: 10 min at 180 °C at 90% microwave power; and the 3rd step: at 100 °C for 10 min at 40% microwave power) in a microwave wet combustion unit resistant to 40 bar pressure (Mertens, 2005a) and then they were determined by reading in the spectrophotometer (Mertens, 2005b). The nitrogen content in the plant and root was determined according to the micro-Kjeldahl wet combustion method (Bremner, 1965). In the method, the samples are converted to ammonium (NH_4) by burning with concentrated H_2SO_4 and it is based on the determination of the total nitrogen as a result of the titration of the ammonia (NH_3) released at the end of distillation in an alkaline environment.

2.12. Statistical analysis

SPSS 25 (IBM, NY, USA) was used for data analysis. The main effects of salinity and biochar and their interactions were analyzed by two-way MANOVA. Duncan's multiple-range test at the 0.05 level of significance was used to determine differences among treatments. The principal component analysis (PCA) method was applied to evaluate the relationships between salt stress and biochar applications. It used BioVinci 3.08.exe version free download from the web for PCA.

3. Results

3.1. Plant growth parameters

The effects of biochar and PGPR applications on plant height, stem diameter, leaf area, plant fresh weight, root fresh weight, plant dry weight, and root dry weight of eggplant seedlings grown under salinity stress conditions are shown in Table 3. The results of the study revealed that salinity stress negatively affected the growth characteristics of eggplant seedlings. Salt irrigation conditions without biochar and PGPR (B0R0) reduced the plant height, stem diameter, leaf area, plant fresh weight, plant dry weight, root fresh weight, and root dry weight of eggplant seedlings by 28.21%, 17.07%, 17.16%, 31.95%, 54.55%, 51.54%, and 40%, respectively, compared to salt-free irrigation conditions. However, both biochar and PGPR treatments eliminated the negative impacts of salinity stress on the growth characteristics of eggplant seedlings (Figures 2–4). The cumulative effects of PGPR and biochar treatments were more pronounced. B2R1 increased the plant height (56.62%), stem diameter (38.24%), leaf area (74.71%), plant fresh weight (86.73%), plant dry weight (87.95%), root fresh weight (84.73%), and root dry weight (77.78%) by several fold compared to the control under salt stress. The effect of treatments and all their interactions on the growth parameters investigated was statistically important, excluding the SxR interaction for stem diameter (Table 3).

Salinity conditions result in increased EL but decreased LRWC. Conversely, both PGPR and biochar treatments reduced EL and increased LRWC. The lowest EL value under salinity conditions was obtained from B2R1, while the highest LRWC was obtained in B0R1. Furthermore, salinity significantly reduced the chlorophyll a, chlorophyll b, and total chlorophyll contents of eggplant seedlings. However, biochar and PGPR combinations elevated the chlorophyll a, chlorophyll b, and total chlorophyll contents of eggplant seedlings grown under salinity stress conditions. The interactions of treatments for EL, LRWC, chlorophyll a, chlorophyll b, and total chlorophyll were statistically significant (Table 4). When comparing salt treatments not amended with biochar and PGPR, salt stress increased the EL value in the leaves by 250.28%, while LRWC, chlorophyll a, chlorophyll b, and total chlorophyll content in the leaves under salt stress were reduced by 16.32%, 27.38%, 66.42%, and 44.19%, respectively. The leaf EL values of salt-stressed plants treated with only biochar decreased by 13.25% in B1 and by 21.69% in B2 compared to the control. On the other hand, the B2 biochar dose applied together with PGPR was more effective in reducing the EL value (44.53% decrease over salt control) and increasing the LRWC content (37.86% increase over salt control). While biochar applications under saline conditions increased the chlorophyll a, b and total contents of the plants compared to the control, PGPR application did not change the plant chlorophyll properties. Under the salted conditions, the highest chlorophyll a and total chlorophyll content was obtained from the B2 biochar dose, while the highest chlorophyll b content was obtained from the B1 biochar dose. All the physiological properties studied were significantly affected by the treatments and interactions, with the exception of the BxR interaction in EL (Table 4).

3.2. H_2O_2, MDA, proline, sucrose, and antioxidant enzymes

The effects of biochar and PGPR applications on leaf H_2O_2, MDA, proline and sucrose content, and CAT, POD, and SOD activities of eggplant seedlings grown under salinity stress conditions are given in Table 5. Salt stressed plants had higher H_2O_2, MDA, proline and sucrose contents than

Table 3. Effects of biochar and PGPR applications on plant height, stem diameter, leaf area, plant fresh weight, root fresh weight, plant dry weight, and root dry weight of eggplant seedlings grown under salinity stress conditions.

Salt (S)	Biochar (B)	PGPR (R)	Plant height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Plant fresh weight ($g\ plant^{-1}$)	Plant dry weight ($g\ plant^{-1}$)	Root fresh weight ($g\ plant^{-1}$)	Root dry weight ($g\ plant^{-1}$)
S0	B0	R0	11.45 h	3.28 f	85.7 hi	2.41 i	0.44 h	2.01 g	0.20 e
		R1	14.67 e	3.69 e	112.27 g	3.78 i	0.65 g	1.98 g	0.17 e
	B1	R0	21.63 c	5.43 ab	268.04 d	13.59 d	1.89 c	3.97 e	0.51 b
		R1	24.18 a	5.48 a	431.47 b	17.09 b	2.28 b	6.76 b	0.56 a
	B2	R0	22.78 b	5.01 c	375.76 c	14.72 c	1.85 c	4.23 d	0.36 c
		R1	24.85 a	5.17 bc	460.37 a	20.33 a	2.51 a	7.67 a	0.56 a
S1	B0	R0	8.22 i	2.72 g	70.99 j	1.64 k	0.20 i	0.98 h	0.12 f
		R1	12.08 gh	2.84 g	100.71 gh	4.48 h	0.44 h	2 .00g	0.19 e
	B1	R0	12.75 fg	3.98 d	170.35 f	7.95 g	0.83 f	3.28 f	0.34 c
		R1	14.77 e	3.70 e	206.80 e	9.44 f	1.13 e	3.24 f	0.34 c
	B2	R0	13.60 f	3.21 f	206.10 e	9.70 f	1.17 e	3.86 e	0.30 d
		R1	18.95 d	3.76 de	280.32 d	12.36 e	1.66 d	6.42 c	0.54 ab
Salt (S)			***	***	***	***	***	***	***
Biochar (B)			***	***	***	***	***	***	***
Rhizobacteria (R)			***	***	***	***	***	***	***
Applications (B/R)			***	***	***	***	***	***	***
Salt * Applications (SxB/R)			***	***	***	***	***	***	***
Salt * Biochar (SxB)			***	***	***	***	***	***	***
Salt * Rhizobacteria (SxR)			**	*NS*	***	***	*	***	**
Biochar * Rhizobacteria (BxR)			**	**	***	***	***	***	***
Salt * Biochar * Rhizobacteria (SxBxR)			**	*	***	***	*	***	***

The data followed by a different letter are significantly different according to Duncan's multiple range test. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS, non-significant. S0: nonsalinity/ S1:100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free/ R1: rhizobacteria formulation applied.

Figure 2. Effects of biochar and rhizobacteria applications on eggplant grown under nonsalty conditions. S0: nonsalty; B0: %0 biochar / B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free / R1: rhizobacteria formulation applied.

Figure 3. Effects of biochar and rhizobacteria applications on eggplant grown under salty conditions. S1:100 mM NaCl; B0: %0 biochar/ B1: %5 biochar / B2: %10 biochar; R0: rhizobacteria free /R1: rhizobacteria formulation applied.

nonstressed plants. The content of H_2O_2, MDA, proline, and sucrose in salt-stressed eggplant leaves without biochar and PGPR increased by 13.60%, 53.70%, 31.82%, and 31%, respectively, compared to the plants with normal irrigation. Biochar and PGPR applications together or separately significantly reduced the H_2O_2, MDA, proline and sucrose contents under salinity stress conditions. The lowest H_2O_2, MDA, proline, and sucrose contents were observed in B1R1, which were reduced by almost 72.95%, 48.41%, 48.28%, and 36.39, respectively. The CAT and SOD activities of eggplant seedlings under salinity stress were 16.74% and 27.09% higher than those under nonstressed conditions. Biochar and PGPR combinations significantly reduced CAT and SOD activities of eggplant seedlings under salinity stress. The CAT and SOD contents in saline conditions decreased by 38.30% and 21.38% with B1R1, while they decreased by 53.20% and 22.46% with B2R1. In contrast, salinity conditions caused decreased POD activity while biochar and PGPR applications differently affected POD activity. However, it was observed that salt stress increased the POD content compared to all other applications in nonsaline conditions. The B0R1 and B1R1 treatments decreased POD activity by 22.81% and 18.41%, respectively, compared to the salt control (S1B0R0). Interactions were significant for H_2O_2, MDA, proline, sucrose, and all antioxidant enzyme activities, except for the SxR interaction for POD (Table 5).

3.3. Hormones

Biochar and PGPR applications and their interactions were significant for the hormone content of eggplant seedlings grown under salinity stress conditions. Salinity stress conditions caused an increase in ABA, GA, and SA contents and a decreasing ZT and JA contents. When the saline and unsalted conditions without amendatory were compared, ABA, GA, and SA contents in salt-stressed plants increased by 44.76%, 42.68%, and 26.30%, respectively, while ZT and JA contents decreased by 28.65% and 24.06%. However, the IAA and CK contents of eggplant seedlings were not affected by salinity stress. Under salinity stress conditions, biochar and PGPR coapplication generally increased the IAA, GA, SA, CK, ZT, and JA, whereas ABA content was reduced by biochar and PGPR coapplication. The most

Figure 4. Postharvest rooted plant images of eggplant seedlings treated with salt, biochar, and rhizobacteria.

Table 4. Effects of biochar and PGPR applications on EL, LRWC, chlorophyll a, chlorophyll b, total chlorophyll of eggplant seedlings grown under salinity stress conditions.

Salt (S)	Biochar (B)	PGPR (R)	EL (%)	LRWC (%)	Chlorophyll a (µg cm^{-2})	Chlorophyll b (µg cm^{-2})	Total chlorophyll (µg cm^{-2})
S0	B0	R0	19.81 f	61.39 e	1.68 ab	1.34 b	3.01 b
		R1	12.75 g	68.02 b	1.69 ab	1.29 b	2.97 b
	B1	R0	12.13 g	63.44 d	1.65 ab	1.99 a	3.64 a
		R1	15.5 g	71.11 a	1.67 ab	1.74 a	3.41 a
	B2	R0	13.31 g	57.33 g	1.66 ab	1.84 a	3.51 a
		R1	14.43 g	67.31 b	1.64 b	2.05 a	3.69 a
S1	B0	R0	69.39 a	51.37 i	1.22 c	0.45 c	1.68 c
		R1	61.13 b	59.2 f	1.19 c	0.39 c	1.58 c
	B1	R0	60.19 b	54.08 h	1.69 a	1.36 b	3.05 b
		R1	45.25 d	65.64 c	1.69 a	1.31 b	3.01 b
	B2	R0	54.34 c	64.78 c	1.65 ab	2.00 a	3.65 a
		R1	38.49 e	70.82 a	1.67 ab	1.73 a	3.41 a
Salt (S)			***	***	***	***	***
Biochar (B)			***	***	***	***	***
Rhizobacteria (R)			***	***	**	**	**
Applications (B/R)			***	***	***	***	***
Salt * Applications (SxB/R)			***	***	***	***	***
Salt * Biochar (SxB)			***	***	***	***	***
Salt * Rhizobacteria (SxR)			***	***	**	**	**
Biochar * Rhizobacteria (BxR)			*NS*	***	***	**	***
Salt * Biochar * Rhizobacteria (SxBxR)			***	***	***	***	**

The data followed by a different letter are significantly different according to Duncan's multiple range test. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS, nonsignificant. S0: non-salinity/ S1:100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free /R1: rhizobacteria formulation applied. EL: Electrolyte leakage, LRWC: Leaf relative water content.

Table 5. Effects of biochar and PGPR applications on leaf H_2O_2, MDA, proline, and sucrose contents, and CAT, POD, and SOD activities of eggplant seedlings grown under salinity stress conditions.

Salt (S)	Biochar (B)	PGPR (R)	H_2O_2 (mmol kg^{-1})	MDA (mmol kg^{-1})	Proline (mmol kg^{-1})	Sucrose (%)	CAT (EU g $leaf^{-1}$)	POD (EU g $leaf^{-1}$)	SOD (EU g $leaf^{-1}$)
S0	B0	R0	43.90 b	24.19 b	0.22 cde	3.00 d	522.19 cd	32575.30 a	1770.24 bc
		R1	28.96 gh	19.04 e	0.19 f	2.93 de	376.83 g	21602.18 f	1643.19 c
	B1	R0	33.23 ef	20.27 cde	0.23 bc	3.42 bc	484.62 e	24889.12 de	1850.85 b
		R1	34.31 e	16.42 f	0.20 ef	2.66 ef	426.47 f	20440.18 f	1373.27 d
	B2	R0	30.25 fg	21.25 cd	0.24 b	3.34 c	513.07 de	27053.84 bcd	1858.84 b
		R1	30.97 fg	20.05 de	0.21 de	1.86 g	275.77 h	27672.26 bcd	1894.73 b
S1	B0	R0	49.87 a	37.18 a	0.29 a	3.93 a	609.06 a	28176.13 bc	2249.82 a
		R1	39.62 c	16.94 f	0.16 g	2.90 de	546.70 bc	21749.90 f	1469.90 d
	B1	R0	40.58 c	24.72 b	0.19 f	2.82 de	530.08 cd	29600.65 b	2283.61 a
		R1	26.13 h	19.18 e	0.15 g	2.50 f	375.75 g	26223.23 cd	1775.68 bc
	B2	R0	37.83 cd	22.07 c	0.23 bcd	3.65 b	574.92 b	27473.67 bcd	1838.46 b
		R1	35.31 de	23.95 b	0.23 bcd	2.41 f	284.98 h	22988.94 ef	1744.6 bc
Salt (S)			***	***	*	**	***	*	***
Biochar (B)			***	***	***	***	***	***	*NS*
Rhizobacteria (R)			***	***	***	***	***	***	***
Applications (B/R)			***	***	***	***	***	***	***
Salt * Applications (SxB/R)			***	***	***	***	***	***	***
Salt * Biochar (SxB)			***	***	***	***	***	***	***
Salt * Rhizobacteria (SxR)			***	***	**	**	**	*NS*	***
Biochar * Rhizobacteria (BxR)			***	***	***	***	***	***	***
Salt * Biochar *Rhizobacteria (SxBxR)			**	***	***	***	***	***	***

The data followed by a different letter are significantly different according to Duncan's multiple range test. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS, nonsignificant. S0: nonsalinity/ S1:100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free/ R1: rhizobacteria formulation applied. H_2O_2: Hydrogen peroxide, MDA: Malondialdehyde, CAT: Catalase, POD: Peroxidase, SOD: Superoxide dismutase.

Table 6. Effects of biochar and PGPR applications on leaf IAA, ABA, GA, SA, CK, ZT, and JA content of eggplant seedlings grown under salinity stress conditions.

Salt (S)	Biochar (B)	PGPR (R)	IAA (µg g^{-1})	ABA (µg g^{-1})	GA (ng g^{-1})	SA (ng g^{-1})	CK (ng g^{-1})	ZT (ng g^{-1})	JA (ng g^{-1})
S0	B0	R0	1.05 i	5.54 b	3.21 h	3.65 h	4.10 g	1.92 i	10.10 h
		R1	5.12 e	2.78 f	6.22 f	8.39 d	9.83 d	2.63 fg	20.46 e
	B1	R0	1.47 h	4.02 e	7.04 d	8.31 d	7.06 f	2.28 gh	14.53 g
		R1	7.36 a	1.73 h	8.84 c	12.23 b	18.47 a	3.54 c	36.18 c
	B2	R0	4.53 f	4.02 e	4.77 g	6.42 e	8.06 ef	3.13 de	22.28 e
		R1	6.61 c	0.45 i	9.65 b	12.78 b	10.96 cd	5.00 a	51.26 a
S1	B0	R0	1.03 i	8.02 a	4.58 g	4.61 g	4.15 g	1.37 j	7.67 i
		R1	6.98 b	4.25 e	6.29 ef	8.91 d	15.27 b	2.91 ef	22.63 e
	B1	R0	1.37 hi	5.04 c	3.64 h	3.44 h	4.91 g	2.07 hi	11.18 h
		R1	6.15 d	2.07 g	6.80 de	9.79 c	11.84 c	3.40 cd	24.85 d
	B2	R0	2.96 g	4.66 d	4.32 g	5.44 f	8.51 e	2.59 fg	16.78 f
		R1	6.42 cd	0.68 i	10.88 a	14.20 a	11.72 c	4.42 b	47.61 b
Salt (S)			**	***	***	***	*NS*	***	***
Biochar (B)			***	***	***	***	***	***	***
Rhizobacteria (R)			***	***	***	***	***	***	***
Applications (B/R)			***	***	***	***	***	***	***
Salt * Applications (SxB/R)			***	***	***	***	***	**	***
Salt * Biochar (SxB)			***	***	***	***	***	*	***
Salt * Rhizobacteria (SxR)			***	***	*	***	**	*	*NS*
Biochar * Rhizobacteria (BxR)			***	***	***	***	***	***	***
Salt * Biochar * Rhizobacteria (SxBxR)			**	**	***	***	***	***	***

The data followed by a different letter are significantly different according to Duncan's multiple range test. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; NS, nonsignificant. S0: nonsalinity/ S1:100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free /R1: rhizobacteria formulation applied. IAA: Indoleacetic acid, ABA: Abscisic acid, GA: Gibberellic acid, SA: Salicylic acid, CK: Cytokinin, ZT: Zeatin, JA: Jasmonic acid.

effective application for decreasing the ABA content of eggplant seedlings under salinity stress conditions was B2R1 (91.52% compared to salt control). Similarly, B2R1 administration increased GA, SA, ZT, and JA contents by 137.55%, 208.02%, 222.67%, and 520.73%, respectively, compared to the salt control. On the other hand, the application that increased the IAA and CK contents the most under salt stress was B0R1 (577.67% and 267.95%, respectively). Separate and combined biochar and PGPR applications in saline and unsalted conditions had a significant effect on plant hormone contents. However, PGPR application in both salty and unsalted conditions was found to be more effective in reducing ABA content and improving other plant hormone contents. Salt, biochar, and PGPR and all their interactions were significantly effective on the hormone content of eggplant seedlings, except for the salt for CK and SxR interaction for JA (Table 6).

3.4. Mineral elements

The effects of salt, biochar, PGPR, and their interactions on the differences in macro- and micronutrient contents of leaves and roots of eggplant seedlings are given in Table 7. In groups with no amelioration, salinity stress was reduced in leaf-root N, P, K, Ca, Mg, S, Mn, Fe, Zn, and B contents of eggplant seedlings by 23.36%–23.33%, 19.23%–19.86%, 23.03%– 25%, 29.84%–25%, 18.18%–17.63%, 18.77%–24.53%, 23.58%–25.15%, 21.17%–23.65%, 14.27%–17.95%, and 22.05%–24.61%, respectively, compared to those without stress. However, biochar and PGPR applications elevated the N, P, K, Ca, Mg, S, Mn, Fe, Zn, and B contents of the leaves and roots of eggplant seedlings under salinity stress conditions. The best leaf and root nutrient content results were obtained from B2R1 application under salt-free and saline conditions. The B2R1 combination application under salty

Table 7. Results of analysis of variance showing the effects of salt, biochar, PGPR, and their interactions on the nutrient element of eggplant seedlings.

Treatments and İnteractions	Leaf											
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	B (mg kg^{-1})	Cl (mg kg^{-1})	Na (mg kg^{-1})
Salt (S)	***	***	***	***	***	***	***	***	***	***	***	***
Biochar (B)	***	***	***	***	***	***	***	***	***	***	***	***
Rhizobacteria (R)	***	***	***	***	***	***	***	***	***	***	***	***
Applications (B/R)	***	***	***	***	***	***	***	***	***	***	***	***
Salt * Applications (SxB/R)	**	***	***	***	***	NS	NS	NS	**	***	***	
Salt * Biochar (SxB)	NS	***	***	***	***	NS	*	NS	NS	*	***	***
Salt * Rhizobacteria (SxR)	NS	*	NS	*	***	NS	NS	NS	**	***	***	***
Biochar * Rhizobacteria (BxR)	***	NS	NS	NS	NS	*	NS	NS	***	NS	***	***
Salt * Biochar * Rhizobacteria (SxBxR)	***	NS	*	NS	NS	NS	NS	NS	**	NS	***	***
Treatments and İnteractions	Root											
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	B (mg kg^{-1})	Cl (mg kg^{-1})	Na (mg kg^{-1})
Salt (S)	***	***	***	***	***	***	***	***	***	***	***	***
Biochar (B)	***	***	***	***	***	***	***	***	**	***	***	***
Rhizobacteria (R)	***	***	***	***	***	***	***	***	***	***	***	NS
Applications (B/R)	***	***	***	***	***	***	***	***	***	***	***	***
Salt * Applications (SxB/R)	**	*	**	***	NS	NS	NS	*	***	***	***	***
Salt * Biochar (SxB)	NS	**	***	**	NS	NS	*	NS	**	*	***	***
Salt * Rhizobacteria (SxR)	NS	NS	NS	***	NS	NS	NS	NS	NS	***	***	*
Biochar * Rhizobacteria (BxR)	***	NS	*	**	***	NS	NS	NS	***	***	***	***
Salt * Biochar * Rhizobacteria (SxBxR)	***	NS	NS	**	*	NS	NS	**	***	NS	***	***

The data followed by a different letter are significantly different according to Duncan's multiple range test. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; NS, nonsignificant. S0: nonsalinity/ S1: 100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free /R1: rhizobacteria formulation applied.

conditions increased the content of plant leaf-root N, P, K, Ca, Mg, S, Mn, Fe, Zn, and B at 79.68–78.26, 61.90–63.38, 80.27–88.88, 79.31–66.66, 72.22–78.63, 81.06–52.27, 81.07–78.86, 52.27–62.62, 70.93–81.25 and 113.91–110.71 rates, respectively, relative to the nonapplied salt control. Conversely, salinity stress increased the Na and Cl contents of leaf and root of eggplant seedlings while biochar and PGPR applications decreased Na and Cl content of the leaves and roots of eggplant seedlings (Figures 5–7).

3.4. Principal component analysis (PCA)

The PCA, based on the measured plant parameters, showed that the treatments were separated into distinct clusters. PC1 explained 62.17% of the variation, whereas PC2 explained only 11.81% of the variation. Plant growth characteristics are grouped to the lower left of the PC1 axis, and root and leaf nutrients are grouped to the upper left of the PC1 axis, excluding Na and Cl. In addition, it was observed that all phytohormones except ABA were located on the upper left side of the PC1 axis together with the nutrients. It has also been seen that antioxidant enzymes, ABA, H_2O_2, MDA, proline, and sucrose properties were grouped towards the lower right part of the PC1 axis, while the EL properties together with the root and leaf Na and Cl contents were grouped towards the upper left part of the PC1 axis. On the other hand, it was determined that the curative applications (figure: biochar doses; color: rhizobacteria) shown in the graph with colors and shapes were grouped on the right side of the PC1 axis, while the control groups were grouped on the left side of the PC1 axis. According to the PCA, we can declare that the features that showed an increase with soil amendment applications (biochar and rhizobacteria) were located on the left side of the PC1 axis, and the features showing a decrease were on the right side of the PC1 axis (Figure 8).

4. Discussion

Salinity is one of the major abiotic stress factors that negatively affects plant growth and limits agricultural production worldwide. Reduction in plant growth and crop productivity induced by salinity might arise because of the changes in numerous physiological and biochemical attributes, i.e. nutrient imbalance the reduction of leaf chlorophyll content, the disruption of the mechanisms of ion extraction, and osmotic regulation. Salinity is expected to increase the salinity risk of soils with drought and consequently plant production will be limited. In this context, it will be an important soil technology approach to put the soil inputs and plant root zone mechanisms into action together to limit the effect of soil salt stress on the plant. In the present study, salinity stress conditions resulted in reduced growth of eggplant seedlings in the absence of PGPR and biochar. Salt stress inhibits the growth and development of plants by causing osmotic and ionic stress (Parida and Das, 2005). Decreasing the amount of water available causes decreased cell expansion and slowed shoot development. In the ion stress phase that occurs during the continuation of osmotic stress, nutrient deficiency or nutritional imbalance occurs in plants when the increased Na and Cl ions in the environment compete with essential nutrients such as K, Ca, and NO_3 (Hu and Schmidhalter, 2005). The main secondary effects caused by NaCl can be regarded as the synthesis of active oxygen species (ROS) impairing membrane function, DNA, protein, and chlorophyll; inhibition of photosynthesis and K^+ uptake; metabolic toxicity; cell death (Botella et al., 2005; Hong et al., 2009).

Biochar improved eggplant seedling performance under salinity stress. The addition of PGPR further reduced the adverse impact of salinity stress. However, the combination of biochar and PGPR applications proved the most effective and further improved the growth of eggplant seedlings under salinity stress conditions. Root growth of plants is inhibited by the accumulation of Na^+ ions in the rhizosphere as a result of salt application. However, with the addition of biochar to the soil, there was a positive increase in root growth. Akhtar et al. (2015a) demonstrated that biochar mitigated the negative effects of soil salinity on potato (*Solanum tuberosum* sp.) and explained this situation by reducing the soil mass density of biochar and reducing the interaction between the root surface and Na^+ ions. The decrease in yield and growth caused by salinity was eliminated by adding biochar to the soil. Our findings on seedling growth are consistent with the findings that an increase in crop yield can be achieved by adding biochar to the soil (Jeffery et al., 2011).

Biochar can affect the physical (water holding capacity, aeration and bulk density), chemical (nutrient holding capacity, electrical conductivity, pH, and cation exchange capacity), and biological (rhizosphere microbial population, microbial C and N biomass, and enzymatic activities) characteristics of soil (Lehmann and Joseph, 2012). Yield increases with biochar application have been documented in the field as well as in controlled environments (Major et al., 2010). In one study, biochar application increased tomato growth and biomass under salinity stress when compared with the control (She et al., 2018).

PGPR inoculation has been acknowledged to regulate abiotic stress management through direct and indirect systems that induce systemic tolerance (Yang et al., 2009; Dimkpa et al., 2009). PGPR supports plant growth and development through diverse mechanisms such as improved nutrient assimilation via biological nitrogen fixation, phosphorus solubilization, or iron acquisition (Ashraf et al., 2013; Kuan et al., 2016). Many PGPRs can have ACC (1-aminocyclopropane-1-carboxylate)

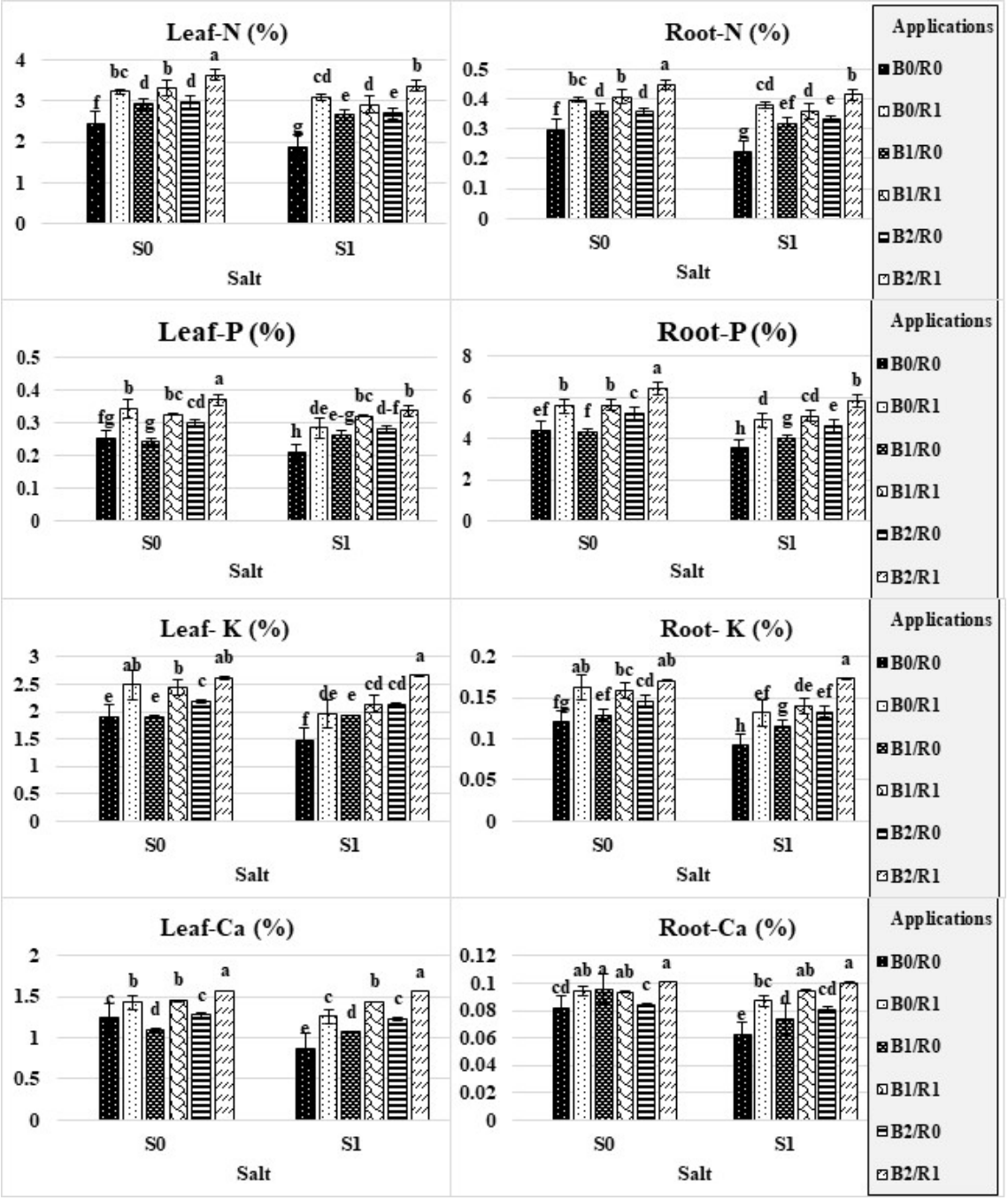

Figure 5. Effects of biochar and rhizobacteria applications on N, P, K, and Ca contents in leaves and roots of eggplants grown under salty conditions. The data followed by a different letter are significantly different according to Duncan's multiple range test. S0: nonsalty/ S1:100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free/ R1: rhizobacteria formulation applied.

deaminase activity, which is responsible for lowering the level of ethylene the plant produces under stress (Singh et al., 2015). These findings are in concordant with previous studies (Yildirim et al., 2006; Karlidag et al., 2010. Karlidag et al., 2013), which showed that PGPR treatments mitigated the negative impacts of salinity stress in several vegetable crops. It has been determined that *P. agglomerans* has IAA, siderophore, and phosphate dissolving activities, and increase plant growth under salty conditions (Silini-Chérif et al., 2012). Furthermore, PGPR could ameliorate the deleterious effect of stress conditions on plant growth by producing ACC deaminase, IAA, GA, SA, and cytokinins

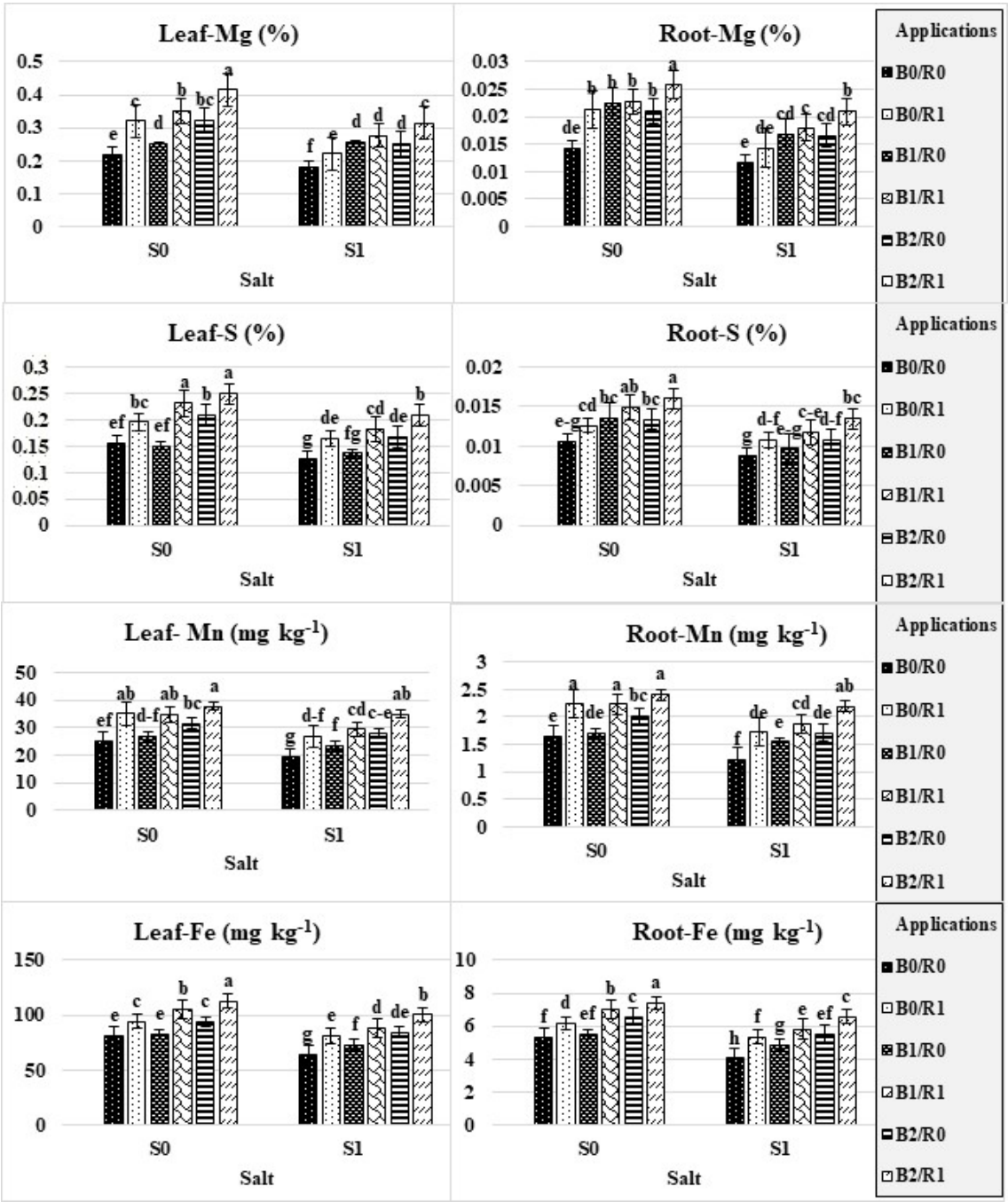

Figure 6. Effects of biochar and rhizobacteria applications on Mg, S, Mn, and Fe contents in leaves and roots of eggplants grown under salty conditions. The data followed by a different letter are significantly different according to Duncan's multiple range test. S0: nonsalty/ S1: 100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: free/ R1: rhizobacteria formulation applied.

(Yildirim et al., 2008, Yildirim et al., 2011; Kang et al., 2014; Ullah et al., 2019). As a result, PGPR strains were used to produce these hormones (Cakmakci et al., 2007; Jha and Saraf, 2012; Turan et al., 2014).

Salt stressed plants had more EC and less LRWC than nonstressed plants. Salt stress triggers a change in lipid composition in the membrane structure and causes membrane damage (Dionisio-Sese and Tobita, 1998; Munns and Tester, 2008). The amount of ion leakage from the leaves was measured to determine the membrane integrity and cell damage. Moreover, salt stress in tomatoes (RomeroAranda et al., 2001; Hossain

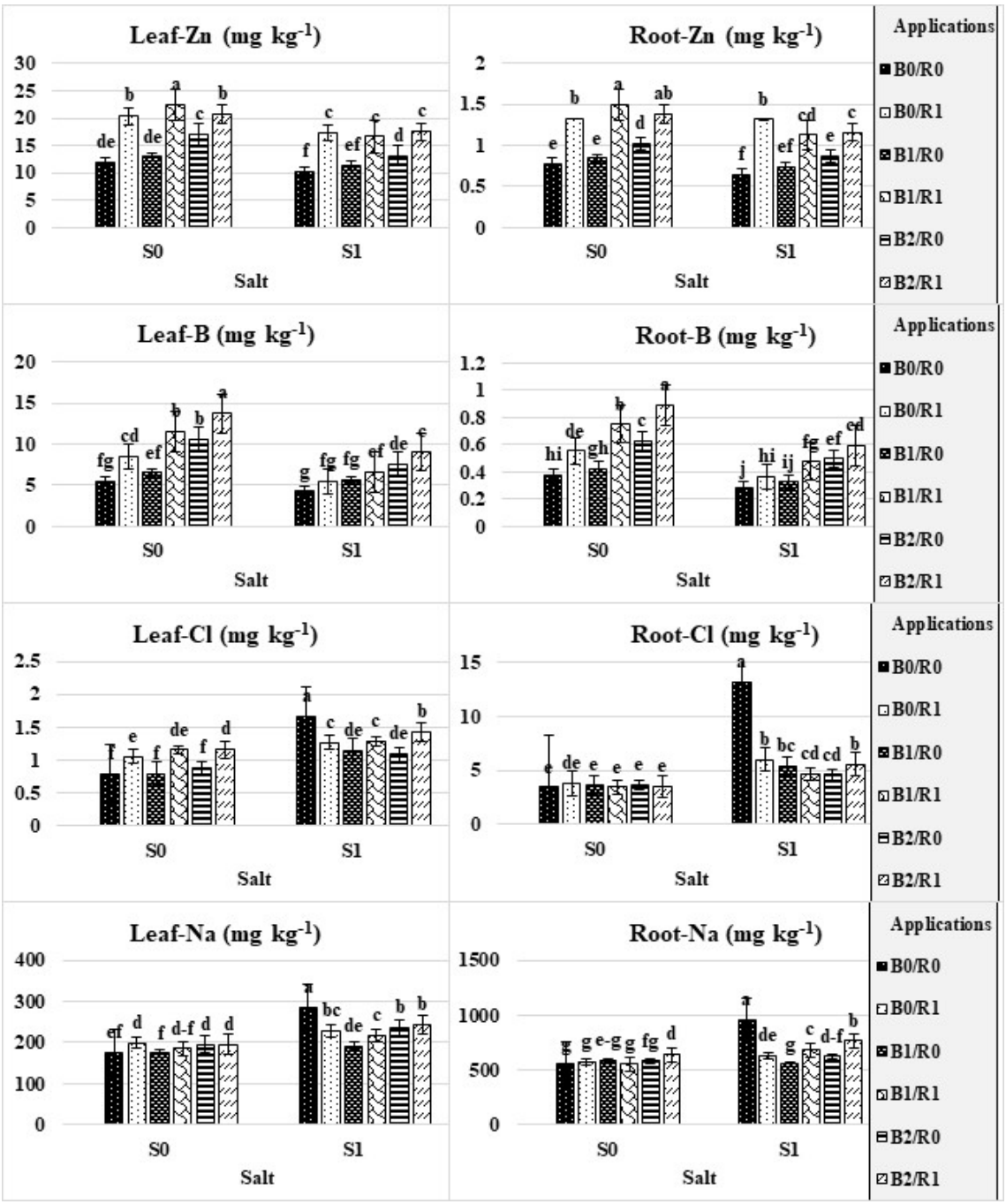

Figure 7. Effects of biochar and rhizobacteria applications on Zn, B, Cl, and Na contents in leaves and roots of eggplants grown under salty conditions. The data followed by a different letter are significantly different according to Duncan's multiple range test. S0: nonsalty/ S1: 100 mM NaCl; B0: %0 biochar/ B1: %5 biochar/ B2: %10 biochar; R0: rhizobacteria free /R1: rhizobacteria formulation applied.

et al., 2012) and melons (Kuşvuran et al., 2011) reduces leaf water potential. Salt stress inhibits the plant's water intake, causing a decrease in plant growth due to osmotic effects and damage to the cells that provide water transport in the leaves due to ion toxicity. In particular, there is a negative correlation between "Na" ion toxicity and water entry into the plant (Munss, 2005). Farooq and Azam (2006) reported that increased salt stress in wheat causes a decrease in LRWC values, but this change may be more pronounced in sensitive genotypes.

Biochar and PGPR applications reduced EC and increased LRWC. PGPRs modulate water potential and

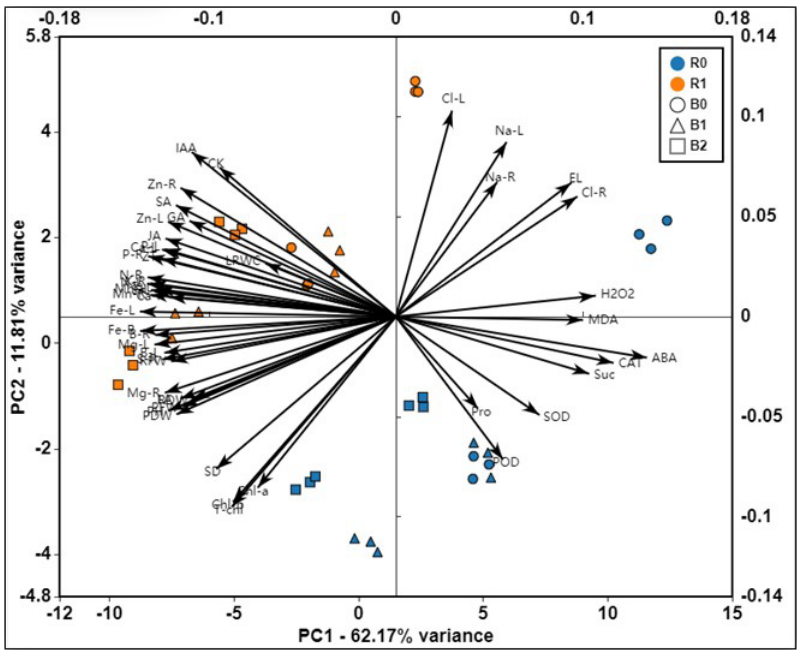

Figure 8. PCA analysis showing the effect of biochar and rhizobacteria applications on eggplant seedlings with and without salt stress. PH: Plant height, SD: Stem diameter, LA: Leaf area, PFW: Plant fresh weight, PDW: Plant dry weight, RFW: Root fresh weight, RDW: Root dry weight, EL: Electrolyte leakage, LRWC: Leaf relative water content, Chl-a: Chlorophyll a, Chl-b: Chlorophyll b, T-chl: Total chlorophyll, H_2O_2: Hydrogen peroxide, MDA: Malondialdehyde, CAT: Catalase, POD: Peroxidase, SOD: Superoxide dismutase, Pro: Proline, Suc: Sucrose, N-L: N-Leaf, P-L: P-Leaf, K-L: K-Leaf, Ca-L: Ca-Leaf, Mg-L: Mg-Leaf, S-L: S-Leaf, Mn-L: Mn-Leaf, Fe-L: Fe-Leaf, Zn-L: Zn Leaf, B-L: B-Leaf, Cl-L: Cl-Leaf, Na-L: Na-Leaf, N-R: N-Root, P-R: P-Root, K-R: K-Root, Ca-R: Ca-Root, Mg-R: Mg-Root, S-R: S-Root, Mn-R: Mn-Root, Fe-R: Fe- Root, Zn-R: Zn Root, B-R: B-Root, CR-R: CR-Root, Na-R: Na-Root.

stomatal opening by affecting hydraulic conductivity and transpiration rate. Bacterial inoculation helped reduce the EL% of plants compared to those that were not inoculated under salt stress conditions (Karlidag et al., 2013). In another study, PGPR applications increased the LRWC in plants compared to the control. PGPR applications protected plants from membrane damage and produced less electrolyte leakage than the control (Kang et al., 2014).

It has been stated that by using biochar, the soil water holding capacity can be increased and the plant can be stimulated to increase the water use efficiency (Baronti et al., 2014). Biochar addition to soil preserves the water balance and leaf turgidity of plants grown under salinity, greatly reducing water waste through stomatal closure and transpiration (Akhtar et al., 2015a). In soil treated with 10% (w/w) biochar, photosynthetic and growth parameters were improved in plants grown in a high salinity medium. The positive impact of biochar in plants grown under saline conditions is usually associated with an increase in photosynthetic activities. Adding biochar to saline soils improved photosynthetic parameters (Thomas et al., 2013; Akhtar et al., 2015a, Akhtar et al., 2015b). Akhtar et al. (2015c) reported that due to high absorption capacity, biochar minimizes the uptake of Na^+ which resulted in less accumulation of salts in plant tissues. Electrolyte leakage was reduced in maize with biochar application in salt-affected soil (Lashari et al., 2014).

Salinity stress negatively affected chlorophyll a, chlorophyll b, and total chlorophyll contents of eggplant seedlings (Table 4). It has been reported that salt stress causes the enrichment of active oxygen species in plants that disrupt the structure of chlorophyll. It has also been found that salt stress causes a decrease in the amount of photosynthetic pigments (chlorophyll and carotenoids) in the light collecting complexes of photosystems (Parida and Das, 2005). Chlorophyll is destroyed because of immoderate salt that causes accumulation of Na, Cl, i.e. ions and reactive oxygen species (ROS) which disturb the cellular metabolism and degenerate cell organelles in tissue (Ashraf and Ali, 2008; Ayaz Tilkat et al., 2019). Additionally, salinity stress causes a decrease in chlorophyll content in plants by decreasing chlorophyll synthesis, increasing chlorophyllase activity, and destroying pigment proteins (Ashraf and Bhatti, 2000; Santos 2004; Anower et al. 2013). However, biochar and PGPR applications improved the chlorophyll content of eggplant seedlings under salinity stress. Likewise, PGPR applications enhanced the chlorophyll content of vegetable crops under salinity stress (Tank and Saraf, 2010; Ilangumaran and Smith, 2017). The researchers stated that the high chlorophyll

pigment content in the inoculated plants was associated with less ethylene synthesis (Habib, 2016) and higher iron, magnesium, and nitrogen absorption (Hosseinzadah et al., 2011) in these plants. Akhtar et al. (2015c) determined that different bacteria and biochar applications caused significant improvements in the growth and physiological characteristics of the corn. Detection related to higher chlorophyll and carotenoid contents in plants treated with PGPR under salty conditions has also been carried by Nadeem et al. (2006). However, Tank and Saraf (2010) stated that PGPR inoculation under salty stress conditions increases chlorophyll content as a result of enlargement in the photosynthetic area of plant leaves as compared with noninoculated plants.

Biochar significantly improved photosynthetic rate, chlorophyll contents, stomatal conductance, relative water contents, and water use efficiency (Akhtar et al., 2014). Our findings showed that the addition of biochar increased the chlorophyll content of the plant. Bruun et al. (2014) stated that biochar increases the moisture-holding capacity of the soil compared to soil without biochar, and this can offer a significant advantage in drought and salty conditions. We observed a substantial increase in chlorophyll content in all treatments with biochar amendment. Akhtar et al. (2015b) have expressed that biochar application enhanced photosynthesis rate as a pointer to the increase in chlorophyll content. They also associate increased chlorophyll content in leaves with increased N levels by the biochar addition.

In this study, it was determined that salinity stress elevated the leaf H_2O_2, MDA, proline, and sucrose contents of eggplant seedlings. Reactive oxygen species (ROS) production increases under salt stress. The increase in MDA content in the tissue is accepted as evidence of tissue injury in plants against salt stress. Many studies have shown a positive relationship between the synthesis of organic substances such as sucrose and proline and stress tolerance (Hasanuzzaman et al., 2013). A similar conclusion regarding the increase in H_2O_2 concentration in plants exposed to salt stress was recorded in cucumbers (Zhu et al., 2004).

Biochar and PGPR applications reduced the H_2O_2, MDA, proline and sucrose contents of eggplant seedlings grown under salinity stress conditions. PGPR applications reduce H_2O_2 and MDA contents in various crops (Ilangumaran and Smith, 2017). The lower MDA content obtained with PGPR inoculation applied to plants exposed to salt stress is in line with previous studies suggesting the use of rhizobacteria to combat oxidative damage caused by salt stress (Bharti et al., 2014; Barnawal et al., 2014).

The current study reveals that PGPR inoculated eggplant seedlings contain lower proline than noninoculated seedlings under NaCl stress (Table 3). In a previous investigation, a notable decrease in H_2O_2 content was noted in seedlings inoculated with PGPR in contrast to noninoculated control seedlings under salinity stress. It could be assumed that PGPR-inoculated seedlings had lower H_2O_2 under salt stress and had, for this reason, not shown many stress symptoms. It has been suggested that biochar can prevent plants from being exposed to excessive salt by decreasing the uptake of Na^+ from the roots or by increasing Na^+ release due to its high adsorption capacity (Akhtar et al. 2015c). In addition, Liang et al. (2006) reported that the addition of biochar to the soil contributes to the cation exchange capacity of the soil; thus, Na^+ ions are adsorbed, and their uptake by plants is prevented. In this study, soil EC in salty/unsalted conditions was 1.45/5.43 dS m^{-1} in soil without biochar addition, 1.95/5.32 dS m^{-1} in soil with 5% biochar addition, and 2.86/4.86 dS m^{-1} in soil with 10% biochar addition. According to the results, biochar amendments under normal irrigation conditions increase soil EC, while biochar amendments in NaCl-added soil reduce soil EC. The results indicated that biochar amendment can assist the reduction in sodium uptake of tomato seedling roots by binding Na^+ due to its high adsorption capacity.

In this study, salinity stress enhanced CAT and SOD activities but reduced the POD activity of eggplant seedlings (Table 5). Accordingly, salt stress increases CAT and SOD enzyme activity in many plants (Hasanuzzaman et al., 2013). Abiotic stress factors including salt stress cause the accumulation of reactive oxygen species (ROS) in plants. These accumulated ROS usually cause oxidative damage by reacting with essential components of plants such as proteins, cell membranes, and lipids. Plants produce many functional antioxidative enzymes such as CAT, SOD, and POD to cope with the oxidative damage caused by ROS (Gill and Tuteja, 2010). Farhangi-Abriz and Torabian (2018) observed that the activities of CAT, APX, POD, and SOD were noticeably increased by NaCl treatments and the use of biochar improved plant growth characteristics by reducing organic osmolytes, antioxidant activities, $O2^-$, MDA, and H_2O_2 concentrations in leaves and roots under salt stress.

Excessive production of ROS may lead to oxidative stress, DNA and protein damage, membrane permeability, lipid peroxidation, loss of cell function and, ultimately, cell death (Das and Roychoudhury, 2014). To scavenge ROS and avoid oxidative stress, plants have antioxidant defense enzymes such as SOD, POD, CAT, and APX (Zhou et al., 2017). A remarkable decrease in SOD and POD activities was distinguished in PGPR inoculated seedlings compared to uninoculated seedlings under salt stress (Table 3). Our results are in accordance with the studies of Manaf and Zayed (2015) and Kang et al. (2014) which reported that PGPR inoculation reduces the detrimental effects of oxidative stress in cowpea and cucumber.

Biochar reduced the CAT, SOD, and POD activities of eggplant seedlings under salinity stress. Biochar application can arrange the production of antioxidant enzymes in plants and therefore can mitigate the effects of salt stress in plants (Thomas et al., 2013). This study shows that biochar efficaciously reduced the ROS generation to mitigate the damage to plant growth and chlorophyll content caused by ROS (Tables 3 and 4). Kim et al. (2016) found that biochar affects oxidative stress and antioxidant enzyme activity and improves plants grown under salinity stress. Hussien Ibrahim et al. (2016) explained that under salinity conditions, CAT, POD, and SOD were significantly reduced with the addition of 5% biochar. Farhangi-Abriz and Torabian (2017) also reported that the activities of antioxidant enzymes such as CAT, APX, POD, and SOD decreased under salty conditions.

Biochar and PGPR applications differently affected the IAA, ABA, GA, SA, CK, ZT, and JA contents of eggplant seedlings grown under salinity stress conditions (Table 6). Salinity conditions significantly increased the ABA, GA, and SA contents, and decreased the ZT and JA contents. However, biochar and PGPR applications usually elevated the hormone content of eggplant seedlings in the presence or absence of salinity conditions except for ABA. Moreover, biochar and PGPR applied plants had lower ABA contents than nonapplied plants under present or absent salinity conditions. The response mechanisms developed by plants against the damaging effects of salinity are adjusted by different external and internal factors. Some phytohormones, which are among the internal factors, have important functions in salt stress tolerance and adaptation (Cao et al., 2007). The amount of ABA increases during salt stress (Szepesi et al., 2009). Antioxidant defense formed in the absence of water is regulated by ABA activity (Hancock et al., 2011). ABA mediates the expression of some protective genes. Therefore, it is a vital cellular signaling molecule (Hasanuzzaman et al., 2013). However, an increase in ABA content in combination with salt stress was suggested to be due to water deficit produced by salts rather than a salt-specific effect (Ghanem et al. 2008). CK is often considered an ABA antagonist (Kaya et al., 2009) and could increase salt tolerance in plants by interacting with other plant hormones, especially auxins and ABA (Iqbal et al., 2006). CK levels are prone to decline under unfavorable environmental conditions. During stress, a reduction in CK supply from the root modifies gene expression in the shoot and thereby reveals apt responses to mitigate the effects of stress (Hare et al., 1997; Kaya et al., 2009). Another phytohormone that increases under salt stress conditions and plays a role in salt tolerance and adaptation is SA (Zahra et al., 2010). In recent years, it has been seen that the endogenous content of SA increases significantly under environmental stresses (Shim et al., 2003). JA is a lipid-derived compound with a signal function in plant responses to abiotic and biotic stresses (Wasternack, 2007). This phytohormone induces a wide range of physiological and biochemical responses in plants (Khan et al., 2012; Nadeem et al., 2016). Although there are few reports on the interaction between auxin levels and salt stress in plants, researchers have determined that IAA content under salt stress conditions is similar to ABA (Ribaut and Pilent, 1991). In addition, auxins are known to have regulatory effects against salt stress (Jung and Park, 2011). Gibberellic acid (GA) regulates plant growth and development. Gibberellic acids are a group of hormones that regulate seed germination, leaf expansion, and root elongation (Kim and Park, 2008). Lower irrigation levels caused a reduction in IAA, GA, and SA contents but increased ABA contents (Samancioglu et al., 2016). Mia et al. (2012) reported that PGPR improves plant growth due to the production of the hormone. Soil bacteria modulate plant hormone status by releasing exogenous hormones, metabolites, and enzymes that may contribute to increased salt tolerance (Ilangumaran and Smith, 2017).

Salinity stress conditions significantly caused to decrease in the plant nutrient element contents of leaves and roots of eggplant seedlings except for Na and Cl, and increased Na and Cl contents. In addition to reducing the water potential, NaCl also affects plant growth by disrupting the ion balance in the cell. A high amount of NaCl intake causes an increase in Na and Cl levels in the cell and a decrease in Ca, K, and Mg concentrations (Parida and Das, 2005). Sodium entering the cell disrupts the membrane potential and facilitates the passive entry of extracellular Cl into the cell through anion channels (Niu et al., 1995; Tuteja, 2007). With the increase in the amount of Na in the external environment, while the entry of Na into the cell increases, the uptake of K into the cell decreases, and the Na/K balance is disrupted accordingly. The reason for this is that Na competes with K for the areas where K will be connected (Tester and Davenport, 2003).

Biochar and PGPR applications increased plant nutrient element contents except for Na and Cl under salinity stress conditions. PGPR treatments increased the plant nutrient element content of various crops under salinity stress (Ilangumaran and Smith, 2017). Phytohormones, especially IAA, produced by rhizobacteria are indulged in root initiation and enhance the root lengths of lateral roots and adventitious roots, which in turn aids the host plant in maximizing nutrient absorption (Patten and Glick, 2002; Jha et al., 2012). PGPR has been known to increase the mineral nutrient exchange of both macro and micronutrients and alleviate nutrient imbalance caused by the high influx of Na and Cl ions. Microbially induced nutrient cycling (mineralization), rhizosphere pH changes (organic acids), and metal chelation (siderophores) increase plant nutrient availability (Dodd and Perez-Alfocea, 2012; Lugtenberg et al., 2013).

Biochar improves plant growth by delivering Ca, Mg, P, K, and S to the plant and reducing Na uptake. Another explanation is that it improves soil's physical, chemical, and biological features (Grattan and Grieve 1998; Cheng et al., 2006; Enders et al., 2012; Peng et al., 2012). Thomas et al. (2013) and Lashari et al. (2014) reported biochar reducing the Na uptake. Farhangi-Abriz and Torabian (2017) found that biochar could mitigate salt effects in common beans grown under salt-affected soil and that decreasing the Na concentration of plant tissues by adding biochar to the soil. Increasing plant growth in soils that added biochar is associated with the improvement of nutrient use efficiency, a positive effect on the chemical and microbial properties of the soil, as well a reduction in the washing of nutrients (Gul et al., 2015). Lehmann et al. (2003) explained the effect of biochar applications on productivity by reducing the nutrient washing applied with fertilizers and increasing fertilizer usage efficiency because of the high water and nutrient retention capability of biochar. They attributed the positive effect of biochar supplementation on crop productivity to the improvement of soil water availability with physical, chemical, and biological soil environment and nutrient availability (Lehmann et al., 2011; Novak et al., 2016). It was first demonstrated in the literature that the combination of biochar and PGPR application has an important effect on improving the negative effects of salinity stress on eggplant plants. In conclusion, it has been found that adding biochar to the soil and PGPR application would be beneficial in improving or reducing the negative effects of salt stress in eggplant. It can be suggested that using biochar and PGPR together is more effective in alleviating the negative effect of salt stress.

Principal component analysis (PCA) is a technique used for converting larger data sets into unrelated variables known as principal components (PCs) by reducing their dimensionality. This method is widely used to uncover the effects on plants of independent different applications (Plaimart et al., 2021). In our study, features that showed an increase with soil amendment applications (biochar and rhizobacteria) were located on the left side of the PC1 axis, and the features showing a decrease were located on the right side of the PC1 axis. On the other hand, when biochar and rhizobacteria applications were applied alone or together, they were grouped on the left side of the PC1 axis compared to those without application. Similarly, the use of biochar to reduce nitrogen use in tomatoes, PCA explained the majority of the difference (PCA1: 73.40% vs PCA2: 12.31%) in terms of their application and the traits examined (Guo el al., 2021).

5. Conclusion

The synergistic use of biochar and PGPR (combination of Bacillus megaterium TV-6D, Paenibacillus polymyxa KIN-37, and Pantoea agglomerans RK92) mitigates the adverse effect of salt stress in eggplant seedlings.

Together, the use of 10% biochar and PGPR is the most effective treatment for eggplant seedlings affected by salt stress. Soil amendments with both biochar doses (5% and 10%) significantly increased plant growth under salty and unsalted conditions. Under saline conditions, synergistic use of PGPR and biochar may be a novel management strategy for sustainable agriculture.

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