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Halotolerant Plant Growth-Promoting Rhizobacteria Induce Salinity Tolerance in Wheat by Enhancing the Expression of SOS Genes

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Abstract

Soil salinity is one of the main yield-limiting factors in various crops. Under different environmental stresses, many rhizobacteria have demonstrated encouraging role in enhancing plant growth and tolerate stress conditions. In this study, three potential 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase- and exopolysaccharides (EPS)-secreting bacterial strains including *Bacillus megaterium*, *B. tequilensis*, and *Pseudomonas putida* have been assessed for their growth-promoting characteristics. These bacterial strains positively affected the physiology, biochemistry, and antioxidant enzymatic activities of wheat plant, under salinity stress. Results of this study depicted that the inoculation of PGPR positively invigorates growth attributes like relative water content and photosynthetic pigments of wheat seedling under saline conditions. Moreover, plants inoculated with PGPR also showed decreased concentration of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). Inoculation of PGPR reduced electrolytic leakage and enhanced enzymatic activity for the scavenging of reactive oxygen species (ROS). These PGPR also increased the production of proline and total soluble sugar. Expression analysis of selected genes by qPCR revealed higher expression of Salt Overly Sensitive (SOS1 and SOS4) genes and predicted their potential role in stress tolerance. These genes can be further overexpressed in wheat plant to tolerate salinity stress. On the basis of these findings, it can be concluded that the priming of seeds with aforementioned PGPR can decrease the adverse effects of salinity on wheat plant.

Keywords Soil salinity · ACC deaminase · Exopolysaccharides · PGPR · Wheat

Introduction

Numerous environmental stresses such as water deficiency, flooding, salinity, and extreme temperature conditions can affect the growth and development of plants and ultimately reduce their yield (Bano and Fatima 2009; Jha et al. 2011). Among these abiotic stresses, soil salinity is one of the most prominent abiotic stresses that restrict crop productivity (Munns and Gilliham 2015). Pakistan has mainly calcareous soils and salinity affects the overall yield of a variety of crops (Shrivastava and Kumar 2015). It has been reported that 6% of the cultivated land in Pakistan is highly saline (Ilangumaran and Smith 2017). More than 20% of cultivated areas, across the globe, have been reported to be under the influence of salt stress and this percentage is increasing, rapidly (Ruan et al. 2010; Lakhdar et al. 2009).

In dry and semi-dry areas of the world, soil salinity adversely disturbs the production of crops. It restricts the growth and production of crops by the generation of reactive oxygen species (ROS), sodium and chloride toxicity, and osmotic and nutrient imbalances (Mishra et al. 2013). Elevated level of ROS under various stresses can threat plant cells by oxidizing proteins, damaging DNA and RNA, doing lipid peroxidation, activating apoptosis, and inhibiting enzymes (Nxele et al. 2017; Ghassemi-Golezani and Farhangi-Abriz 2019). Under salinity stress, plants imitate various alterations in their homeostasis, mostly, because of the decrease in accessibility of water, inadequate absorption, and distribution of ions, which ultimately causes nutritive

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inequity (Hessini et al. 2019). Plants synthesize various compatible solutes like proline and total soluble sugars to maintain an osmotic balance (Hmidi et al. 2018).

Many studies have described the use of different plant growth-promoting rhizobacteria (PGPR) to tolerate salinity stress. PGPR progress the development of plants by colonizing their roots (Lugtenberg and Kamilova 2009). They can decrease the severity of plant damage under various environmental stresses by the changing various biochemical, molecular, and physiological processes of the host plant (Meena et al. 2015). ACC deaminase-producing halotolerant microorganisms can be used for seed priming and bio-augmentation to create stress resistance and enhance the production of crops (Li et al. 2017). Moreover, root-colonizing bacteria along with extracellular polysaccharide matrix (EPS) defend plants against salt stress by decreasing the uptake of sodium ions and increasing the water-holding capacity. Exopolysaccharides-secreting PGPR provide protection against various abiotic and biotic stresses (Kumar et al. 2015).

Various mechanisms have been adapted by PGPR to cope with various stresses. Active salt-tolerant ACC deaminase-producing microbes are used to improve stress tolerance and plant yield by the bio-augmentation of seeds (Ilangumaran and Smith 2017). The ACC deaminase is released as root exudate and is converted into α-ketobutyrate and ammonia, producing ethylene, which has a significant impact on plant growth and function under stress (Zhang et al. 2018). Furthermore, exopolysaccharides maintain higher moisture content and plant growth under stress conditions. Exopolysaccharides provide safety in dehydration, microbial accumulation, plant–microbe interaction, surface attachment, and bioremediation (Naseem et al. 2018). PGPR also promotes plant growth and reduces salt stress by producing the phytohormones and increasing the intake of nutrients. The drastic effects of synthetic fertilizers on crop productivity have also been reduced by PGPR (Kumar et al. 2015). The IAA raises the discharge of plant root exudates that expeditiously fill in as an energy provider for PGPR and improve their growth and colonization (Etesami et al. 2015). Besides providing phosphorus to the plants, phosphate-solubilizing bacteria also improve the growth of plant by promoting nitrogen fixation, increasing accessibility of trace elements, and producing phytohormones (Kumar et al. 2013). Higher accumulation of proline and total soluble sugar is observed in plants under stress condition. Proline is hydroxyl radical scavenger that also adjusts the osmotic pressure in the cells to tolerate stress (Gururani et al. 2013). Increased total soluble sugar content is another important resistance approach of plants in salt toxicity (Upadhyay et al. 2012). Chlorophyll content is considered as a stability indicator under different stresses. Carotenoids are non-enzymatic scavengers of ROS and they are present in substantial amounts in plants (Ashraf 2009). Salinity stress causes the intense oxidative damage to the plants by forming ROS. Oxidative stress resistance is empathetically related to activities of ROS-scavenging enzymes like SOD, POD, and CAT (Miller et al. 2008).

During the interaction of plants with various mutualistic and symbiotic microorganisms, plant undergoes various cellular and molecular changes. These changes might be associated with various interactions between plants and microorganisms. PGPR also induce various changes that affect the development and nutrition of plants (Bashan et al. 2004). With considerable advancements in our knowledge, halotolerant rhizobacteria are being studied to identify genes that are responsible for salinity stress tolerance. Ionic stress at cellular level causes negative impacts on crop yield and production. In plants, numerous genes that are upregulated under stress conditions have been reported to be involved in various metabolic pathways and they regulate the mechanism of transcription, signal transduction, and ion transport (Deinlein et al. 2014). Salt overlay sensitive (SOS) pathways and various ion transporters help in the alleviation of ionic stress in plants (Zhu 2000). SOS1 is an important plasma membrane sodium and hydrogen ions antiporter that helps the plants to cope with salinity stress. Pyridoxal-5-phosphate is an important cofactor for various enzymes. Its synthesis is regulated by pyridoxal kinase that is encoded by SOS4 gene. Moreover, SOS4 gene is also related with the production of IAA (Shi et al. 2002; Mahajan et al. 2008).

The aim of the current study was to screen out the ACC deaminase- and EPS-producing bacterial strains. These bacterial isolates were also screened for their plant growth-promoting activity and their salt stress tolerance potential was tested in wheat. To understand the probable intricate mechanisms, adapted by PGPR to ameliorate salinity tolerance in wheat plants, the expressions of SOS1 and SOS4 genes were also analyzed.

Materials and Methods

Collection of Rhizobacterial Strains

Three plant growth-promoting rhizobacterial strains (*Bacillus megaterium* MPP7, *Bacillus tequilensis* MPP8, and *Pseudomonas putida* MPP18) were obtained from Molecular Plant Pathology Lab, Quaid-i-Azam University, Islamabad, Pakistan. These strains were formerly isolated from the rhizosphere of *Justicia adhatoda, Chenopodium murale*, and *Cenchrus ciliaris*, growing in Khewra salt mine, Pakistan (32°37′44.1″ N and 73°00′47.1″ E). Previous studies have characterized *B. megaterium* (Dahmani et al. 2020), *B. tequilensis* (Kang et al. 2019), and *P. putida* (Kumar et al. 2016) as PGPR.

Bioassays of Isolated Bacterial Strains

ACC Deaminase Activity

Activity of 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) of all three bacterial strains was assessed by quantifying α-ketobutyrate. The bacterial strains were cultured in DF salt minimal medium supplemented with 0, 25, 50, and 100 mM concentration of NaCl and 3 mM ACC. Culture broth were positioned in a shaker incubator at 30 ± 2 °C and 150 rpm for 1 day. The ACCD activity was assessed by following the procedure of Penrose and Glick (2003). Optical density values were recorded at 540 nm. A standard curve of α-ketobutyrate concentration was plotted against absorbance value of each tested sample and finally the α-ketobutyrate was quantified in µM/mg protein/h.

Exopolysaccharide Production

To quantify the production of exopolysaccharide, the bacterial strains were cultured in ATCC no. 14 broth. This culture media was prepared by adding KH_2PO_4 (0.2 g), K_2HPO_4 (0.8 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaSO_4 \cdot 2H_2O$ (0.1 g), $FeCl_3$ (10 mg), $Na_2MoO_4 \cdot 2H_2O$ (10 mg), yeast extract (0.5 g), sucrose (20 g), and agar (15 g) in distilled water (1000 ml). Before autoclave, pH of media was adjusted to 7.2. Bacterial strains were cultured and placed in shaker incubator for 48 h at 30–35 °C and 200 rpm. The bacterial suspensions were centrifuged at 9000 rpm for 10 min after adding 1 mM EDTA to produce pellet. Supernatant containing exopolysaccharides and chilled acetone were mixed in 1:3 ratio and again centrifuged at 15,000 rpm for 3 min to precipitate exopolysaccharides mass, as pellet. It was then washed with sterilized water and allowed to dry. The purified EPS was quantified gravimetrically using an analytical balance (Zainab et al. 2020).

Synthesis of Indole Acetic Acid

IAA synthesis potential of selected three bacterial isolates was assessed by following the protocol of Gordon and Weber (1951). For this purpose, LB broth was prepared and supplemented with varying NaCl concentrations (i.e., 0, 25, 50, and 100 mM) and L-tryptophan (100 mg/l). The culture was incubated in shaker incubator at 35 °C ± 2 °C and 150 rpm for 1 day and each culture (5 ml) was centrifuged at 15,000 rpm for 2 min. Supernatant and Salkowski's reagent were mixed in 1:2 ratio and placed in dark for 30 min. IAA production was checked by Salkowski's reagent. Color development was considered as an indicator of IAA production. The OD of solution (supernatant mixed with Salkowski's reagent) was measured at 535 nm. To calculate IAA production, standard curve was drawn with 10–100 µg/ml IAA.

Phosphate Solubilization

For the screening of phosphate-solubilizing bacteria, plate assay method was used. Bacteria were allowed to grow on Pikoyskaya media, supplemented with various salt concentrations (i.e., 0, 25, 50, and 100 mM) and the Petri plates were positioned in an incubator at 27–28 °C ± 2 °C for 7–8 days (Pikovskaya 1948; Fischer et al. 2007). After clear zone appearance around the bacterial strains, phosphate solubilization index was calculated by the following formula:

PSI = Colony diameter + Halozone diameter ÷ colony diameter

Salt Tolerance Assay of Selected Bacterial Strains, In Vitro

To see the salt tolerance, selected bacterial strains were separately inoculated into the LB broth, amended with 0–100 mM concentration of NaCl (Barra et al. 2016) in 100 ml flasks. The inoculated flasks were positioned in shaker incubator at 35 °C ± 2 °C for 1 day at 250 rpm. The growth of bacteria under saline conditions was observed for next seven days by measuring the OD of growth media at 600 nm.

Salt Tolerance Assay of Selected Bacterial Strains, In Vivo

For this analysis, a pot experiment was conducted (in three replicates) in a complete randomized design (CRD). Seeds of wheat variety Morocco were obtained from National Agriculture Research Center (NARC), Islamabad, Pakistan. Bacterial suspensions were prepared in LB broth by placing inoculated media in incubator shaker at 35 °C ± 2 °C and 120 rpm. After 2 days, broth cultures were centrifuged at 3000 rpm for 10 min and pellets were re-suspended in distilled water to get an optical density (OD) of 1, at 600 nm. Bio-priming was performed by soaking certified wheat seeds (Morocco variety) in the selected bacterial suspensions (Naseem and Bano 2014). A mixture of sand, clay, and peat moss (1:1:1 ratio) was sieved through 2 mm mesh to remove soil micro-organisms and gravel. Following the standard protocols of McLean, the pH and EC of the experimental soil were measured. In each treatment, salt stress was induced with different concentrations of NaCl (0 mM, 25 mM, 50 mM, and 100 mM). Each pot was filled with 200 g of autoclaved soil and seeds were sown in sets of the following treatments: soil containing non-primed (normal) seeds (C);

soil containing seeds primed with *B. megaterium* (B_1), soil containing seeds primed with *B. tequilensis* (B_2), and soil containing seeds primed with *P. putida* (B_3).

Germination Percentage

Seeds were said to be germinated when the 2 mm radical emerged from the seed coat. The germination percentage was recorded by the following formula of Manmathan and Lapitan (2013):

$$\text{Germination percentage} = \text{germinated seeds} \div \text{total seeds} \times 100$$

Biochemical and Physiological Parameters of Plants

After 21 days of sowing, following biochemical and physiological parameters were studied.

Photosynthetic Pigments

Fresh leaves (0.1 g) were grinded in 80% acetone and kept in dark for 24 h. For the determination of carotenoid and chlorophyll contents, absorbance of the extracts was measured at 645 nm (for chlorophyll a), 663 nm (for chlorophyll b), and 480 nm (for carotenoids) (Stockburger and Mitchell 1999; Saeidi and Zabihi-e-Mahmoodabad 2009).

Relative Water Content, Relative Electrolytic Leakage, and Salt Tolerance Index

Relative water contents (RWC) of fresh leaves were assessed by measuring the turgid weight of fresh leaves, followed by its drying in hot air oven, till constant dry weight was obtained. RWC were calculated by following the formula of Whetherley (1950). The relative electrolytic leakage (REL) was measured by the standard protocol of Lutts et al. (1996) and salt tolerance index (STI) was calculated by the following formula:

$$\text{Salt tolerance index} = \text{biomass of plant under stress} \div \text{Biomass of non - stressed plant} \times 100$$

Growth Attributes

Lengths of freshly harvested shoots and roots of wheat plants were measured with the help of measuring tape. Fresh weights of the plants were measured using electrical balance after uprooting the whole plants. The dried weights of the plants were measured after drying them in an oven at 70 °C for 72 h.

Osmoprotectants

Proline contents of wheat leaves were estimated by the procedure of Bates et al. (1973). The absorbance of obtained mixture was measured at 520 nm, and the amount of proline was determined in µg/g FW. The total soluble sugar contents were assessed by following the protocol of Hahm et al. (2017). The optical density (OD) was measured at a wavelength of 620 nm, and based on the constructed standard curve ranging from 0 to 10 mg of dextrose sugar, the total soluble sugar contents (µg/g FW) were measured.

Antioxidant Enzymes Assay

The assessment of Superoxide dismutase (SOD) was performed by the method of Beauchamp and Fridovich (1971). Peroxidase (POD) activity was determined by the method of Vetter et al. (1958), with little modifications (Gorin and Heidema 1976). Catalase activity (CAT) was analyzed by calculating the value of hydrogen peroxide disappearance (Luck 1974).

Oxidative Burst

Malondialdehyde (MDA) activity was measured by the protocol of Yasin et al. (2018) and absorbance was recorded at 532 and 600 nm. The production of H_2O_2 was assessed by mixing 0.2 g of leaf sample in 5 ml of 0.1% chilled trichloroacetic acid (Loreto and Velikova 2001). The absorbance was measured at a wavelength of 390 nm.

Table 1 Primer sequences of selected genes for real-time PCR

Gene name	Primer	Sequence	Reference
SOS1	Forward	5′-GTTGTCGGTGAGGTCGGAGGG-3′	Ramezani et al. (2013)
	Reverse	5′-TCATCTTCTCCTACCGCCCTGC-3′	
SOS4	Forward	5′-ATCCAGTCCCACACCGTCCA-3′	
	Reverse	5′-GCTGATTGCCATTGAGAACCTGTC-3′	

Expression Analysis of Stress-Related Genes

The total RNA of control, salinity-stressed, and PGPR (*B. tequilensis*)-inoculated wheat plants was extracted by CTAB method (Yu et al. 2017). Quantitative real-time PCR was used for the expression analysis of selected salt stress-related genes (SOS1 and SOS4) using forward and reverse primers (Table 1). Actin was used as a housekeeping gene. Three replicates were taken from each treatment. Protocol of Ho-Kim et al. (2008) was used for the preparation of PCR mixture. Standard thermal cycling conditions were 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 15 s, 57 °C for 15 s, and 72 °C for 45 s.

Statistical Analysis

The experiments were performed in triplicates and their mean and standard error were calculated using Excel 2016. The data were subjected to one-way ANOVA, followed by Tukey's least significant difference (LSD) method using Statistix version 8.1. Furthermore, principal component analysis (PCA) was performed by XLSTAT 2016 to compare different experimental treatments.

Results

Characterization of Plant Beneficial Traits

All tested bacterial strains were able to produce ACC deaminase, EPS, and IAA and were also able to solubilize inorganic phosphate, even under salt stress condition (Fig. 1). The production of ACCD by the selected bacterial strains was ranging from 0.52 to 1.83 µM/mg protein/h. Among all tested strains, *B. tequilensis* was found to be more proficient and showed highest ACCD activity at 0 mM (1.83 µM/mg protein/h) and 25 mM salt concentration (1.70 µM/mg protein/h). In comparison, at 100 mM NaCl concentration, the strain synthesized 0.95 µM/mg protein/h. The range of IAA production by all three selected PGPR was 89.44–79.4 µM/ml. Moreover, *B. tequilensis* synthesized maximum amount of IAA in tryptophan-supplemented media, under salt stress. The maximum PSI of *B. tequilensis*

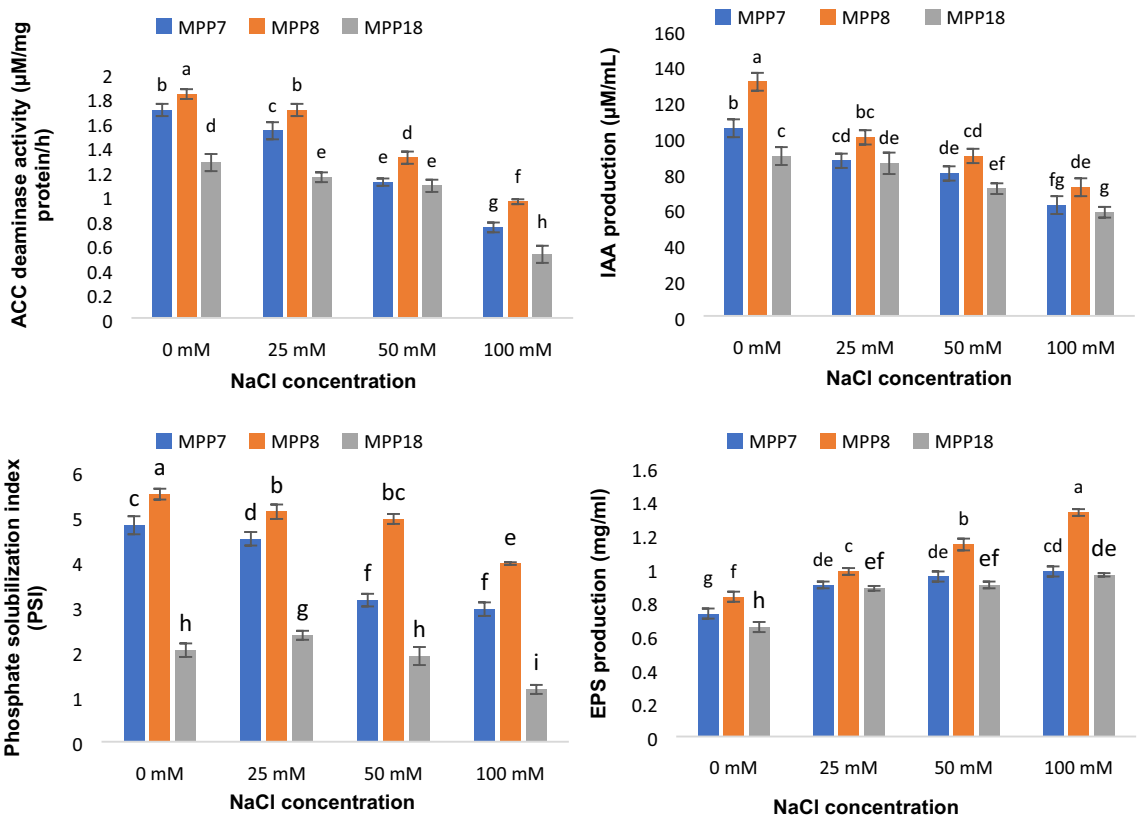

Fig. 1 Analysis of plant growth-promoting properties of *B. megaterium* (MPP7), *B. tequilensis* (MPP8), and *P. putida* (MPP18). Under different salt stress conditions, ACC deaminase activity (**a**), IAA production (**b**), phosphate solubilization index (**c**), and EPS production (**d**) were observed. Values are described as means and bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test

MPP8 was observed at 0 mM salt concentration (5.49) followed by 25 mM concentration (5.1) and 100 mM concentration (3.95). Under varying salt concentrations, strain *B. tequilensis* MPP8 was found to be more efficient in accumulating EPS than *B. megaterium* and *P. putida*. Maximum increase in EPS accumulation (1.33 mg/ml) was observed at 100 mM concentration by *B. tequilensis.*

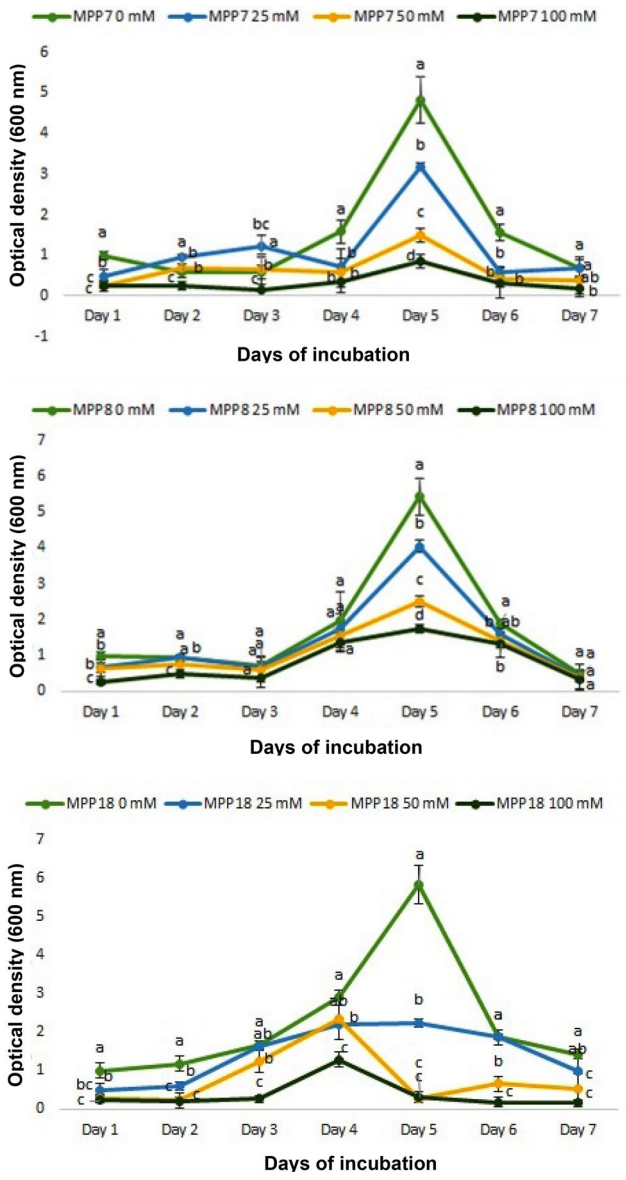

Fig. 2 Growth curve of *B. megaterium* (MPP7), *B. tequilensis* (MPP8), and *P. putida* (MPP18) under different salinity stress levels (0 mM, 25 mM, 50 mM, and 100 mM). Bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test

Salt Tolerance Assay of Selected Bacterial Strains, In Vitro

The growth rate of *B. megaterium* (MPP7), *B. tequilensis* (MPP8), and *P. putida* (MPP18) was successfully observed under different NaCl concentrations (0 mM, 25 mM, 50 mM, and 100 mM) for seven days (Fig. 2). All the selected bacterial strains exhibited variable potential of salt tolerance. Till 5th day of incubation, both species of *Bacillus* revealed highest growth rate and it declined, thereafter. *P. putida* showed highest growth till 4th day of incubation, under different concentrations of NaCl.

Germination Percentage

Bacterial strains were successfully inoculated with seed priming and germinated in experimental soil. The texture of the soil was loamy with 6.3 pH and 0.005 ds/m EC. These characteristics declared this soil to be ideal for plant growth. In the bacterial inoculated treatments, the germination rate of seeds was observed to be more than the control. Increasing concentration of salt negatively affected the seed germination. The minimum germination rate was observed in control treatment at 100 mM concentration of salt. Increase in germination rate was observed in bacterial inoculated treatments. The germination rate was in the subsequent order $B_2 > B_1 > B_3 > C$ and the maximum germination percentage was observed in B_2 treatment, even at 100 mM salt concentration (Fig. 3).

Biochemical and Physiological Parameters of Plants

Different biochemical and physiological parameters helped us to understand the response of plants under various treatments. Significant elevations of proline and sugar contents were observed with increasing concentrations of NaCl in all bacterial inoculated treatments (B_1, B_2, and B_3). Among these, B_2 treatment resulted in maximum increase of both proline and sugar contents (Fig. 4a).

Salinity stress directly affected the growth of wheat seedlings. Among all treatments, the minimum root length and shoot length were observed in control (C treatment) at 100 mM NaCl concentration. The maximum root length and shoot length were observed in B2 treatment, under different concentrations of salt (Fig. 4b). It was obvious from the growth analyses that the inoculation of bacterial strains significantly increased the fresh and dry weight of plants in both stressed and non-stressed conditions (Fig. 4c).

The application of bacterial strains triggered the production of photosynthetic pigments and these were decreased under salinity stress condition. Similar effects of salt stress on carotenoid content were also observed (Fig. 4d).

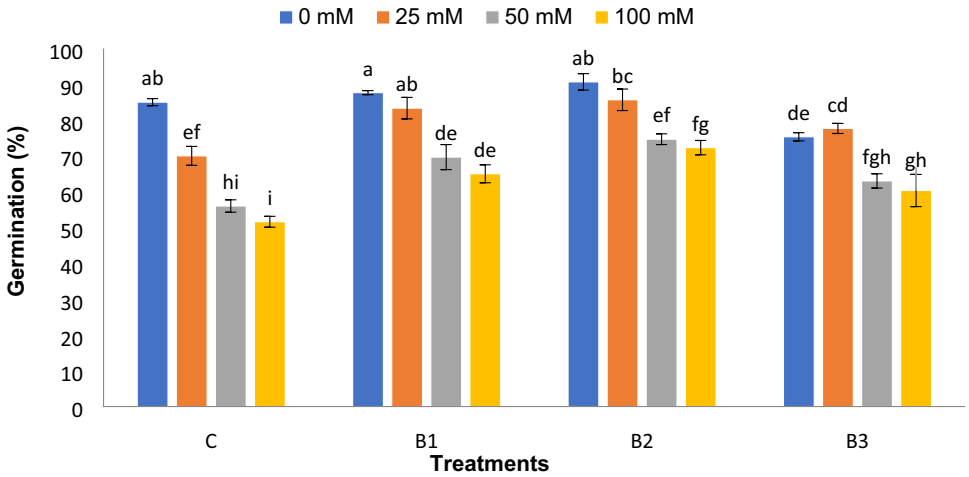

Fig. 3 Influence of various treatments on seed germination. Four different treatments including soil containing non-primed (normal) seeds (C), soil containing seeds primed with *B. megaterium* (B_1), soil containing seeds primed with *B. tequilensis* (B_2), and soil containing seeds primed with *P. putida* (B_3) were used under various concentrations (0–100 mM) of NaCl. Bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test

RWC of wheat plants was reduced significantly with the increasing concentration of NaCl. By the application of PGPR, noticeable increase in RWC under salt stress condition was observed. The maximum RWC was observed in treatment B2, even under higher salt stress conditions (Fig. 4e). Salinity stress significantly increased electrolytic leakage. Priming of seeds with bacterial strains resulted in significant reduction of electrolytic leakage in all treatments (Fig. 4e). The salt tolerance index of wheat plants was significantly reduced under salinity conditions. However, PGPR inoculation increased the salt tolerance index and the maximum salt tolerance was observed in *B. tequilensis*-treated plants (Fig. 4f).

Antioxidant Enzymes Activity

The production of antioxidant enzymes was significantly increased in PGPR-inoculated plants, under salt stress conditions (Fig. 5). Among all bacterial strains, the highest enzymatic activities were exhibited by *B. tequilensis* at 100 mM salt stress.

Oxidative Burst

In the present study, under 100 mM salt stress condition, increased production of MDA and H_2O_2 was observed in wheat seedlings. Though the inoculation of all PGPR decreased their production, *B. tequilensis* (B_2) was more useful in reducing the levels of MDA and H_2O_2 (Fig. 6).

Expression Analysis of Stress-Related Genes

For better understanding of plant defense mechanism, the expression levels of two stress-related genes viz. SOS1 and SOS4 were observed in wheat plants under salinity and PGPR treatments. In this trial, only the most efficient strain (*B. tequilensis* MPP8) was used. Application of PGPR significantly affected the relative expression of SOS1 and SOS4 genes in the leaves of wheat seedlings (Fig. 7). A higher expression of these genes was observed after salt stress and bacterial inoculation. As compared to control, the application of *B. tequilensis* enhanced the expression of SOS1 and SOS4 protein genes and depicted their potential role in salinity tolerance.

Principal Component Analysis (PCA)

Principal component analysis (PCA) confirmed the role of PGPR in the growth of wheat plant under different stress conditions (Fig. 8). The biplot among the charted statistics (F1 and F2) showed 83.66% differences (F1 presented about 61.58% and F2 presented about 22.08% differences). Blue color dots are indicating correlation among the different experimental treatments and red dots are representing the correlation among different variables. Positively correlated variables were found to be located near to each other in the same quadrant. Biplot revealed that plant growth attributes, photosynthetic pigments, RWC, and STI are positively correlated with each other and negatively correlated with all other parameters.

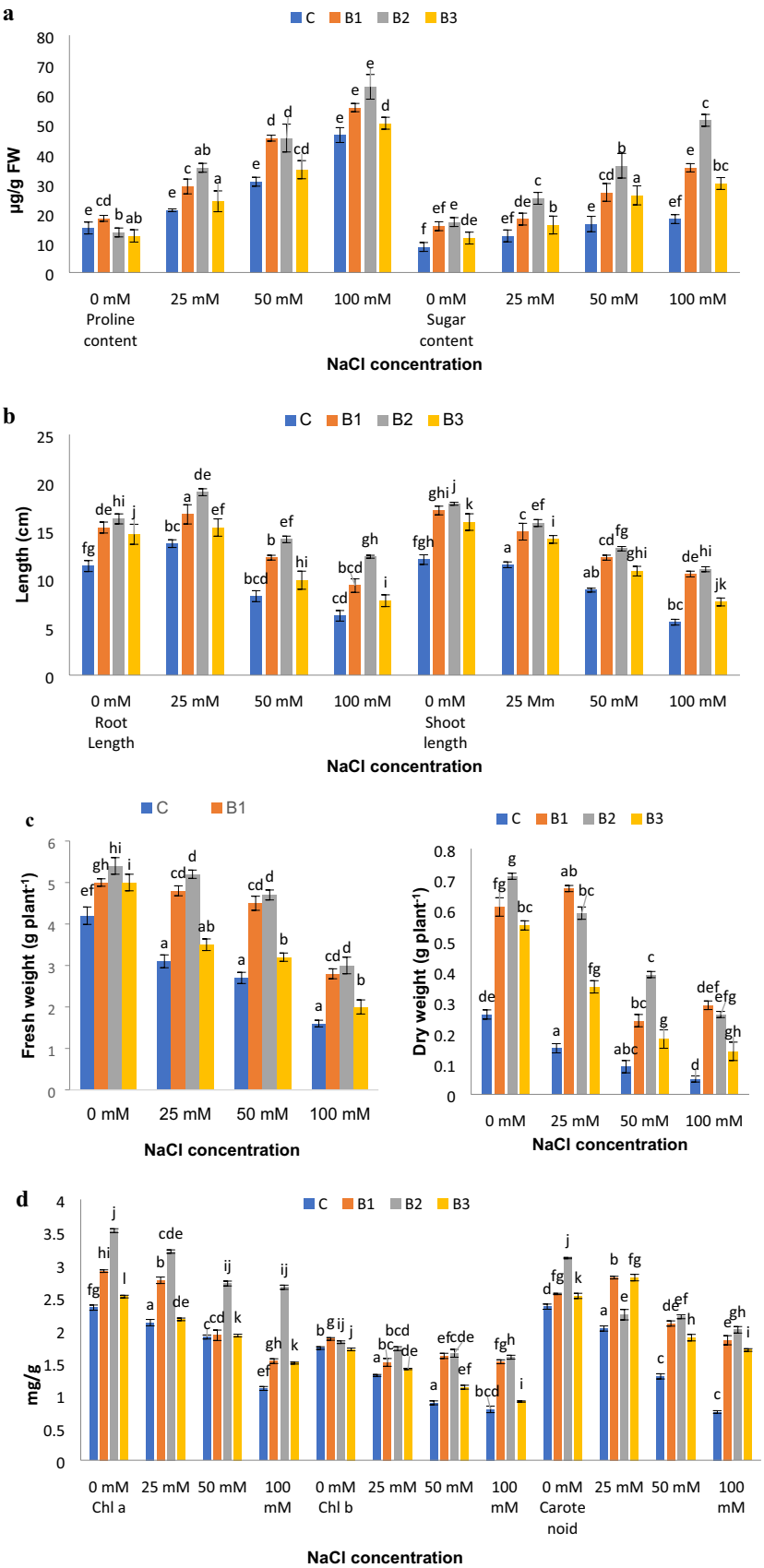

Fig. 4 Effects of different treatments on various biochemical and physiological parameters of PGPR-inoculated wheat seedlings under various levels of salinity stress (0 mM, 25 mM, 50 mM, and 100 mM). Four treatments including soil containing non-primed (normal) seeds (C), soil containing seeds primed with *B. megaterium* (B_1), soil containing seeds primed with *B. tequilensis* (B_2), and soil containing seeds primed with *P. putida* (B3) were used. Bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test. *RWC* Relative water content, *REL* Relative electrolyte leakage

Fig. 4 (continued)

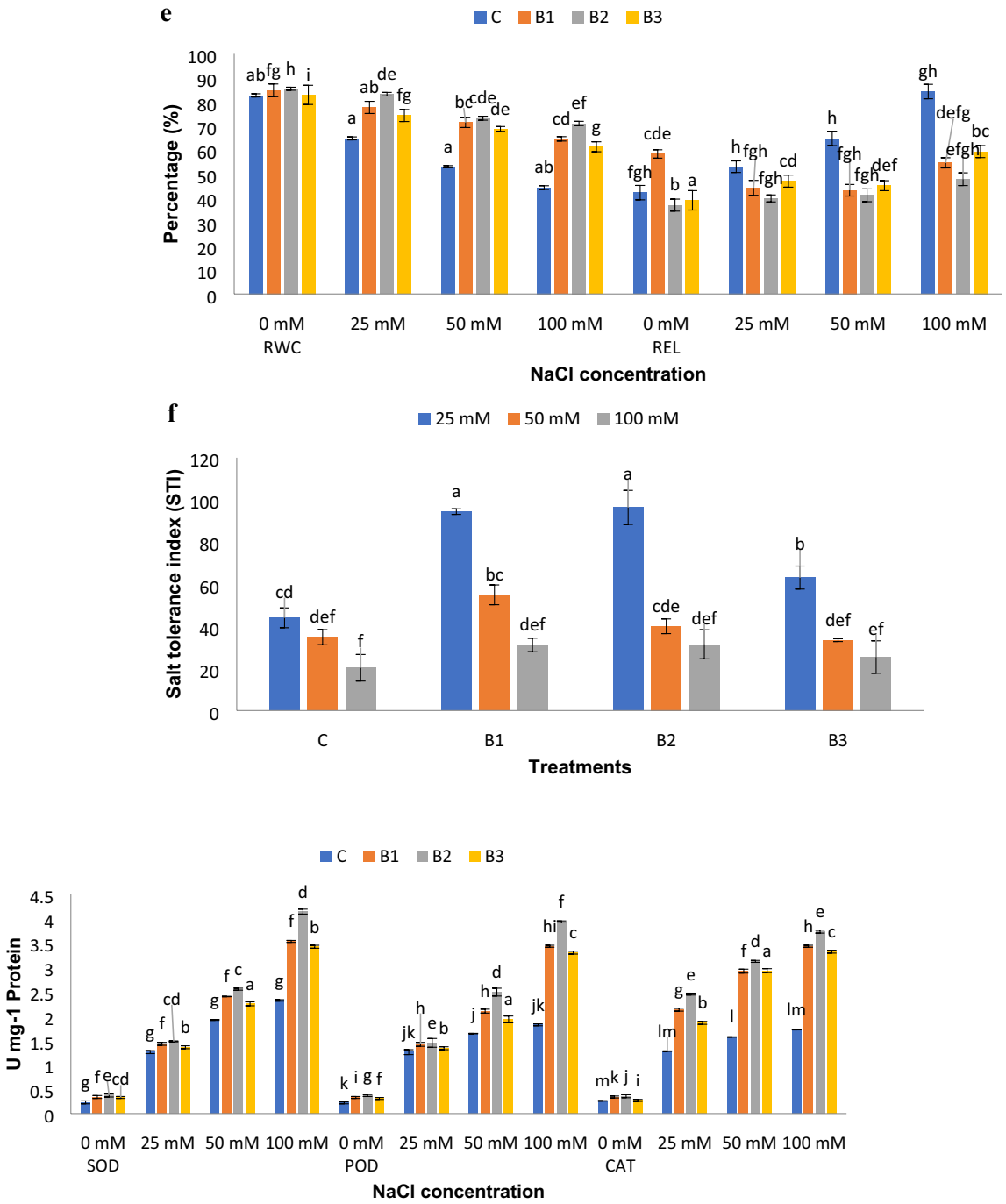

Fig. 5 Antioxidant enzymatic activities in PGPR-inoculated wheat seedlings under various levels of salinity stress (0 mM, 25 mM, 50 mM, and 100 mM). Four treatments including soil containing non-primed (normal) seeds (C), soil containing seeds primed with *B. megaterium* (B_1), soil containing seeds primed with *B. tequilensis* (B_2), and soil containing seeds primed with *P. putida* (B_3) were used. Bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test

Discussion

Plant growth-promoting rhizobacteria help to promote the growth of plants under various biotic and abiotic stresses (Dimkpa et al. 2009). This study has validated the efficiency of rhizobacteria in inducing salinity tolerance by promoting various plant growth-promoting traits.

The selected bacterial strains efficiently produced ACC deaminase. It has been reported earlier that ACC

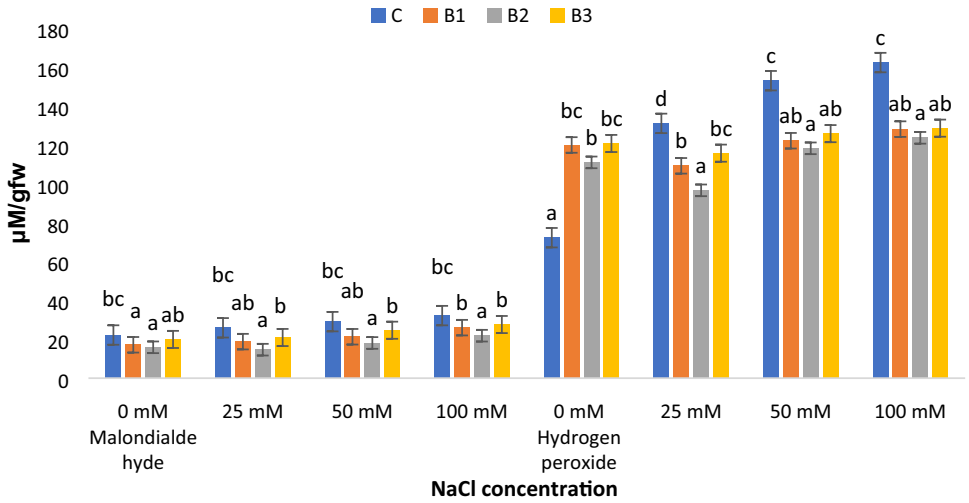

Fig. 6 Changes in malondialdehyde and hydrogen peroxide production in PGPR-inoculated wheat seedlings under various levels of salinity stress (0 mM, 25 mM, 50 mM, and 100 mM). Four treatments including soil containing non-primed (normal) seeds (C), soil containing seeds primed with *B. megaterium* (B_1), soil containing seeds primed with *B. tequilensis* (B_2), and soil containing seeds primed with *P. putida* (B_3) were used. Bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test

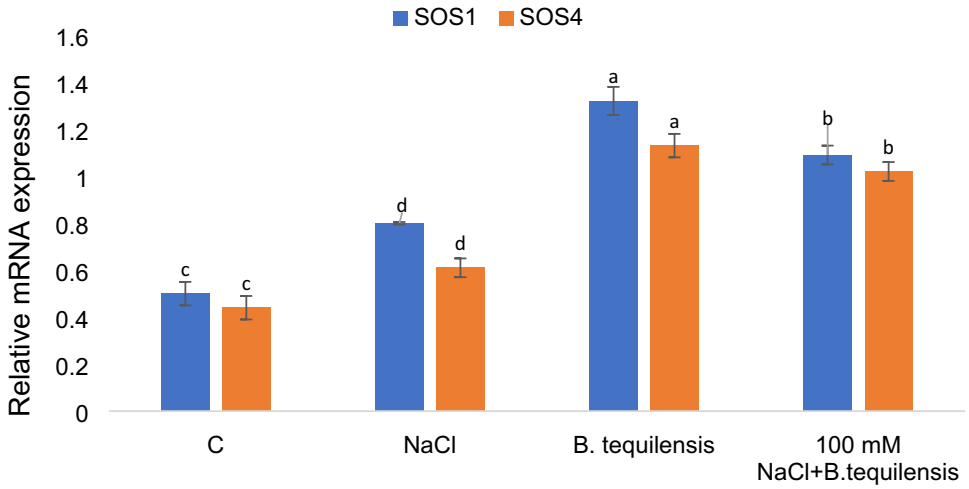

Fig. 7 Relative expression of SOS1 and SOS4 genes in the leaves of wheat plants. Relative expression was observed in plants grown without PGPR and salt stress (C), plants grown under salt stress (NaCl), plants inoculated with halotolerant PGPR (*B. tequilensis*) and plants grown under salinity stress and inoculated with *B. tequilensis* (100 mM NaCl + *B. tequilensis*). Bars denote standard errors. Dissimilar alphabets demonstrate significantly different values ($p < 0.05$) from each other, as calculated by Tukey's least significant difference (LSD) test

deaminase-producing Bacillus strains efficiently improved the growth of wheat plants under salinity stress (Din et al. 2019). The findings of the current analysis showed an increased production of EPS under salinity stress and suggested the protective role of halotolerant bacterial strains (Li et al. 2017). The auxin-synthesizing bacteria have been previously reported to enhance root growth and uptake of nutrient, which help plants to cope with the salinity stress (Yasin et al. 2018). Various species of *Bacillus, Azotobacter*, and *Pseudomonas* have been stated to synthesize auxin (Cassán et al. 2014; Verma et al. 2018). The current study showed that all the tested strains have the ability to produce IAA, indicating their potential use as PGPR. All the selected bacterial strains were able to solubilize phosphate which is an important plant growth-promoting trait. Our results depicted that among all the studied bacterial strains, *B. tequilensis* produced the highest levels of ACCD, EPS, and IAA. *B. tequilensis*

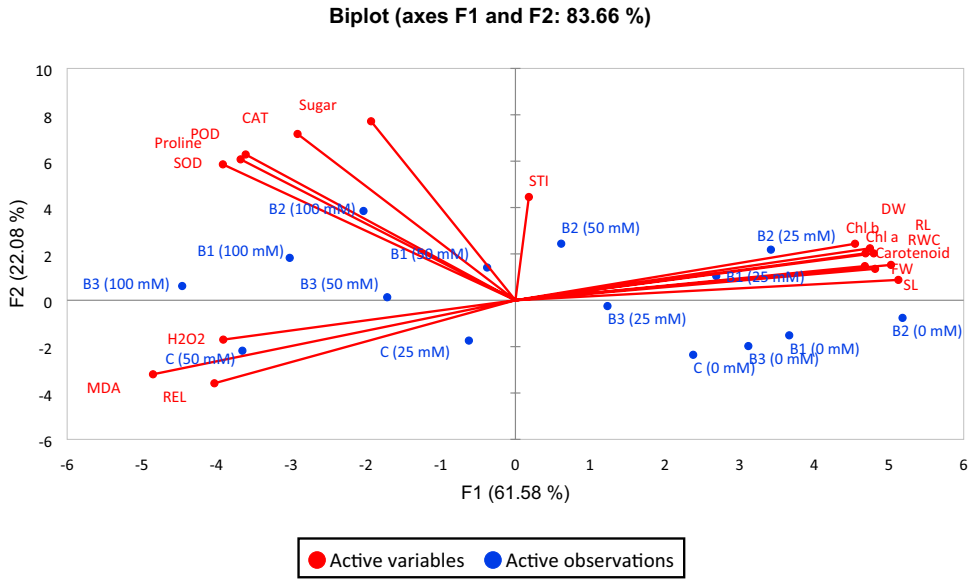

Fig. 8 Pearson correlation biplot among the charted statistics (F1 and F2). Blue color dots are indicating correlation among the different experimental treatments and red dots are representing the correlation among different variables

also exhibited efficient phosphate-solubilizing ability and proved it to be the best PGPR.

Seed priming with PGPR increased the germination percentage and improved various growth attributes of wheat plant. In the current research, both sugar and proline contents were increased in bacterial inoculated wheat seedlings grown under saline conditions. Consequently, the PGPR inoculants enhanced the growth of plant under various levels of salinity stress by improving metabolic resistance mechanisms (Ilangumaran and Smith 2017).

In the current study, salinity stress significantly reduced chlorophyll content of plants, while bacterial inoculation significantly increased chlorophyll a and chlorophyll b contents. Reduction in chlorophyll a and b content is an indication of photo-oxidation (Rahdari et al. 2012). Scientists have previously stated improved production of photosynthetic pigments in bacterial inoculated plants, under salt stress conditions (Sapre et al. 2018). In this study, carotenoid contents were noticeably increased due to bacterial application under stressed and non-stressed conditions. High carotenoid content attributes to genotype tolerance since they are responsible for breakdown of singlet oxygen (Efeoğlu et al. 2009).

Salinity results in osmotic stress by reducing the RLWC (Fahad et al. 2015). Our results showed improved RLWC in plants, treated with PGPR, while RLWC were decreased in diseased plants. In previous studies, substantial decrease in RLWC has been repeated under the stress conditions (Dekov et al. 2000; Nayyar and Gupta 2006). Our findings also revealed relative electrolyte leakage under stress conditions, which might have enhanced POD and CAT activity. Among all treatments, bacterial inoculation diminished the adversity of stress by decreasing electrolytic leakage. Previously, Bacillus sp. has also been reported to decrease electrolytic leakage and imparting membrane stability (Vardharajula et al. 2011). The outcomes of the current analysis revealed that STI of wheat seedlings was significantly reduced in salt stress condition. However, PGPR inoculants showed elevated STI value, correspondingly. Previous studies have also reported increased STI value in *Capsicum annum* (Yasin et al. 2018).

After the inoculation of PGPR under salinity stress, all three inoculated bacteria showed considerable increase in both root and shoot length and fresh and dry weights of root and shoot. Application of PGPR has been reported to increase root shoot length and overall plant vigor (Farooq and Bano 2013). Karlidag et al. (2013) also reported improved growth attributes due to bacterial inoculation, under salt stress.

In the current study, antioxidant enzymes activities (SOD, POD, and CAT) in wheat plants inoculated with PGPR were significantly increased, when compared with their respective un-inoculated control plants. Our results are supported by the results of Hmaeid et al. (2019), who also reported that the activities of ROS-scavenging enzymes were enhanced in PGPR-inoculated *Sulla carnosa,* under the influence of salinity stress.

Under the influence of excess salt, plants start producing excessive level of MDA. The results of this study depicted that MDA content was high in plants under the salinity stress. Bacterial inoculation alleviated the stress tolerance potential in wheat plants by decreasing the level of MDA. Our results are similar to the findings of Singh and Jha (2017), who also noted that decrease in malondialdehyde production in *S. maltophilia* SBP-9 inoculated wheat plants, under salinity stress. Hydrogen peroxide level was increased in the plants under salt stress but bacterial inoculated plants showed low production of hydrogen peroxide. PGPR has been well documented to lower the levels of lipid

peroxidation, superoxide anions, and hydrogen peroxide and stimulate defense mechanisms (Gupta et al. 2017).

The SOS protein family is clearly shown to be able to mediate salt tolerance directly and indirectly (Ramezani et al. 2013). The findings of the current research showed high expression of SOS genes in the plants inoculated with halotolerant *B. tequilensis,* under salinity stress and described their potential role in salinity tolerance. It has previously been reported that the rice SOS genes play a significant role in the adaptive mechanism to salinity tolerance (El Mahi et al. 2019).

Conclusion

This is the first comprehensive study in which three halotolerant ACCD- and EPS-producing PGPRs including *Bacillus megaterium, B. tequilensis*, and *Pseudomonas putida* have been studied on wheat seedlings, simultaneously. These bacterial inoculants showed ability to solubilize phosphate and produce indole-3-acetic acid to maintain plant growth. Bacterial inoculation increased salinity stress tolerance in wheat by increasing expression levels of ROS-scavenging enzymes. Higher expression of SOS1 and SOS4 genes also predicted their potential role in stress tolerance. These genes can be further overexpressed in wheat plant to tolerate salinity stress. All these results make this study very reliable and novel and provide an environmental solution to salinity stress.

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Author Contributions UH executed the experiments and wrote the manuscript; MFHM designed the experiments, supervised the work, read and approved the final manuscript; FL and MK did initial characterization of bacterial strains; MA, AK, and HJC did data analysis; KT and SSB did statistical analysis; MA revised manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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