SALT TOLERANT RHIZOBACTERIA: FOR BETTER PRODUCTIVITY AND REMEDIATION OF SALINE SOILS

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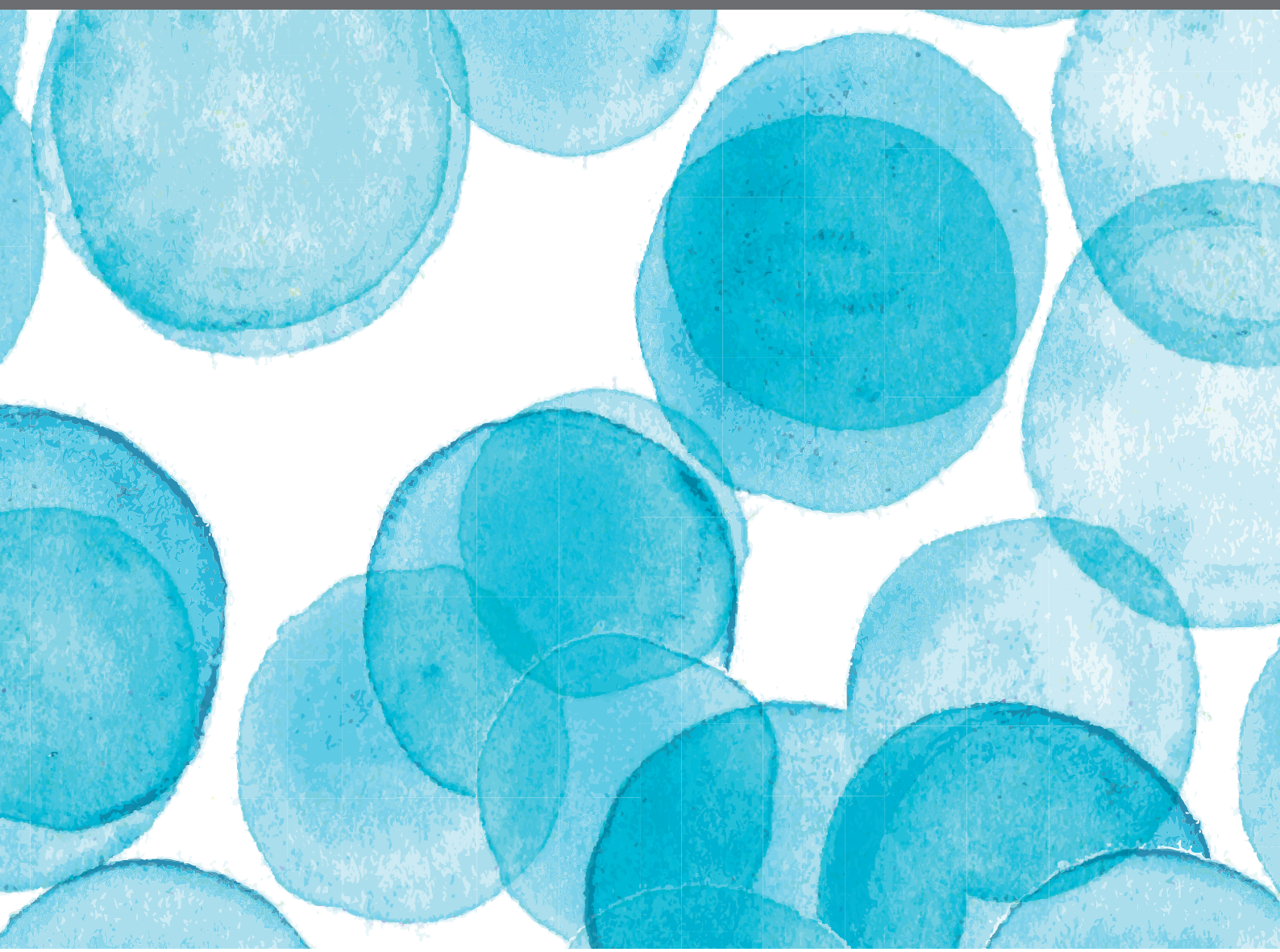

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SALT TOLERANT RHIZOBACTERIA: FOR BETTER PRODUCTIVITY AND REMEDIATION OF SALINE SOILS

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Table of Contents

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EDITORIAL
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Editorial: Salt Tolerant Rhizobacteria: For Better Productivity and Remediation of Saline Soils

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Keywords: saline soils, salt tolerant rhizobacteria, plant growth promoting rhizobacteria, bioremediation, agro-ecosystems

Editorial on the Research Topic

Salt Tolerant Rhizobacteria: For Better Productivity and Remediation of Saline Soils

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Soil salinity has been recognized as a major issue particularly in arid and semi-arid areas of the world and is one of the main constraints that undermine plant growth and agricultural productivity. The pace at which saline soils are increasing around the globe has posed a serious threat to food security, the environment, and biodiversity. The Research Topic entitled "Salt Tolerant Rhizobacteria: For Better Productivity and Remediation of Saline Soils" is focussed on reviews and research articles on major challenges caused by soil salinization in agroecosystems and its remediation using salt-tolerant rhizobacteria as sustainable solutions to increase the productivity of these degraded lands.

The electrical conductivity (EC) of saline soils is either equal to or exceeds 4dS/m. Saline soils have a higher concentration of the salts Na^+, Cl^-, Ca^{2+}, HCO^{3-}, Mg^{2+}, NO^{3-}, and SO_4^{2-}, which prove detrimental to the microbial communities of the soil. The problems associated with salt accumulation are visible in several vital functions of soil such as poor water holding capacity and structural stability, reduced infiltration rate, disturbed pH, decreased levels of nutritional content, and lower organic matter. A study by Wang et al. explored the soil factors determining the structure and composition of bacterial communities in saline soils of Songnen Plain, China to dig out the potential of microbial resources. Systemic analysis using high throughput sequencing (Illumina MiSeq sequencing) revealed that the EC of the soil is one of the direct environmental factors that control the distribution of bacterial communities. The above concept was also explained in another research article by Yang et al., in which a metagenomic approach and *NifH* Illumina sequencing has been carried out for the characterization of rhizosphere and nodule microbiomes of wild salt-tolerant soybean growing in saline-alkaline soils of China. The study provides a systematic and functional understanding of the plant root microbiome under saline-alkaline conditions. Yang and Sun, have presented a similar correlation by showing how changes in soil properties regulate soil fungal communities and affect their distribution patterns and ecological functions.

There are myriad impacts on crops grown in salt stress conditions which have been interestingly detailed in a review by Kumar et al.. Such crops show signs of nutrient deficiency, ionic toxicity, oxidative stress, reduced photosynthetic activity, and decreased germination rate resulting in lower

agricultural productivity (Kumar et al.). To obviate this problem, there is a need to adopt methods that are organic in origin, cost effective, and above all, ensure environmental sustainability. In this context, plant growth promoting rhizobacteria (PGPR) especially salt-tolerant or halotolerant PGPR have been identified as potential tools to alleviate salinity stress by eliciting tolerance in crops against high salt concentrations.

The mini review by Bhat et al., explains, salinity stress and plant productivity can be managed by halotolerant PGPR. The manuscript describes that halotolerant PGPR, due to their catabolic versatility and efficient root colonizing abilities, help in rejuvenating plant health through an array of mechanisms. These include nutrient acquisition, metal chelation, maintenance of water balance and ionic homeostasis, the production of phytohormones, exopolysaccharides (EPS), volatile organic compounds (VOCs), and antioxidative enzymes, triggering stress responsive genes under high salt concentrations. The article by Kaushal has reviewed similar aspects of PGPR by stating that microbially inoculated plants show well-established salt tolerance and endurance mechanisms (STEM), which include the aggregation of osmolytes, activating antioxidant machinery, recovery of nutritional status, and ionic homeostasis protecting the symbiotic partner under salt/ osmotic stress conditions. This theory was supported in a study by Nawaz et al., where they determined the potential of PGPR inoculation on two wheat genotypes (Aas-11; salt tolerant and Galaxy-13; salt sensitive). The outcome of the study revealed that PGPR inoculation significantly enhanced the physio-chemical attributes in both salt-tolerant and salt-sensitive wheat genotypes. In the same context, Singh et al., in their study showed that seeds bioprimed with selected strains, both individually or in combination, conferred better germination and vigor in maize plants. The authors showed that there is microbe based stress amelioration in maize plants making them ecologically fitter to survive and grow in saline-sodic soils.

A study by Taj and Challabathula also suggests that PGPR can increase the level of photosynthetic electron transport rate, and enhance carboxylation efficiency in their host plants during salinity stress. Recently, molecular tools have helped us to better understand the mode of actions of salt tolerant PGPR. Work by Nawaz et al., demonstrates the impact of acyl homoserine lactone (AHL) (bacterial signal molecules) producing PGPR *Aeromonas* sp., on two wheat genotypes. The study revealed that the exogenous application of AHL improved root parameters in both salt-sensitive and salt-tolerant genotypes. Principle component analysis (PCA) also showed the effectiveness of inoculation response of AHL producing *Aeromonas* in comparison to non-producing strains. The study opens a gateway for future exploration of AHL producing PGPR to exploit their role in the alleviation of salt stress and plant growth improvement. A comprehensive understanding of the complexities of signal transduction pathways can give new insights into biochemical and molecular mechanisms in response to salinity stress.

The beneficial characteristics of halotolerant PGPR make them excellent green solutions to enhancing the productivity of crops in saline agro-ecosystems in a sustainable manner. In the manuscript presented by Meena et al., the impact of inoculation of a halotolerant methylotrophic actinobacterium

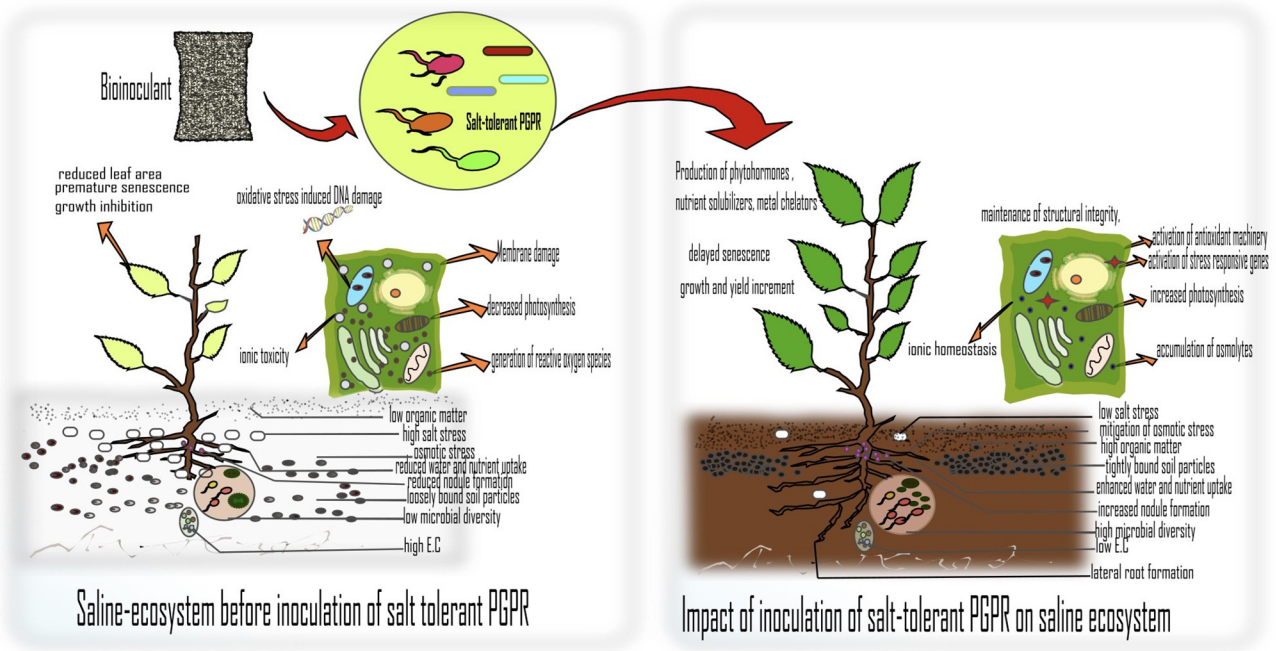

FIGURE 1 | Mechanisms of improving plant health and remediation of saline soils by inoculation of PGPR.

(*Nocardioides* sp. NIMMe6; LC140963) and the seed coating of its phytohormone-rich bacterial culture filtrate extract (BCFE) on wheat seedlings was investigated. The results suggested that bacterial inoculation mitigated saline stress in plants whereas seed priming with BCFE improved physiological status, enhanced oxidative enzymes, and resulted in gene modulation. The complete profiling of metabolites and the genes involved (of both the symbiotic partners) at inter and intra-cellular level will pave way for the development of reliable products for salt affected agro-ecosystems.

Salt specific metabolites and gene triggers will also be explored in the near future, as researchers explore tailor made halotolerant PGPR based bioinoculants. Vaishnav et al. examined the role of salt-tolerant PGPR *Sphingobacterium* BHU-AV3 on tomato plants under 200 mM NaCl concentration. The inoculated plants showed decreased levels of oxidative stress, lipid peroxidation, ROS, and cell death and enhanced levels of antioxidant enzymes and energy metabolism thus suggesting an overall plant protection strategy under salt stress. The potential of halotolerant PGPR under salt stress has also been reported on legumes. The research article by Alexander et al. shows that the inoculation of *Arachis hypogaea* by halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 improved its photosynthetic pigments, auxin levels, and total amino acid content in salt stress conditions (100 mM NaCl) as compared to untreated plants.

The role of plant-microbiome under diverse conditions is now becoming more and more obvious. It is important to explore this intricate biological coordination and determine the functional mechanisms at metabolic and genetic levels to use the system optimally in stressed habitats. Concerted research toward novel bioformulations for stressed agro-ecosystems, their field screening, improved delivery systems, and higher shelf life, are needed. The use of microbial metabolites and additives such as EPS, biosurfactants, and nutrients need to be explored for bioinoculants developed specifically for saline soils. Engineered nanomaterials are now being considered as next generation carrier materials for the development of tailor made nano-formulations that can work with precision to increase site specific and controlled nutrient availability in stressed soil conditions.

The proper monitoring and mapping of salt stressed habitats, involving as wide as satellite imaging and as narrow as micro-ecosystems in soils (for the level of ions, nutrients, and microbial communities) is also of importance so that these systems can be properly and timely managed. These will provide insights about the existing microbial communities, which are unique in their characters and can be helpful for agriculture in saline areas. Soil engineering through tools like metabolomics and metagenomics can help decipher novel microbial metabolites from the rhizospheric ecobiome. The repeated use of green products will be crucial to increasing soil organic matter, nutrient content, and microbial load in salt stressed soils through the process of rhizoremediation (**Figure 1**). This Research Topic thus summarizes how the co-evolutionary relationship between rhizobacteria and their host plants can help increase the yield of crops in salt degraded lands and provide new possibilities for exploration and research that can altogether change the future of bioformulations for sustaining the agro-ecosystems in general and for remediation of stressed ecosystems in particular.

AUTHOR CONTRIBUTIONS

NA conceptualized the idea. Figure was drawn by NA and IM. All the authors helped in writing the manuscript.

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Exploring Soil Factors Determining Composition and Structure of the Bacterial Communities in Saline-Alkali Soils of Songnen Plain

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Songnen Plain is originally one of the three major glasslands in China and has now become one of the three most concentrated distribution areas of sodic-saline soil worldwide. The soil is continuously degraded by natural and anthropogenic processes, which has a negative impact on agricultural production. The investigation of microbial diversity in this degraded ecosystem is fundamental for comprehending biological and ecological processes and harnessing the potential of microbial resources. The Illumina MiSeq sequencing method was practiced to investigate the bacterial diversity and composition in saline-alkali soil. The results from this study show that the change in pH under alkaline conditions was not the major contributor in shaping bacterial community in Songnen Plain. The electrical conductivity (EC) content of soil was the most important driving force for bacterial composition (20.83%), and the second most influencing factor was Na^+ content (14.17%). Bacterial communities were clearly separated in accordance with the EC. The dominant bacterial groups were *Planctomycetes*, *Proteobacteria*, and *Bacteroidetes* among the different salinity soil. As the salt concentration increased, the indicators changed from *Planctomycetes* and *Bacteroidetes* to *Proteobacteria* and *Firmicutes*. Our results suggest that *Proteobacteria* and *Firmicutes* were the main indicator species reflecting changes of the main microbial groups and the EC as a key factor drives the composition of the bacterial community under alkaline conditions in saline-alkali soil of Songnen Plain.

Keywords: bacterial community, driving force, electrical conductivity, saline-alkali soil, Songnen Plain

INTRODUCTION

Salinity and/or sodicity is one of the main problems causing soil degradation, which is a grievous environmental problem with negative impacts on agricultural sustainable development. All over the world, over 800 million ha of land is estimated to be affected by salinity, which includes saline and alkaline soil (Yadav et al., 2011). It is reported that ~20% of the agricultural

land worldwide is salt affected, and there is an increase in saline land of 1–1.5 × 10^6 ha worldwide every year (Sumner, 2000; Munns and Tester, 2008). If this continues, about half of cultivable land will be taken out of production by the middle of the 21st century (Mahajan and Tuteja, 2005). As one of the three most concentrated alkaline soil distribution areas worldwide, the Songnen Plain is located in the northeast of China (Wang et al., 2009). In this region, several environmental problems such as soil salinization, alkalinization, and desertification occur due to soil parent material, hydrological conditions, and overgrazing (Zhou et al., 2011). The annual rainfall exceeds the potential evaporation and poor management such as overgrazing and irrational utilization are common in the area (Gao and Liu, 2010; Liu et al., 2011a); thus, the affected alkali-saline area has been increasing in size by 20,000 ha per year (Zhang et al., 2015). Not only about two-thirds of the land in this area is salinized, but also increased by 1.5–2% annually (Ma et al., 2015). Under increase aridity and soil alkalization, large areas of croplands have been abandoned (Shang et al., 2003) and large proportions of grasslands have degraded seriously to unprecedented levels (Li et al., 2014).

Soil microorganisms participate in multiple aspects in the adjustment of ecological processes, for instance, degradation of organic matter, nutrient element transformations, enzyme production, and maintenance of soil quality (Sleator et al., 2008). Given that microorganisms are rapidly affected by the changes of their environment (Jiang et al., 2012), some biological characteristics (such as microbial diversity, composition, and structure) of soil are often considered to be sensitive and early indicators of dynamic environmental changes and soil ecological stress status (Li et al., 2011; Liu and Kang, 2014). For instance, soil microbial diversity was shaped by land-use changes, such as urbanization, agriculture, deforestation, and desertification. Many studies have shown that current environmental factors, for example, nutritional status, metalloid contamination, soil pH, the plant secretion (Hansel et al., 2008; Rousk et al., 2010; Xiong et al., 2010), and geographic distance (Xiong et al., 2012), influence structure and composition of microbial community.

Among the environmental factors, the pH was proposed to be a driver force for bacterial horizontal distribution in the soil (Shen et al., 2013; Liu et al., 2014). As soil salinization and alkalinization frequently co-occur, meta-analysis has been conducted merely on microbial diversity and composition (including bacteria and archaea) in saline soil habitats (Lozupone and Knight, 2007; Ma and Gong, 2013). Salinity is a dominant factor in determining bacterial community composition (Lozupone and Knight, 2007), however, relatively little is known about how the bacterial community composition response to the salinity gradients. Especially, the microbial structure under different salinities at similar pH has not been investigated.

Several recent studies on the composition of soil microbial diversity in saline soils revealed that soil salinization had negative effects not merely on soil biochemical properties, but also on the structure of microbial communities (Foti et al., 2007; Yuan et al., 2007; Hidri et al., 2013; Wang et al., 2014; Zhang et al., 2015). Regarding the soil microbial community in saline soils of Songnen Plain, only some representative new species of halophilic and halotolerant bacteria and archaea have been reported by pure culture methods (Wu et al., 2008; Wang et al., 2010; Liu et al., 2011b; He et al., 2014; Pan et al., 2016). These microorganisms have gradually formed adaptations, including unique structures and physiological functions, such as the accumulation of osmotic adjustment-related substances (Yan and Marschner, 2012). However, physical and chemical properties in saline-alkaline environments and microbial composition in Songnen Plain have not been sufficiently explored.

Systematic analysis of microbial diversity and composition in saline-alkaline soil of Songnen Plain is essential for gaining insight into the biological and ecological processes, saline adaption mechanisms, and digging the potential microbial resources from such environments. We collected 29 soil samples with different salinities across Songnen Plain and performed high throughput sequencing (Illumina MiSeq sequencing) to investigate the microbial diversity and composition in this under-studied system and to identify the key factors controlling the distribution of bacterial communities. The aim was to clarify the direct effects of environmental factors in shaping bacterial communities under geographic scale.

MATERIALS AND METHODS

Site Description

The study area locates in the Songnen Plain (42°30′–51°20′N, 121°40′–128°30′E), Northeast China and belongs to a transitional zone between semi-humid and semi-arid regions, and is typically influenced by continental monsoon climate with mean annual temperature of 4.7°C (Shang et al., 2003; Yang et al., 2010). The average annual evaporation in this region is four times greater than the annual precipitation. The groundwater average mineral content is 2–5 g/L, with a maximum of 10 g/L, and the major anions present are CO_3^{2-} and HCO_3^- (Zhang, 2014). The saline-sodic soils are characterized by a high pH (up to 10) and a large exchangeable Na percentage (Chi and Wang, 2010). The dominant zonal soils include meadow carbonate chernozem, deep chernozem, sodium carbonate-salinized soil, and dark chestnut soil. These soils are mainly distributed in the west part of Jilin and Heilongjiang provinces (Chi et al., 2011), including Zhenglai, Da'an, Changling, Qianguo, and Tongyu prefectures in Jilin province, and Zhaoyuan, Zhaozhou, Dumeng, Daqing, and Anda prefectures in Heilongjiang province (Wang et al., 2009). There are no naturally growing tree species because of salinization, only some salt/sodium-tolerant grass species such as *Leymus chinensis*, *Puccinellia tenuiflora*, and *Suaeda corniculata* are able to grow in the study area.

Site Selection and Soil Sampling

A total of 29 soil samples (with a mean depth of 0–15 cm) with GPS located site information from 12 counties (cities)

across Jilin and Heilongjiang provinces were randomly collected in September 2013 (**Figure 1**). At each site, the analyzed soil samples mixture was from three soil samples collected in the vertices of 1 m side equilateral triangle. Then, the soil sample mixture from each site was divided into two subsamples: one was air dried, 2 mm sieved for soil physical and chemical analysis, and the other subsample was stored at −80°C for subsequent high-throughput sequencing.

Soil Physical and Chemical Analysis

Soil organic carbon (SOC) was measured on a Total Organic Carbon (TOC) Analyzer (Multi N/C2100, Analytik-Jena, Germany). The electrical conductivity of the saturated paste extraction (EC) was measured using a conductivity meter (DDS-307A, REX, Shanghai), and pH was determined with air-dried soil (soil:water, 1:5) by pH meter (PHS-3C, REX, Shanghai). Soil exchangeable sodium(ammonium acetate exchange method) was determined by an atomic absorption spectrophotometer (TAS-990, Persee, Shanghai). Potentiometric titration was used to determine CO_3^{2-}, HCO_3^-, Cl^-, and SO_4^{2-} with air-dried soil (soil:water, 1:5). The cations Ca^{2+} and Mg^{2+} were detected using Ethylenediaminetetraacetic acid (EDTA) titration and Na^+ and K^+ were measured using an atomic absorption spectrophotometer (TAS-990, Persee, Shanghai). Total content of dissolved salt (TDS) was determined by the drying-weighing method. The soil:water 1:5 tilt rate was placed in an oven at 105°C until constant weight was reached.

Soil DNA Extraction

The total DNA was extracted from 0.25 g soil samples using the Power Soil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA), followed by electrophoresis using a 1% agarose gel. The quality and quantity of DNA extracts (final volume, 100 µl) were examined using a Nano Drop spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA).

Illumina Sequencing for Communities of Bacteria

The primer pair 515F (5′-GTGCCAGCMGCCGCGG-3′)/907R (5′-CCGTCAATTCMTTTRAGTTT-3′) was used to amplify the V4-V5 region of bacterial 16S rRNA genes (Yao et al., 2017). A 6-bp unique barcode unique to each sample was added into the reverse primers. Amplicon sequencing was performed using the Illumina MiSeq platform at Majorbio Inc. (Shanghai, China).

The sequencing data analysis was performed according to previous study (Zhu et al., 2016). Briefly, raw high-throughput sequencing data was processed using QIIME toolkit and the UPARSE pipeline (Caporaso et al., 2010; Edgar, 2013). After filtering DNA sequences using quality files, the remaining sequences were trimmed to remove barcodes and forward primers. The low quality (quality score < 20, length < 300 bp) sequences were excluded. The sequencing data were pre-treated to remove the chimeras from the datasets. After optimizing the sequences, the UPARSE pipeline was used to make an operational taxonomic unit (OTU) table. The identity threshold to bin the sequences into OTUs was 97%, and the most

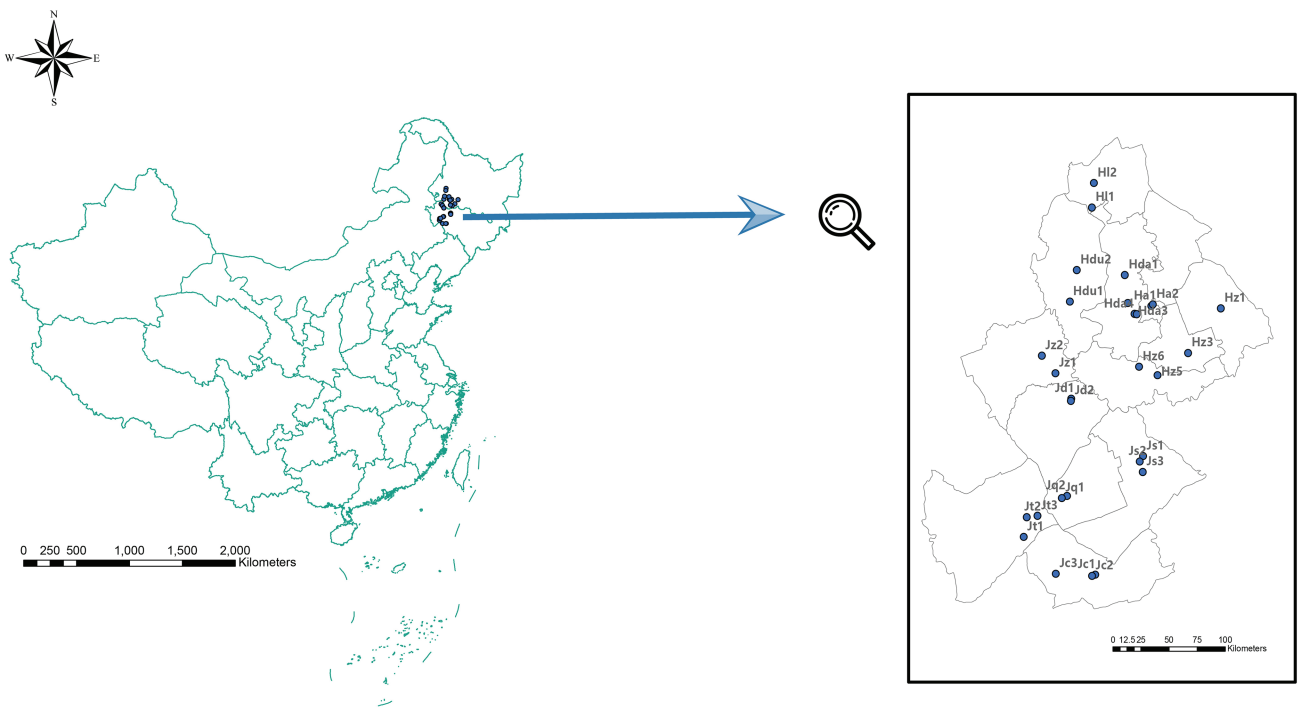

FIGURE 1 | Sampling positions in Jilin Province [including Zhenglai (Jz), Da'an (Jd), Qianguo (Jq), Changling (Jc), Songyuan (Js), and Tongyu (Jt) prefectures], and in Heilongjiang Province [including Dumeng (Hdu), Lindian (Hl), Daqing (Hda), Anda (Ha) Zhaodong (Hz1), and Zhaozhou (Hz3) prefectures].

abundant sequence from each OTU was selected as the representative sequence for that OTU. The assignment of taxonomic data to microbial representative sequences was based on the Ribosomal Data Project (RDP) database. All sequencing data were deposited in the NCBI Sequence Read Archive (SRA) database (accession number: SRP172399).

Statistical Analysis

Analysis of variance was performed for each measured soil variable, and variance was compared between groups by a Fisher's least significant difference test ($\alpha = 0.05$). The richness index (Chao1 index), α-diversity (Shannon diversity) index, and rarefaction curves of soil samples were calculated by QIIME with normalized data. Non-metric multidimensional scaling (NMDS), and one-way PERMANOVA were used to analyze the β-diversity of bacteria in different treatments according to Bray-Curtis distance and Canonicla Correlation Analysis (CCA) was used to examined the relationships between the environmental factors and bacterial community structure with the R (2.15.3) packages ape and vegan. Aggregated boosted tree (ABT) analysis (De'ath, 2007), was performed using the gbmplus package (with 500 trees used for the boosting, 0.02-fold shrinkage rate and three-way interactions) to determine the relative influence of environmental variables on bacterial community composition (NMDS axis 1).

Network analyses were used to dissect the interrelationship and interaction between bacterial species along the different electrical conductivity (EC) gradients. All pair wise Pearson correlation coefficients were calculated by Mothur (version 1.29.2) for analysis of the networks. The correlation coefficient of Pearson correlation was further filtered with the cut-off as an absolute value of 0.6–0.93. After applying multiple hypothesis correction by the BH method (Benjamini and Hochberg, 1995), edges with adjusted values of p below 0.05 were kept and were further used to improve the veracity of the networks. Interactive networks were visualized by Gephi with a Fruchterman-Reingold layout (Bastian et al., 2009). The average clustering coefficient, average path length, and modularity of the network were calculated (Newman, 2006). We referred to the active species as the species that most strongly interacts with the other species within the networks (Magurran and Henderson, 2003; Montoya et al., 2006; Barberán et al., 2012). According to the network analysis, we selected the first 10 hubs under different salinity treatments. Times of iteration were 10,000. The indicator status of OTUs from each of the salinity treatments was assessed, and indicator of a treatment was conducted under the significance threshold of 0.05.

RESULTS

Physical and Chemical Properties for All Collected Samples

To explore the soil factors that affect bacterial community composition, we first survey the physical and chemical properties in over 29 soil samples in different salinities collected from Jilin and Heilongjiang provinces (**Figure 1**). In all the studied sites, soil samples showed differences in pH. One sample from Ha2 had a pH of 8.84, whereas the pH of others was ranged from 9.89 to 10.66. However, the remaining physical and chemical properties, including various ion concentrations (Cl^-, Na^+, SO_4^{2-}, CO_3^{2-}, HCO_3^-, Ca^{2+}, Mg^{2+}, and Na^+), SOC, EC, and SAR, varied more substantially than the pH. In particular, the lowest concentration of SO_4^{2-} was 0.480 g/kg from Ha1, and the highest was 21.6 g/kg in Hda3, a difference reached 48 times. The same conspicuous change was observed in EC, the highest EC (Hda4: 10.867 ms/cm) was 26.3 times higher than the lowest value (Jc1: 0.413 ms/cm) (**Supplementary Table S1**).

Bacterial Community Analysis

The widespread change of the physical and chemical properties leads us to ask whether these properties would be associated with bacterial community in the salinity soils. The soil bacterial communities under different salinization levels were compared by sequencing of the bacterial 16S rRNA amplifications. Therefore, we sought to identify environmental factors that contribute to the ecological variation of bacterial community by analyzing characteristics of different soil samples (**Supplementary Table S1**), and creating ABT models to evaluate the relative impact of environmental factors on the bacterial composition NMDS axis 1. The NMDS result showed that the bacterial community structure was significantly separated in three EC levels (PERMANOVA, $F = 0.2248$, $p < 0.001$) (**Figure 2**). Soil EC was the most important driving force for microbial composition (20.83%, **Figure 3**), and the second most influencing factor was Na^+ (14.17%) followed by Cl^-. Given the key role of salinity in shaping the bacterial community, we tentatively divided all samples into three groups depending on the EC level as low (L)-, medium (M)- and high (H)-level treatment. The L, M,

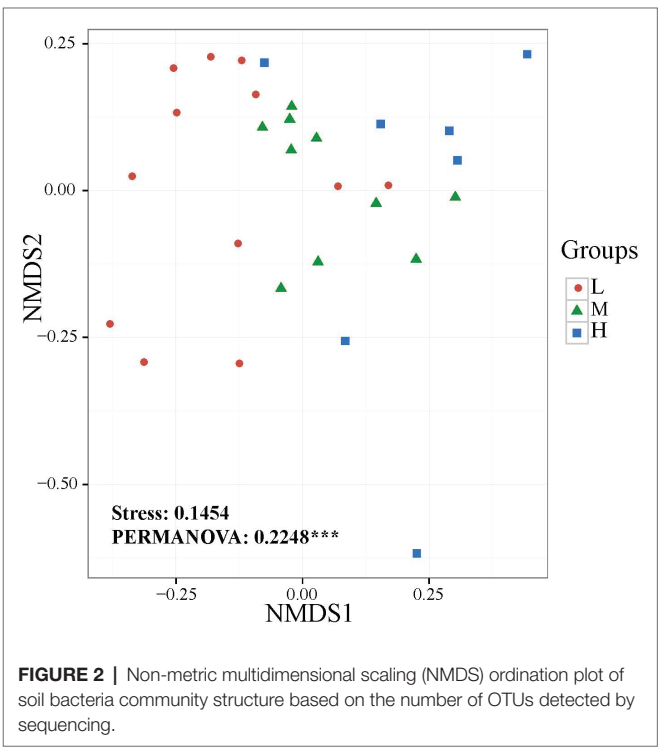

FIGURE 2 | Non-metric multidimensional scaling (NMDS) ordination plot of soil bacteria community structure based on the number of OTUs detected by sequencing.

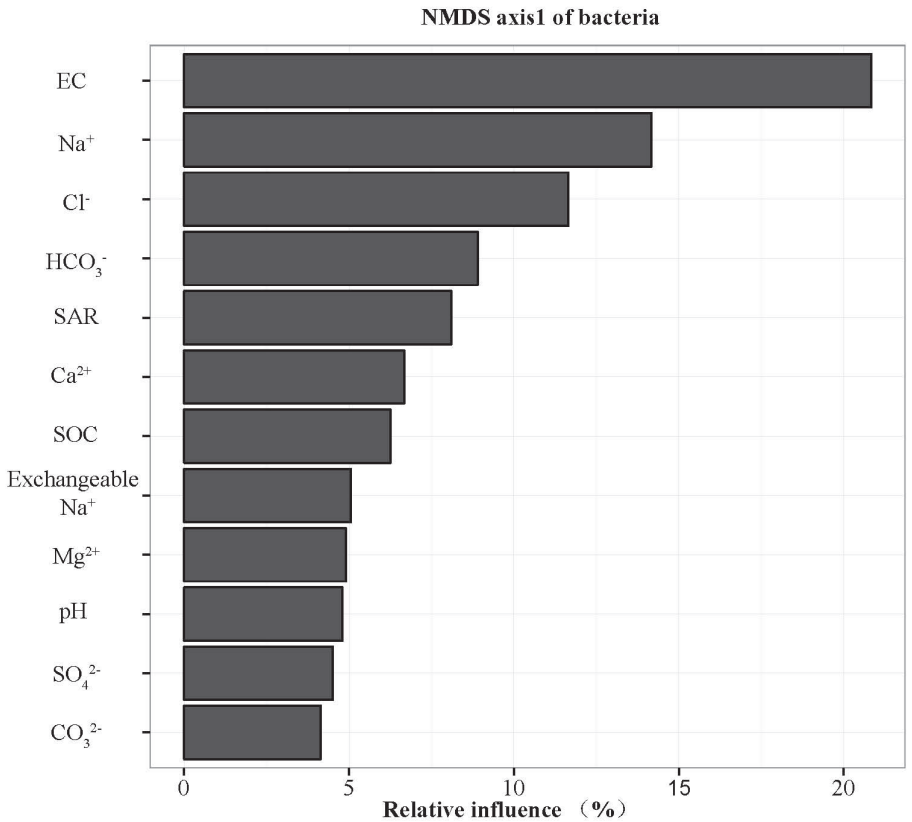

FIGURE 3 | Relative variable importance plot (%) of environmental driving factors for composition of bacteria by ABT models.

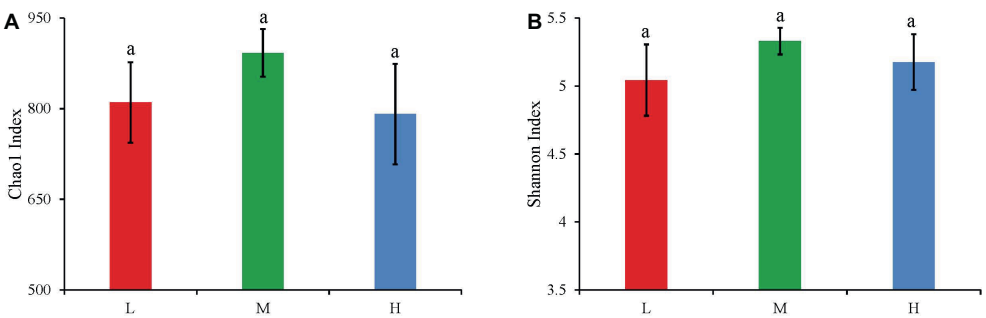

FIGURE 4 | The Chao1 index **(A)** and Shannon index **(B)** of bacteria in three salinity rates. The values are the means of three replicates, error bars represent standard errors (L: $n = 12$; M: $n = 10$; H: $n = 7$), and different letters above the bars indicate significance differences at the $p < 0.05$ level. The L, M, and H treatments represent EC of 0–2, 2–4, and >4 ms/cm, respectively.

and H treatments represent EC of 0–2, 2–4, and >4 ms/cm, respectively. However, there was no significant diffidence in the α-diversity, including Chao1 index and Shannon index, among the three different salinity levels (**Figure 4**).

The Co-occurrence of Bacteria in Three-Salinity Gradient Treatments

Bacterial species usually work corporately to regulate soil properties. Based on the abovementioned statement about dividing the samples into three groups, we divided all samples into three salinity treatments (L, M, and H treatments) and the three treatment groups were used for network analysis. The network parameters of the three salinity levels were showed significant differences (**Figure 5**). In the L treatment, the network had 1,208 nodes and 29,183 edges, and the modularity was 0.72, with 10 modules. For the M treatment, the network presented 982 nodes and 12,819 edges, and the modularity was 0.82, with 9 modules. Finally, 878 nodes and 10,871 edges were found in the H treatment, for which the modularity was 0.74, with 7 modules. Nodes, edges, modularity, and modules all declined with increasing salinity,

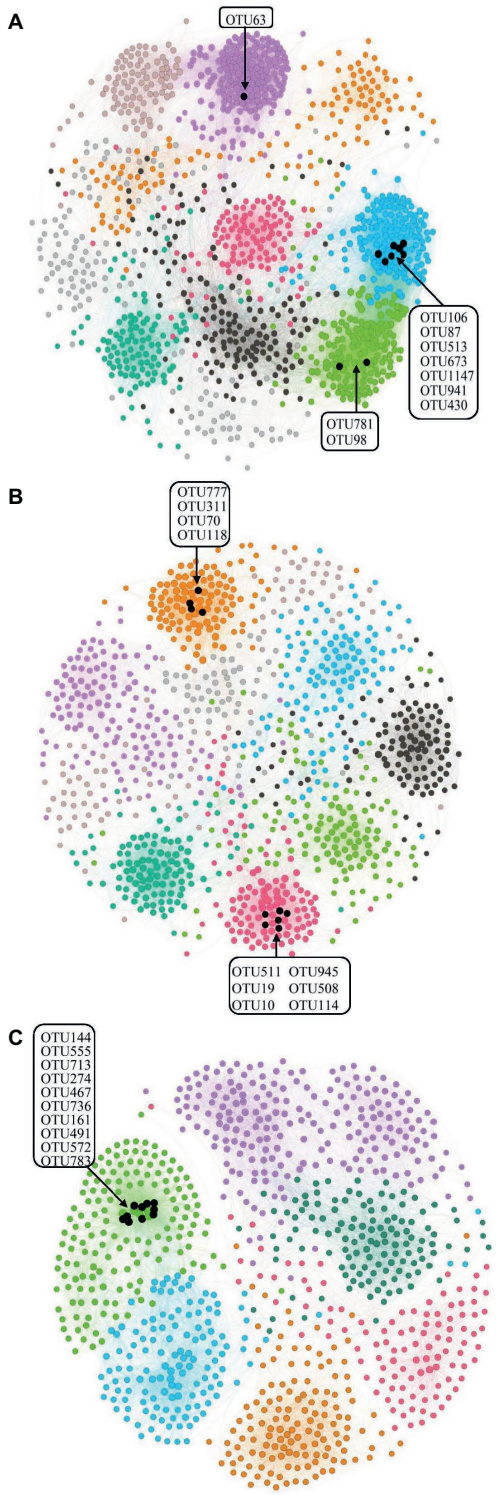

FIGURE 5 | Network analysis of the three different salinity rates treatments based on Pearson correlations. The different color edges belong to different modules. **(A)** Low-salinity treatment (EC: 0–2) with modularity resolution of 0.72 and 10 modules; **(B)** middle-salinity treatment (EC: 2–4) with modularity resolution of 0.82 and 9 modules; and **(C)** high-salinity treatment (EC: >4) with modularity resolution of 0.74 and 7 modules. The black nodes indicate the OTUs belonging to active hubs in the network.

suggesting that a higher concentration of salt led to reducing connectivity of the bacterial network (**Figure 5**).

We defined the 10 nodes with largest number of edges as network hubs, which were active mediators in the bacterial community network (**Supplementary Table S2**). Taken as a whole, *Planctomycetes*, *Proteobacteria*, and *Bacteroidetes* were the three dominant phyla among the different salinity rate treatments, including 63.3% network hubs at the phylum level (**Figure 6**). Half of the network hubs in the L treatment belonged to *Planctomycetes* and *Bacteroidetes*, such as OTU513, OTU106, OTU63, OTU781, and OTU1147. The hubs of M treatment had similar phylogenetic classifications to the L treatment, with half assigned to *Planctomycetes* and *Bacteroidetes*. However, at the highest salinity, more than half hubs were classified as *Proteobacteria*, for instance, OTU713, OTU274, OTU572, OTU555, and OTU783. However, only one indicator (OTU144) belonged to the classification of phyla at low salt concentrations, while a second indicator (OTU777) belonged to the classification of phyla in moderate salt concentrations, which were identified as *Planctomycetes* and *Bacteroidetes*, respectively. As the salt concentration increased, the indicators changed from *Planctomycetes* and *Bacteroidetes* to *Proteobacteria* and *Firmicutes*. **Supplementary Figure S2** shows that the *Rhodospirillaceae*, belonging to a family of *Proteobacteria*, had a strong correlation with the EC (R^2 = 0.3365, p < 0.001). At the higher taxonomic level, the genus of *Marinicella*, assigned to *Proteobacteria*, also had a positive correlation with EC. The results revealed that multiple microbes belonging to *Proteobacteria* are well adapted to the high-salinity environment.

DISCUSSION

Driving Force for Microbial Community Composition in Saline Soils

We collected a total of 29 soil samples from Jilin and Heilongjiang provinces to investigate the distribution characteristics of microbes and reveal the general rule of microbial community composition under different salinities. Saline soils are characterized by high salt concentrations as well as an uneven temporal and spatial water distribution. The high salt concentrations shape the special environment patterns for microbes, causing the microbes in saline soils to vary from those found in the non-saline environment (Canfora et al., 2014). Firstly, we have analyzed the physical and chemical properties among the samples with different salinities. Although pH is a potential important determinant of salinity, we found that pH does not vary substantially among these samples, while the concentration of CO_3^{2-} varies greatly, from 0.01 to 2.82 g/kg. Besides, the concentration of other ions, SOC, EC, and SAR are all different to some extent in the 29 soil samples, encouraging us to investigate whether the variation of physical and chemical properties was associated with salinity.

Identifying environmental factors contributing to the variation of microbial communities is a central aim in ecology. Several studies have shown that environmental factors, including soil pH (Rousk et al., 2010) and trophic status (Hansel et al., 2008;

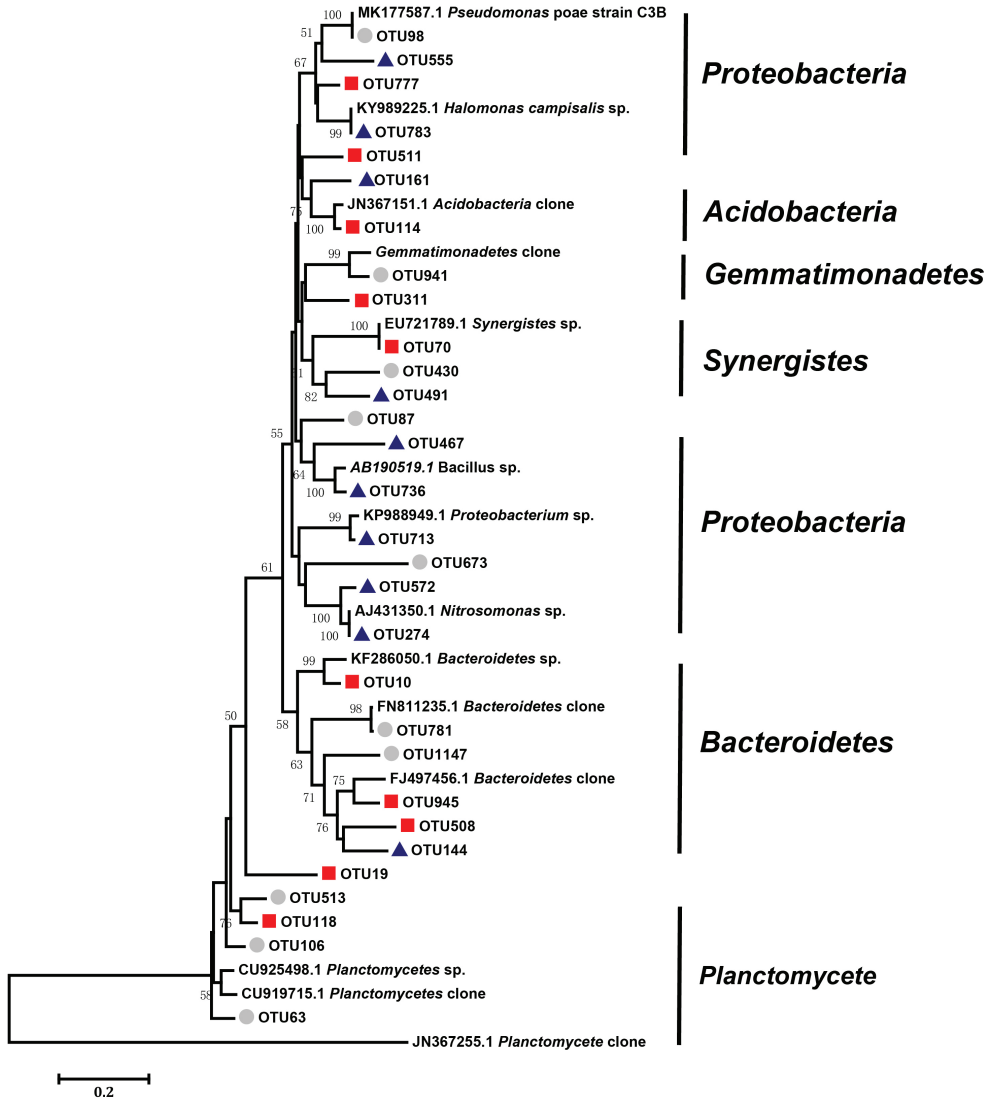

FIGURE 6 | Neighbor-joining analysis of key OTUs forms the networks of three different salinity treatments (low, middle, and high). Bootstrap values higher than 40% are indicated at branch nodes. The scale bar represents 2% nucleic acid sequence divergence. The key OTUs as circles represent OTUs in the low-salinity treatment (EC: 0–2); the key OTUs as squares represent OTUs in the middle-salinity treatment (EC: 2–4); and the key OTUs as triangles represent OTUs in the high-salinity treatment (EC:>4).

Xiong et al., 2010) affect the microbial community. As pH is the most important driver in bacterial community change (Shen et al., 2013; Liu et al., 2014), the explanation of pH for community variation only accounted for 4.80% in this study. ABT models were used to analyze the influence of the environmental factors on the bacterial community composition. EC content of soil was the most important driving force for microbial community composition (**Figure 3**) rather than soil pH. In CCA analysis, the EC value was the highest factor explaining the bacterial community by Monte Carlo permutation (**Supplementary Table S3**). This result showed gradient distribution of salinity along our sampling sites. Thus, EC could be the major factor controlling the differences. It has been reported recently that both low and high EC will affect bacterial growth in high-salinity soils (Rath et al., 2019), the influence of EC might be different among bacterial species, supporting our finding that EC is a causal factor for bacterial community in our salinity soil samples.

A previous study has shown that bacterial composition was associated with pH changes in both acidic soils and alkaline lake sediment (Xiong et al., 2012). While the current study shows that pH is unlikely to be the principal contributor to the considerable microbial community changes according to the ABT model. It should be noted that effect of pH in shaping bacterial communities is affected by local features. It is well known that salinization consists of salt accumulation by one or more natural processes including high salt content of the parent material or in groundwater, and human interventions

such as inappropriate irrigation practices or inappropriate use of fertilization regimes. However, no matter which way, Na^+ is a type of salt, which is the main component. For alkaliphilic bacterial taxa, the Na^+ could serve as important proton replacement to cope with the high external pH (Canfora et al., 2014). As the relative abundance of these microbes are associated with the carbon mineralization rate in the soil (Fierer et al., 2007), we speculate the changes of dominant taxa that occur across the salinity gradient probably play a role in regulating ecosystem functions.

Changes in Diversity and Structure of Microbial Community in Saline Soils

In general, soil microbial diversity changes distinctly in response to environmental variations (Liu and Kang, 2014). However, in this study, the α-diversity did not change remarkably, whereas there was significant variation in β-diversity under the different salinity. Moreover, some studies have reported that α-diversity and β-diversity did not change simultaneously β-diversity transforms prior to α-diversity (Van Diepen et al., 2011; Xiong et al., 2012; Zhu et al., 2016). Furthermore, some researchers have suggested that the change of community structure (β-diversity) was typically correlated with shifts in functional behavior (Van Diepen et al., 2011; Fierer et al., 2013; Griffiths and Philippot, 2013). We speculated that the function responds sensitively and rapidly in the environment, which shows through β-diversity rather than α-diversity. In summary, these results suggest that the microbial community functions and structure respond initially to the environment variations, and then it takes a longer time for microbial α-diversity to change in the saline-alkali soils.

It has been a great challenge to dissect the association between microbial community structure and soil ecosystem. In this study, we used co-occurrence network analysis based on correlation to thoroughly dissect the microbial associations under salinity (Barberán et al., 2012; Faust and Raes, 2012; Friedman and Alm, 2012). Network analyses were conducted to reveal positive and negative interactions among different OTUs. A microbial community in the low-salinity soil was found to have a network with higher connectivity, suggesting more operational community with a greater number of functionally interrelated members (10 major modules) compared to the microbial community network in the high-salinity soil (7 major modules) (**Figure 5**). Given that the highly connected microbes within a module that co-occur might share similar ecological characteristics within communities (Chaffron et al., 2010; Williams et al., 2014; Yao et al., 2014), our results suggest that the high-salinity soil could harbor less ecologically similar functional groups.

Another important benefit of network analysis in microbial ecology studies is the ability to identify central organisms in maintaining soil ecosystems, according to the network theory, the "hub" species, as hotspots of connections in the microbial network, are the most important mediators for the complicated interactions among different species constituting the soil ecosystem (Montoya et al., 2006). According to the method described by the previous study (Zhu et al., 2018), 10 nodes with the most edges were defined as network hubs. *Planctomycetes*, *Proteobacteria*, and *Bacteroidetes* were the predominant phyla occupying 63.3% of the network hubs among the different salinity treatments (**Figure 6**). Ma and Gong (2013) reported that six phyla (*Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, and *Bacteroidetes*) contained 90% of the bacterial sequences in saline soils; here, we detected five out of the six major phyla, with the exception for *Acidobacteria*. *Proteobacteria*, one common bacterial taxa in saline soil identified by a previous study (Valenzuela-Encinas et al., 2009), was the common denominator in our experimental sites, being especially dominant in highly saline soils, with 50% frequency in the indicators. In addition, *Proteobacteria* was reported as "salinity related" in a previous study (Yang et al., 2018), our results confirmed this finding, as *Rhodospirillaceae* and *Marinicella*, assigned to *Proteobacteria*, had good correlation with salt concentration. In addition, *Firmicutes* can also be considered special indicators specifically for the high salinity rate soil, which was absent in various hyper saline environments in previous studies (Demergasso et al., 2004; Ramette, 2007). *Bacillus* stands out among the prevailing genera assigned to *Firmicutes*, as it has shown to be an important resource for exploring halophilic enzymes and metabolic pathways for pollutant remediation in saline soil (Liszka et al., 2012). Species within *Proteobacteria* and *Firmicutes* may be good indicators, reflecting changes of the main microbial groups in saline-alkali soil. Although other studies have reported *Gemmatimonadetes* and *Bacteroidetes* to be an important participant in biogeochemical transformations in soils under salinity (Fierer et al., 2012; Ma and Gong, 2013), they were not detected in the high salinity soils of the current study. Different regions form different ecological environments, resulting in various microbial compositions. Therefore, our result suggests the requirement of future study on a wide range of spatial scales.

Our examination on the variability of the microbial community in saline soils successfully revealed microbial community subdivision across micro-environmental gradients. We expect future studies using metagenome sequencing data could identify similar patterns of bacterial composition variation at finer taxonomic resolution.

CONCLUSION

Taken together, this study presents an attempt to explore bacterial composition in saline-alkali soils across Jilin and Heilongjiang provinces. We show that bacterial β-diversity and community structure correlate with the salt gradient. We demonstrate that EC, instead of pH predicts bacterial community structure in saline-alkaline soils. Microbes belonging to the phyla *Proteobacteria* and *Firmicutes* were predominant and may be good groups of indicator species, reflecting changes of the main microbial groups in saline-alkali soil. Our results revealed local geochemical features as driving force of bacterial composition in the soil, whereas the EC as a key dominant factor in regulating microbial composition at a regional spatial scale. Correlating population of microbes with environmental parameters could facilitate reconstructing the formation of bacterial communities under specific environmental conditions like salinity. In this

study, we provide a framework for future research to deeply analyze microbial composition in extreme environments.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the NCBI Sequence Read Archive (SRA) database/SRP172399.

AUTHOR CONTRIBUTIONS

DW and FC contributed to the conception of the study. SW and LS contributed significantly to analysis and manuscript preparation. WL, XH, WZ, and JB performed the collection of soil samples and data analyses. NL revised the manuscript. LC played an important role in interpreting the results. CZ helped perform the analysis with constructive discussions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.02902/full#supplementary-material

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Sphingobacterium sp. BHU-AV3 Induces Salt Tolerance in Tomato by Enhancing Antioxidant Activities and Energy Metabolism

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Salt tolerant bacteria can be helpful in improving a plant's tolerance to salinity. Although plant–bacteria interactions in response to salt stress have been characterized, the precise molecular mechanisms by which bacterial inoculation alleviates salt stress in plants are still poorly explored. In the present study, we aimed to determine the role of a salt-tolerant plant growth-promoting rhizobacteria (PGPR) *Sphingobacterium* BHU-AV3 for improving salt tolerance in tomato through investigating the physiological responses of tomato roots and leaves under salinity stress. Tomato plants inoculated with BHU-AV3 and challenged with 200 mM NaCl exhibited less senescence, positively correlated with the maintenance of ion balance, lowered reactive oxygen species (ROS), and increased proline content compared to the non-inoculated plants. BHU-AV3-inoculated plant leaves were less affected by oxidative stress, as evident from a reduction in superoxide contents, cell death, and lipid peroxidation. The reduction in ROS level was associated with the increased antioxidant enzyme activities along with multiple-isoform expression [peroxidase (POD), polyphenol oxidase (PPO), and superoxide dismutase (SOD)] in plant roots. Additionally, BHU-AV3 inoculation induced the expression of proteins involved in (i) energy production [ATP synthase], (ii) carbohydrate metabolism (enolase), (iii) thiamine biosynthesis protein, (iv) translation protein (elongation factor 1 alpha), and the antioxidant defense system (catalase) in tomato roots. These findings have provided insight into the molecular mechanisms of bacteria-mediated alleviation of salt stress in plants. From the study, we can conclude that BHU-AV3 inoculation effectively induces antioxidant systems and energy metabolism in tomato roots, which leads to whole plant protection during salt stress through induced systemic tolerance.

Keywords: antioxidants, proteins, salt stress, *Sphingobacterium*, tomato

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INTRODUCTION

Soil salinity is one of the major abiotic stresses that severely affect seed germination rate, plant growth, and productivity. Worldwide, around 20% of cultivated land and almost 50% of irrigated land is affected by salt (Machado and Serralheiro, 2017). Soil salinity restricts plant growth via osmotic and ionic stress. For instance, soluble salts present in the soil induce osmotic stress in roots, which hinders water acquisition in plant cells, and at high concentrations of salts, accumulation

of sodium and chloride ions in plant cells cause ionic stress and can lead to nutrient deficiency. In addition, ionic stresses disturb the equilibrium of reactive oxygen species (ROS) in plant cells, which directly causes oxidative stress (Vaishnav et al., 2016a,b).

Salinity response in plants is a complex mechanism involving regulation of both physiological and molecular processes. The modification or activation of different metabolic processes during salt stress is controlled by the plant's innate immunity and the habitat-imposed immunity provided by associated microbes (Vaishnav et al., 2019). A group of plant-beneficial microbes are known as plant growth-promoting rhizobacteria (PGPR), and they provide various benefits to the plants under both biotic and abiotic stresses. Application of salt-tolerant PGPR is a sustainable and cost-effective solution for cultivation in saline soil. Salt-tolerant PGPR can survive in saline soil and help plants tolerate salinity by several synergistic mechanisms, i.e., increasing osmolyte accumulation, phytohormone signaling, nutrient uptake, and antioxidant capacity (Sharma et al., 2016). A positive outcome of a plant's interaction with beneficial microbes during salt stress is a promising way to improve crop productivity in saline soils. However, there is a need to understand the mechanisms of beneficial interaction between plant and microbes to alleviate stress.

How plant-associated microbes modulate host physiology to withstand stress conditions is a topic of interest. Recently, some attempts have been made to understand plant responses to salt stress with microbial inoculation, and these suggest the involvement of antioxidative machinery, osmolyte accumulation, and phytohormone signaling (Cao et al., 2017; Chanratana et al., 2019; Orozco-Mosqueda et al., 2019; Vimal et al., 2019; Yoo et al., 2019). However, targeting a single response and single plant tissue will miss the broader effect of plant–microbe interaction and also limit our understanding of stress signaling. In this context, advanced molecular tools and technologies will facilitate the characterization of plant–microbe interactions and may expand our understanding. Protein and gene transcript studies can provide meaningful insights for describing the interactions of plants with beneficial microbes under stress conditions (Cheng et al., 2012; Singh et al., 2017; Jaemsaeng et al., 2018; Kumar et al., 2019). Investigating and analyzing the plant's root protein can provide a clear picture of the changes occurring at the time of microbe interaction. Only a few reports are available on combinational effects of beneficial microbes and salt stress through protein study, and these show that most of the expressed proteins in plants under salinity stress are related to transcription and translation factors, photosynthesis, lignin biosynthesis, and antioxidative and defense proteins (Cheng et al., 2012; Vaishnav et al., 2015; Singh et al., 2017). However, plant growth response to microbial application may vary with the experimental conditions, microbial diversity, and plant functional groups (Afroz et al., 2013).

Tomato is a vegetable crops grown all over the world for its nutritional value. Tomato is highly sensitive to salinity stress, which affects germination, vegetative growth, fruit set, development, ripening of fruit, and fruit quality. Previously, some attempts have been made to induce salt tolerance in tomato by inoculating other PGPR strains (Mayak et al., 2004; Tank and Saraf, 2010; Palaniyandi et al., 2014; Cordero et al., 2018). However, the molecular mechanism of PGPR-mediated salt tolerance in tomato plants is poorly explored. Therefore, the purpose of the present work is to (i) examine the plant growth-promoting properties of the strain *Sphingobacterium* sp. BHU-AV3; (ii) document the changes in tomato root proteins in response to salt stress when inoculated with the salt-tolerant strain BHU-AV3; (iii) compare the root and leaf tissues for contents of ions, proline, and different isoforms of antioxidative enzymes induced during salt stress upon inoculation of the strain BHU-AV3. This study extends our understanding of microbially mediated systemic tolerance in plants and motivates us to use microbial inoculants for the reclamation of salt lands.

MATERIALS AND METHODS

Isolation, Identification, and NaCl Tolerance of the Bacterial Strain

BHU-AV3 was isolated from an agricultural field of Banaras Hindu University, Varanasi, India, on nutrient agar medium (NA) supplemented with 2% sodium chloride (NaCl). The molecular characterization of the BHU-AV3 bacterium was done by 16S rRNA gene sequencing using universal primers 27F (5′AGAGTTTGATCCTGGCTCAG 3′) and 1492R (3′ACGGCTACCTTGTTACGACTT 5′). The sequence was analyzed by Nucleotide BLAST (BLASTn) and further verified through the EzTaxon database. The salt tolerance ability of the BHU-AV3 bacterium was determined through inoculation in nutrient broth medium (NB) supplemented with 0.1–0.85 M NaCl and incubated at 28 ± 2°C.

Determination of Plant Growth-Promoting (PGP) Activities

One-day-old bacterial culture (1.1×10^8 CFU) was used for the detection of PGP activities. Phosphate solubilization activity was determined by the modified method of Mehta and Nautiyal (2001). Bacterial culture was grown in NBRIP-BPB medium supplemented with phenol red dye (0.001%) and incubated at 28 ± 2°C for 3–4 days. The change in medium color from red to yellow indicated Pi solubilization.

Siderophore production was estimated on chrome azurol S agar (CAS) medium. BHU-AV3 culture was inoculated on CAS agar plates and incubated at 28 ± 2°C for 72 h. After incubation, the formation of orange halos around bacterial colonies represents a positive result for siderophore production (Schwyn and Neilands, 1987).

Indole-3-acetic acid (IAA) production by BHU-AV3 bacterium was determined as per the method of Gordon and Weber (1951). One-day-old bacterial culture was inoculated in NB medium containing Tryptophan (200 μg/mL) and incubated for 72 h with shaking (120 rpm) at 28 ± 2°C. Thereafter, complete culture was centrifuged and the supernatant collected. A volume of 1 mL of supernatant was mixed with 3 mL of Salkowski's reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35%

$HClO_4$) and kept in the dark for 30 min. The development of a pink color represents a positive result for IAA production.

Plant Growth Assay With BHU-AV3 Inoculation

A loopful bacterial culture (24 h old) was dissolved aseptically in phosphate buffer saline (pH 7.4) and maintained at 10^8 CFU mL^{-1}. The bacterial cells were collected and resuspended in 1% of sterilized carboxymethyl cellulose (CMC) solution. Tomato seeds (cv. Kashi amrit) were surface-sterilized by 1.0% NaOCl for 1 min followed by 70% ethanol for 3 min and then rinsed with sterile distilled water three times. Sterilized seeds were dried on pre-sterilized blotting paper. The dried seeds were soaked into a bacterial suspension for priming, while only CMC-treated seeds served as a control. The primed seeds were kept in an incubator at 28 ± 2°C for 24 h. After incubation, seeds were sown in earthen pots filled with sterile soil. There were four treatment groups, i.e., (1) control, (2) salt (NaCl), (3) bacterial (BHU-AV3) inoculation, and (4) bacterial inoculation + salt. The complete experiment had a randomized block design, where three replications for each treatment were present under controlled conditions. Seven days after germination, salt treatment was given by irrigation with 50 mM NaCl for 4 days in the respective treatments. Seedlings were harvested at 21 days and evaluated for plant growth parameters.

Evaluation of Physiological Responses of Plants to Salt Stress

Different physiological traits were measured to quantify the impact of salinity on tomato plants, as reported by Negrão et al. (2017).

Total free proline content and ion measurement were performed in root and leaf tissues. Proline content was measured according to the method of Bates et al. (1973). The chromophore-containing toluene was measured at 520 nm. The amount of proline was determined in μg/g fresh weight (FW) from a standard curve.

For Na^+ and K^+ ion measurement, samples were dried in a hot air oven at 60°C for 3–4 days and then ground into a fine powder. A 1-g dry powder sample was extracted with 5 ml of HNO_3 at 37°C overnight. The filtered solution was diluted by distilled water and used for flame photometer analysis. Ion contents were measured in mg/g dry weight (DW).

Chlorophyll estimation was performed by the method of Moran and Porath (1980). The total chlorophyll content was quantified using the following formula, and the amount was expressed as μg Chlorophyll/g FW.

$$\text{Chlorophyll content} = [(ABS_{664} \times 7.04) + (ABS_{647} \times 20.27)] \times 5/\text{sample weight(g)}$$

The relative water content (RWC) was measured in plant leaves according to the protocol of Sade et al. (2015). Leaf samples were immediately placed in polythene bags after plucking to minimize water loss through transpiration. Samples were weighed to measure fresh weight (FW) and then kept in distilled water for 8 h. The leaf samples were placed between blotting papers to absorb excess water and then again weighed for turgid weight (TW). The samples were then oven-dried (60°C for 48 h) and again weighed to obtain dry weight (DW). The RWC was calculated by the following formula:

$$\text{RWC}(\%) = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$$

In situ Detection of ROS, Lipid Peroxidation, and Cell Death

In situ detection was estimated by the modified method of Ray et al. (2016). ROS production in the form of superoxide radicals was detected in tomato plant leaves. Leaves were kept in 25 mL of nitroblue tetrazolium (NBT) solution (10 μg/mL NBT dissolved in 50 mM phosphate buffer, pH 7.6) for 3 h in the dark. For lipid peroxidation, leaves were stained with Schiff's reagent for 1 h, and aldehyde formation was detected as the end product of lipid peroxidation. Cell death was analyzed by immersing leaves in 0.1% Evan's blue solution for 15 min. After staining, leaves were boiled in 95% (v/v) ethanol for 30 min in a water bath. Thereafter, leaves were kept in 40% glycerol before examination.

Antioxidant Enzyme Activity Assays and Zymography

Antioxidant enzyme activities were performed in root and leaf tissues of tomato plant. The crude protein was extracted according to the method of Qureshi et al. (2013). One gram of tissue was homogenized in 4 mL of 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetate (EDTA), 2% polyvinylpyrrolidone (PVP), and 1 mM phenylmethylsulfonyl fluoride (PMSF). The crude extract was centrifuged at 15,000 × *g* for 20 min at 4°C, and the supernatant was then collected; this was used as an enzyme extract. Total protein content was estimated according to the Bradford (1976) method.

Determination of peroxidase (POD) enzyme activity and zymography of its isoforms were performed as per the method of Kumari et al. (2015). POD activity was expressed in U/mg protein. POD isoforms were identified by native-polyacrylamide gel electrophoresis (PAGE) on 10% acrylamide gel. Polyphenol oxidase (PPO) activity was quantified as per the method of Weisany et al. (2012), and activity was expressed in U/mg protein. PPO isoforms were identified according to the method of Ramamoorthy et al. (2002). The native-PAGE gel was immersed in 0.1% p-phenylene diamine for 30 min. Thereafter, the solution was discarded and the gel was exposed to 20 mM catechol.

Superoxide dismutase (SOD) activity was determined according to the method of Qureshi et al. (2013), in which photo-reduction of nitroblue tetrazolium chloride (NBT) was measured at 560 nm. A 50% photo-reduction of the NBT was measured as one unit of SOD enzyme, and the activity was expressed in U/mg protein. Zymography of SOD isoforms was performed as per the method of Kumari et al. (2015).

Extraction of Total Proteins and 1D SDS-PAGE, Trypsin Digestion, and In-Gel Extraction of Peptides for MALDI

Total proteins were extracted from root tissue by the phenol extraction method (Isaacson et al., 2006). The soluble fraction of extracted proteins was subjected to separation on 10% polyacrylamide gel using SDS-PAGE (Laemmli, 1970). The differentially expressed protein bands in bacteria-inoculated salt-stressed plants (T4) were observed and selected for trypsin digestion. Bands were excised from the gel and further processed for trypsin digestion as per the method of Mahajan et al. (2014). Peptides were extracted and submitted for peptide mass fingerprint (PMF) using a MALDI-TOF mass spectrometer. The generated data were searched for on the Swiss-Prot database using the MASCOT search engine (Matrix Science, London, United Kingdom).

Expression Analysis of the m-RNA Genes of Selected Proteins Using qRT PCR

Root tissues were crushed in liquid nitrogen immediately after harvesting, and RNA was extracted with Trizol (Merck GeNei). The extracted RNA was treated with DNase, which was further used for cDNA synthesis. RNA was reverse transcribed by using Avian Myeloblastosis Virus (AMV) reverse transcriptase and Oligo $(dT)_{18}$ primer. qRT–PCR was performed in a real-time PCR system (Bio-Rad Laboratories) using Eva Green SYBER Master Mix. The primers of selected genes were designed through IDT software; details are given in **Table 1**. The delta–delta CT method was applied to compare relative expression. The actin gene was chosen for normalization.

Statistical Analysis

The data obtained were subjected for significance analysis through analysis of variance (ANOVA) testing. Then, *post hoc* testing was performed using the DMRT test. All analysis described was performed in SPSS software version 11.5.

RESULTS

Molecular Characterization and PGP Attributes of BHU-AV3 Isolate

As part of a program to discover salt-tolerant PGPR, BHU-AV3 isolate was recovered from an agricultural field. The isolate is most closely related to *Sphingobacterium*, with 99% identity based on 16S rDNA gene sequence analysis. The sequence data of BHU-AV3 has been deposited to the GenBank database with the accession number MK588751 (**Figure 1**). Screening of the PGP traits of strain BHU-AV3 revealed characteristics of phosphate solubilization, siderophore production, and indole-3-acetic acid (IAA) production (**Figure 2**). The strain BHU-AV3 is moderately halophilic, as it tolerates NaCl up to 4% (w/v).

Effect of BHU-AV3 on Plant Growth Parameters Under Salt Stress

Salt stress caused a significant negative effect on plant growth parameters. All growth parameters were reduced under salt stress as compared to the control condition. However, BHU-AV3 was able to protect plants from severe damage due to salt toxicity (**Figure 3**). BHU-AV3-inoculated plants (T4) registered significantly enhanced shoot and root length (44 and 51.3%) as compared to T2 treatment. Similarly, bacterial inoculation significantly enhanced plant biomass as compared to un-inoculated plants under salt stress. T4 treatment showed an increase of 54% biomass compared to the un-inoculated control plants (T2) under salt stress (**Table 2**). Under non-salt conditions (T1 and T3), both treatments showed similar trends in plant growth parameters.

Effect of BHU-AV3 on Physiological Response to Salt Stress

A significant decrease in chlorophyll content was observed under salt stress. However, the BHU-AV3-inoculated plants (T4) had significantly higher (56%) chlorophyll content compared to un-inoculated plants (T2). The relative water content in tomato plants was remarkably reduced under saline conditions. Nevertheless, T4-treatment plants accumulated more water content (91%) compared to T2-treatment plants (**Table 2**). In addition, in terms of ion contents, Na^+ content was significantly increased under salt stress conditions. In T2 treatment, plant roots had 190% higher Na^+ content with respect to non-salt treatment (T1), whereas BHU-AV3-inoculated plant root (T4) had only 125% higher Na^+ content than non-salt treatment (T3). On the other hand, the K^+ content was significantly decreased in both salt treatments (T2 and T4). However, in T4 treatment, the plants exhibited a smaller decrease in K^+ content (29%) compared to T2 plants (50%) from their respective controls. Hence, a 5-fold increment in Na^+/K^+ was recorded in T2 plant roots, whereas only a 2.2-fold increment was found in T4 plant roots compared to T1- and T3-treatment plants,

TABLE 1 | Details of primers used in the qRT-PCR study.

Gene name and accession number	Forward primer	Reverse primer
Catalase (*CAT*) (NM_001247898)	TCGCGATGGTGCTATGAACA	TGTCTTGCCTGTCAGGTTCC
Enolase *(PGH1)* (NM_001247151)	GGCAGGTTGGGGTGTAATGA	CAATCTCAACACTTGGAACTGC
ATP synthase (KY887588)	GGTGAACGTACTCGGGAAGG	TGCTTGGACGAAACGGAAGA
Thiamine (*ThiC*) (NM_001317405)	CTTTCCGGGGATGAACCACA	ATTGGCTCCAACTCAGGGTG
Elongation factor-1α (*EF-1α*) (XM_004240531)	GTGCATTTGATGAGCACGGA	AGCAGTGACCAAGACTGTGT
Actin (NM_001330119)	TGGCTCCTAGCAGCATGAAG	ACACTACAATTGCATCTCTGGTC

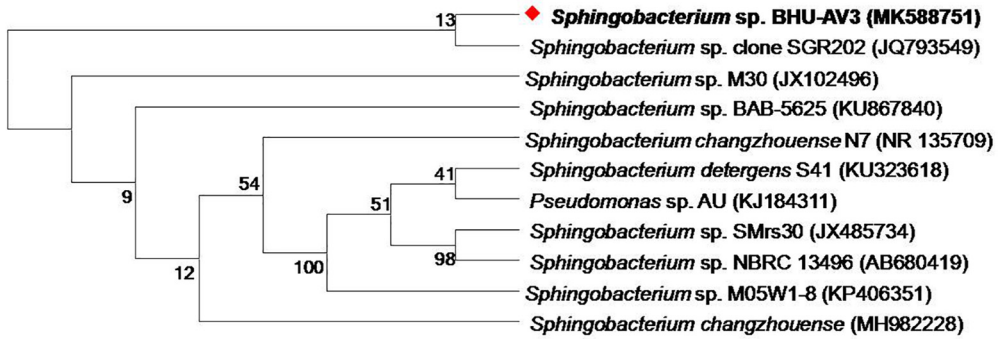

FIGURE 1 | Phylogenetic tree constructed by using neighbor-joining analysis between BHU-AV3 isolate and reference bacterial sequences retrieved from GenBank based on 16S rDNA sequences. The tree shows the phylogenetic position of BHU-AV3 isolate among the genus of *Sphingobacterium*. GenBank accession numbers are given in parentheses. Numbers at nodes indicate percentages of bootstrap support, based on 1,000 resample datasets. Evolutionary analyses were conducted in MEGA7.

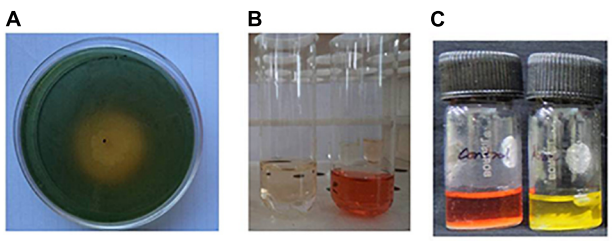

FIGURE 2 | Qualitative estimation of plant growth-promoting properties of BHU-AV3 isolate. **(A)** Siderophore assay – yellow zone indicates positive result; **(B)** IAA production assay – development of pink color indicates positive result; **(C)** Pi solubilization test – development of red to yellow color indicates positive result.

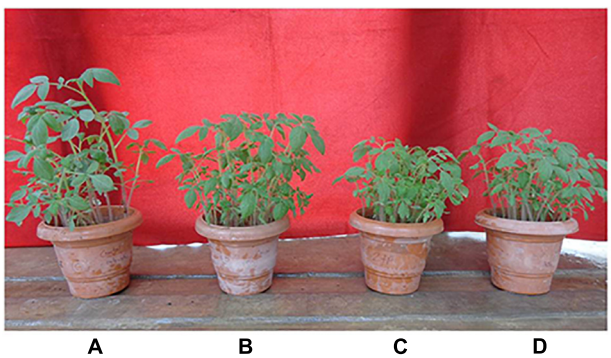

FIGURE 3 | Effect of bacterial (BHU-AV3) inoculation on tomato plant growth under salt stress. **(A)** Control plants; **(B)** bacteria (BHU-AV3)-inoculated plants; **(C)** salt (NaCl) treatment; **(D)** bacterial inoculation + salt.

respectively. T4-treatment plant leaves showed significantly less accumulation of Na^+ content (36%) as compared to T2-treatment plant leaves (**Table 3**). Proline content was increased in both salt stress treatments (T2 and T4). Remarkably and in contrast to T2 treatment, T4-treatment plant roots had an increment (40%) in proline content. However, leaf tissue accumulated less proline content in T4 (27%) as compared to T2 plants (**Table 3**). These results suggest that strain BHU-AV3 relieved the negative effect of Na^+ ion toxicity on tomato plant physiology under salt stress. All of these physiological parameters were observed at constant levels in non-salt stress treatments (T1 and T3).

Effect of BHU-AV3 on Antioxidant Enzyme Activity in Response to Salt Stress

Comparative study of antioxidant enzyme activities was performed in root and leaf tissues with different treatments. Salt stress generally stimulates the antioxidant system throughout the plant. BHU-AV3 strain-inoculated roots (T4) showed higher POD (50%), SOD (29%), and PPO (16%) as compared to T2-treated plant roots. In leaf tissue, un-inoculated plants (T2) displayed significant increases in all enzymatic activities compared to T4 plants. Under non-salt conditions, both treatments (T1 and T3) maintained the same level of POD, SOD, and PPO activities (**Figures 4–6**). Further, the impact of salt stress on isozyme profiles was also explored. Native PAGE coupled with activity localization showed multiple isoforms of POD, PPO, and SOD in roots, while in leaves, faint bands were observed. Examination of POD isozyme profiles in the roots revealed five isoforms (POD1-5), and the activity of POD3, POD4, and POD5 was higher in T4 plants compared to T2 plants. In leaves, only one isoform, POD5, was detected in all treatments and exhibited higher expression in T4 plants (**Figure 7**). In the case of PPO activity, a total of six isoforms (PPO1-6) were expressed. Of these, PPO1 was expressed in root tissue only, whereas PPO5 and PPO6 were expressed only in leaves. In roots, the expressions of PPO2, PPO3, and PPO4 isozymes were found to be highest in T4 treatment compared to other treatments. In leaves, PPO2–PPO6 isoforms were highly expressed in the T4 treatment compared to the others (**Figure 8**). As shown in **Figure 9**, only two prominent SOD bands were detected in roots, while no band was found in leaves. The expression of SOD1 was

TABLE 2 | Effect of bacterial (BHU-AV3) inoculation on tomato plant growth parameters under salt stress conditions.

Treatments	Shoot length (cm)	Root length (cm)	RWC (%)	Chlorophyll (μg/g FW)	Biomass content (g)
T1	18.6 ± 1.2^{a}	6.7 ± 0.8^{a}	85.6 ± 3.7^{a}	80.9 ± 4.3^{a}	0.28 ± 0.03^{a}
T2	9.0 ± 0.6^{c}	3.4 ± 0.4^{c}	34.0 ± 2.6^{c}	45.0 ± 3.2^{c}	0.13 ± 0.04^{c}
T3	16.5 ± 0.9^{a}	7.8 ± 0.6^{a}	88.6 ± 3.1^{a}	84.6 ± 2.9^{a}	0.31 ± 0.03^{a}
T4	13.0 ± 0.7^{b}	5.2 ± 0.7^{b}	65.0 ± 2.9^{b}	70.0 ± 2.0^{b}	0.20 ± 0.02^{b}

T1, control; T2, salt (NaCl); T3, bacterial (BHU-AV3) inoculation; and T4, bacterial inoculation + salt. Values represent the mean ± SD, n = 3. Different superscript letters in the same column are significantly different (p = 0.05, DMRT analysis was carried out).

TABLE 3 | Effect of bacterial (BHU-AV3) inoculation on ion and proline content in different tomato plant tissues under salt stress conditions.

Treatments	Na^+ (mg/g DW)		K^+ (mg/g DW)		Na^+/K^+		Proline (μg/g FW)	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
T1	6.1 ± 0.7^{c}	3.3 ± 0.4^{c}	10.7 ± 0.5^{a}	8.7 ± 0.6^{a}	0.5 ± 0.2^{c}	0.3 ± 0.01^{c}	87.0 ± 3.2^{c}	45.0 ± 2.6^{c}
T2	17.7 ± 1.2^{a}	11.8 ± 0.8^{a}	5.0 ± 0.3^{c}	3.2 ± 0.7^{c}	3.5 ± 0.6^{a}	3.6 ± 0.8^{a}	131.0 ± 4.3^{b}	114.0 ± 4.1^{a}
T3	5.8 ± 0.6^{c}	3.6 ± 0.6^{c}	11.5 ± 1.0^{a}	9.5 ± 0.5^{a}	0.5 ± 0.2^{c}	0.3 ± 0.01^{c}	83.0 ± 2.4^{c}	42.0 ± 3.1^{c}
T4	13.1 ± 1.9^{b}	7.6 ± 1.2^{b}	8.1 ± 0.6b	7.0 ± 0.9b	1.6 ± 0.5^{b}	1.0 ±0.2b	184.0 ± 3.5^{a}	83.0 ±5.2b

T1, control; T2, salt (NaCl); T3, bacterial (BHU-AV3) inoculation; and T4, bacterial inoculation + salt. Values represent the mean ± SD, n = 3. Different superscript letters in the same column are significantly different (p = 0.05, DMRT analysis was carried out).

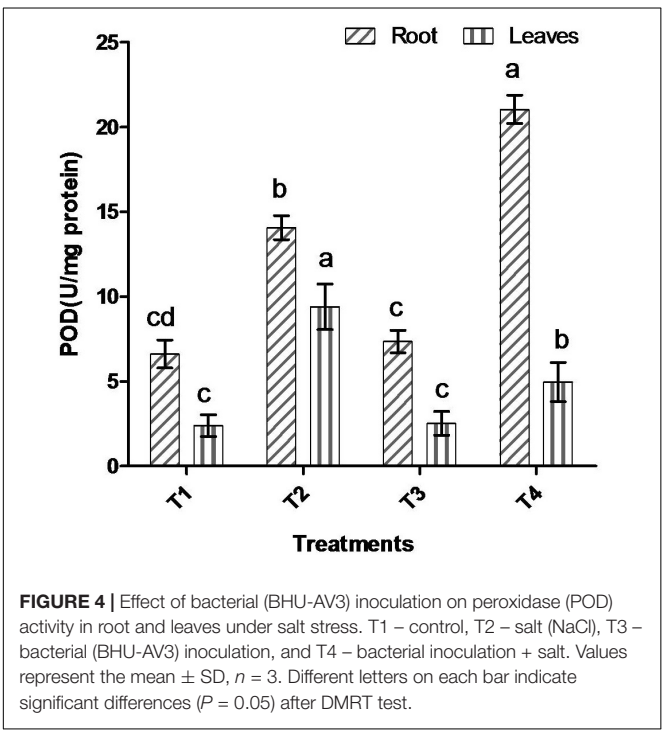

FIGURE 4 | Effect of bacterial (BHU-AV3) inoculation on peroxidase (POD) activity in root and leaves under salt stress. T1 – control, T2 – salt (NaCl), T3 – bacterial (BHU-AV3) inoculation, and T4 – bacterial inoculation + salt. Values represent the mean ± SD, *n* = 3. Different letters on each bar indicate significant differences (*P* = 0.05) after DMRT test.

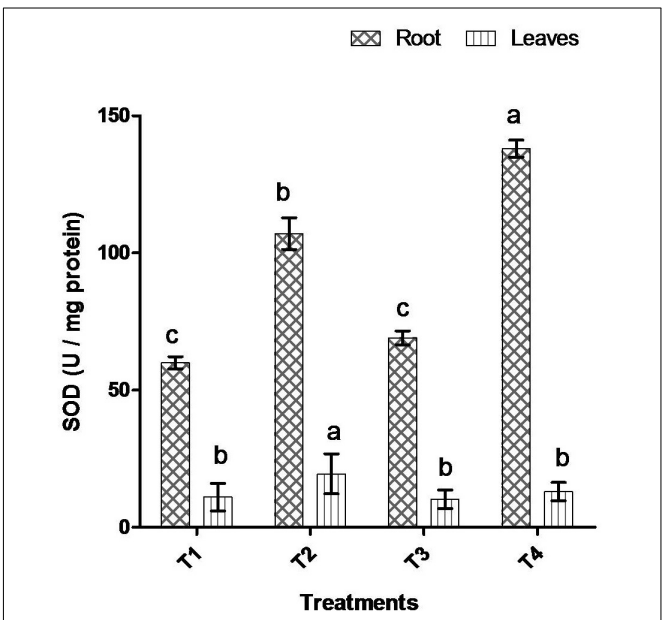

FIGURE 5 | Effect of bacterial (BHU-AV3) inoculation on superoxide (SOD) activity in root and leaves under salt stress. T1 – control, T2 – salt (NaCl), T3 – bacterial (BHU-AV3) inoculation, and T4 – bacterial inoculation + salt. Values represent the mean ± SD, *n* = 3. Different letters on each bar indicate significant differences (*P* = 0.05) after DMRT test.

higher in T4 plants compared to other treatments, while the SOD2 isoform was expressed only in T4 plant root.

Effect of BHU-AV3 on *in situ* ROS Detection and the Effect of ROS on Plant Cells

Superoxides were detected as bluish spots due to formazan formation on the leaf surface. In the presence of salt, leaves had higher staining, indicative of the production of ROS; however, plants inoculated with the strain BHU-AV3 (T4) showed lighter staining than un-inoculated plants (T2). In cell death analysis, un-inoculated plant leaves had more area of necrotic lesions in indigo blue spots compared to inoculated plants under salt stress. Lipid peroxidation was estimated through detection of malondialdehyde contents as pink spots on the leaf surface.

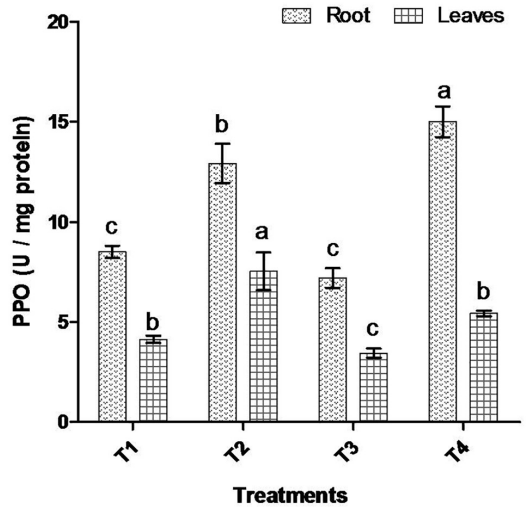

FIGURE 6 | Effect of bacterial (BHU-AV3) inoculation on dismutase polyphenol oxidase (PPO) activity in root and leaves under salt stress. T1 – control, T2 – salt (NaCl), T3 – bacterial (BHU-AV3) inoculation, and T4 – bacterial inoculation + salt. Values represent the mean ± SD, $n = 3$. Different letters on each bar indicate significant differences ($P = 0.05$) after DMRT test.

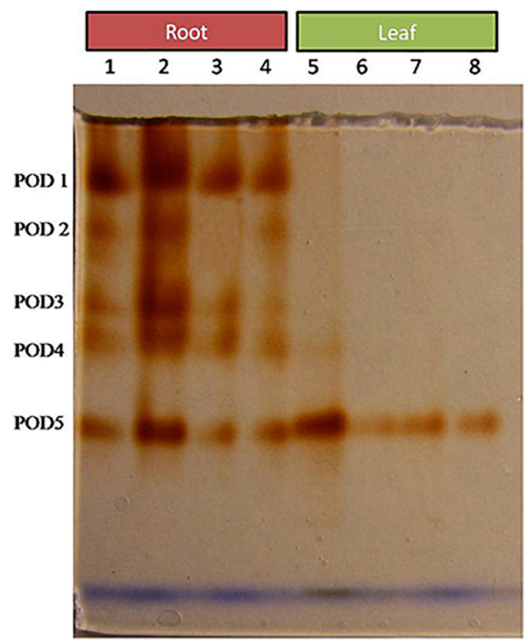

FIGURE 7 | Zymography of POD isoforms expressed in root and leaves upon bacterial (BHU-AV3) inoculation under salt stress. Lanes 1 and 5 – salt treatment; Lanes 2 and 6 – bacterial (BHU-AV3) inoculation + salt; Lanes 3 and 7 – bacterial inoculation; Lanes 5 and 8 – Control. POD 1–5 represents number of isoforms expressed.

T2-treated plant leaves exhibited a higher number of pinkish spots compared to T4-treated plants (**Figure 10**). These results suggest that strain BHU-AV3 reduced ROS content in salt-stressed tomato plants.

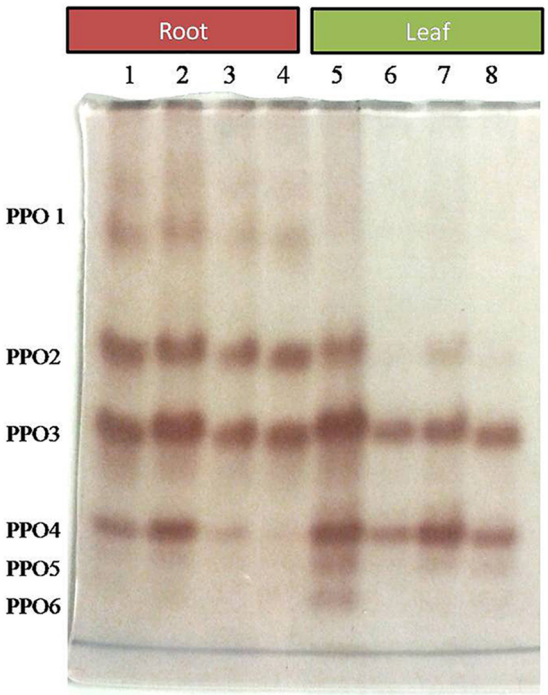

FIGURE 8 | Zymography of PPO isoforms expressed in root and leaves upon bacterial (BHU-AV3) inoculation under salt stress. Lanes 1 and 5 – salt treatment; Lanes 2 and 6 – bacterial (BHU-AV3) inoculation + salt; Lanes 3 and 7 – bacterial inoculation; Lanes 5 and 8 – Control. PPO 1–5 represents number of isoforms expressed.

Effect of BHU-AV3 on Root Protein Profiling in Response to Salt Stress

The differential expression of proteins produced in roots upon inoculation with BHU-AV3 under salt stress was investigated using a non-targeted approach. A total of 11 different protein bands were detected in tomato plant roots. The five highly expressed proteins in the T4 treatment were identified by MALDI-TOF/MS. Proteins were identified based on a high MASCOT score and peptide match (**Table 4**). The proteins expressed in T4 treatments were enolase, involved in the glycolytic pathway, ATP synthase, associated with energy metabolism, thiamine biosynthesis protein, elongation factor 1 alpha (EF1-alpha), involved in protein biosynthesis during the translation process, and catalase, associated with the ROS-scavenging process under stress conditions.

Correlation of Protein Data With Gene Expression Analysis

To correlate the protein data, gene expression analysis through qRT-PCR was performed for all five selected proteins. The mRNA expression of selected proteins was up-regulated in bacterially inoculated plant roots under salt stress (T4) in a similar way as determined by the 1-D PAGE analysis. In T2 treatment, plants exhibited less expression of all the tested mRNA genes compared to T4 plant roots (**Figure 11**).

DISCUSSION

While salt-tolerant PGPRs have received scientific attention due to their application in the reclamation of saline land, the molecular mechanism underpinning PGPR-mediated salt stress alleviation in plants has not yet been investigated systematically. Understanding of the molecular mechanisms involved is essential for developing salt-tolerant crop varieties (Chinnusamy et al., 2005; Vaishnav et al., 2014; Qin et al., 2016). In the present study, experiments were conducted to test the response elicited in tomato plants by inoculation with salt-tolerant BHU-AV3 under salt stress. The challenge by 200 mM NaCl resulted in reduced growth and biomass, imbalance of ions, decreased water content, and production of reactive oxygen species (ROS) in tomato plants. However, tomato plants inoculated with BHU-AV3 exhibited less senescence under 200 mM NaCl stress, positively correlated with the maintenance of ion balance, chlorophyll content, relative water content, and a low ROS level in plant cells.

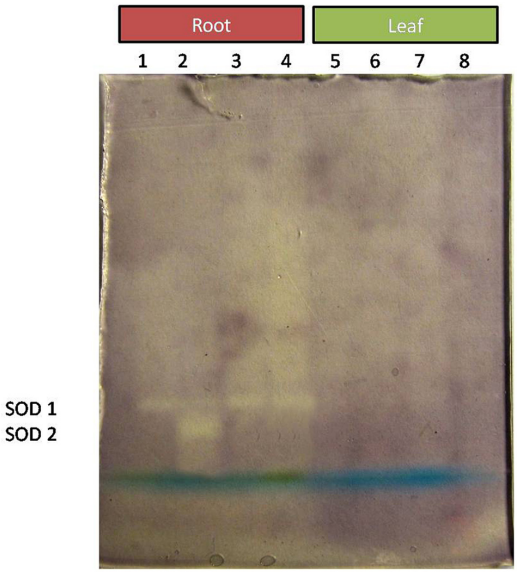

FIGURE 9 | Zymography of SOD isoforms expressed in root and leaves upon bacterial (BHU-AV3) inoculation under salt stress. Lanes 1 and 5 – salt treatment; Lanes 2 and 6 – bacterial (BHU-AV3) inoculation + salt; Lanes 3 and 7 – bacterial inoculation; Lanes 5 and 8 – Control. SOD 1–5 represents number of isoforms expressed.

Phylogenetic analysis of BHU-AV3 isolate using 16S rDNA sequencing revealed a similarity of the isolate with *Sphingobacterium* sp. Further, BHU-AV3 exhibited plant growth-promoting abilities that indicate the potentiality that the BHU-AV3 isolate would promote plant growth under nutrient-limited conditions. *Sphingobacterium* spp. are reported to have beneficial PGP traits, with plant growth-promotion abilities under different stress conditions (Marques et al., 2010; Ahmed et al., 2014; Cardinale et al., 2015; Rolli et al., 2015). In addition, the isolate BHU-AV3 was also found to tolerate salt stress up to 4% NaCl concentration. *Sphingobacterium* spp. are also reported to participate in soil remediation processes (Lodewyckx et al., 2002; Miliute et al., 2015).

BHU-AV3-inoculated plants had a higher biomass content under salt stress compared to un-inoculated plants, which is probably due to IAA production and nutrient solubilization activity of the BHU-AV3 strain in soil. Several other findings

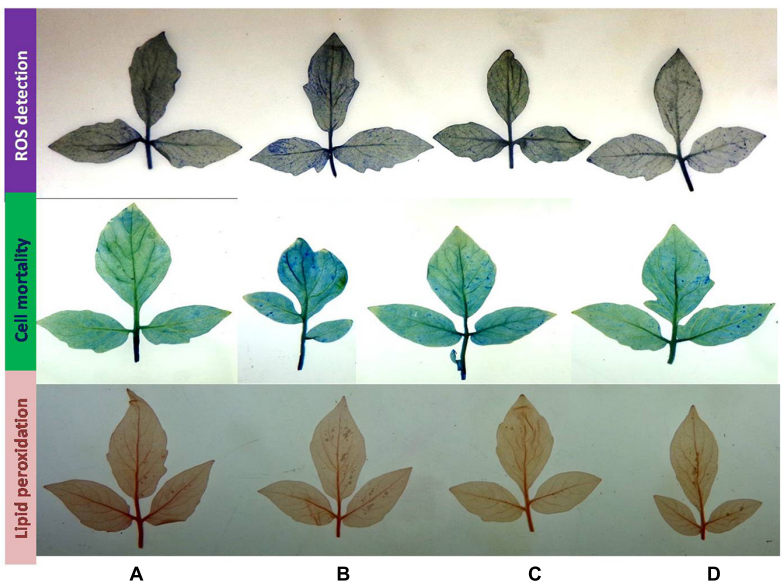

FIGURE 10 | Histochemical analysis of cell death, ROS production, and lipid peroxidation in tomato plant leaves upon bacterial (BHU-AV3) inoculation under salt stress. ROS detection – blue spots show production of superoxide radicals; cell mortality – light blue spots show cell mortality; lipid peroxidation – red spots show lipid peroxidation. **(A)** Control; **(B)** salt (NaCl); **(C)** bacterial (BHU-AV3) inoculation; **(D)** bacterial inoculation + salt.

TABLE 4 | Differentially expressed proteins in tomato plant roots upon bacterial inoculation (BHU-AV3) under salt stress conditions.

Band number	UniProtKB* Accession	Homologous protein**	mW[#] (Da)	pI[$]	PLGS[$$] Score	Coverage[¥] (%)	Matched[£] Peptides	Molecular function[¥¥]	Biological[£] function
1	B9TU32	Thiamine biosynthesis protein ThiC variant L1 OS *Solanum lycopersicum* GN thiC PE 3 SV 1	72573	6.0337	836.6132	32.9231	9	ADP-ribose pyrophosphohydrolase activity	Response to vitamin B1
2	P30264	Catalase isozyme 1 OS *Solanum lycopersicum* GN CAT1 PE 2 SV 1	56470	6.5764	1237.124	38.2114	24	Catalase activity	Hydrogen peroxide catabolic process
3	Q2MI93	ATP synthase subunit beta chloroplastic OS *Solanum lycopersicum* GN atpB PE 3 SV 1	53433	5.0967	651.9818	28.1124	8	ATP binding	ATP synthesis coupled proton transport
4	P17786	Elongation factor 1 alpha OS *Solanum lycopersicum* PE 2 SV 1	49256	9.4614	651.9798	30.3571	14	GTPase activity	Translation
5	P26300	Enolase OS *Solanum lycopersicum* GN PGH1 PE 2 SV 1	47768	5.5746	1519.975	32.4324	12	Phosphopyruvate hydratase activity	Glycolytic process

*Accession number according to the UniProt database. **Homologous protein identified in the UniProt database. #mW, molecular weight. $pI, isoelectric point. $$Ion score of identified protein using UniProt database. ¥Sequence coverage; proteins with <25% sequence coverage were excluded from the result. £Proteins with more than three matched peptides were considered. ¥¥ Molecular Function, category using molecular functional classification by UniProt database. ££Biological Function, category using biological functional classification by UniProt database.

FIGURE 11 | Effect of bacterial (BHU-AV3) inoculation on expression of selected proteins' mRNA in roots under salt stress. T1 – control, T2 – salt (NaCl), T3 – bacterial (BHU-AV3) inoculation, and T4 – bacterial inoculation + salt. Values represent the mean ± SD, $n = 3$. Different letters on each bar indicate significant differences ($P = 0.05$) after DMRT test.

are also available on PGPR producing IAA and nutrient solubilization activities that helped increase plant growth and biomass accumulation in plants as an adaptive response to salt stress (Ali et al., 2014; Qin et al., 2014; Hahm et al., 2017; Pandey and Gupta, 2019). In the present work, a comparative study was performed in root and leaf tissues to evaluate the differences in physiological responses to salt stress upon inoculation with BHU-AV3. Interestingly, there were obvious differences in ion, antioxidant enzyme, and free proline contents in the leaves and roots of salt-stressed plants. A high salt concentration outside the root is known to cause ion imbalances in plants (Flowers and Yeo, 1986). Studies have reported a reduced level of internal K^+ at high external NaCl concentrations (Horie et al., 2011; Wakeel, 2013). Due to this, an increased ratio of Na^+/K^+ was observed, which reduced plant growth and caused ionic toxicity (Hariadi et al., 2010; Abdelaziz et al., 2019). Our study revealed that salt stress induced a significant increase in Na^+ content and Na^+/K^+ compared to the non-salt stress condition. It was observed that BHU-AV3-inoculated plant roots exhibited lower accumulations of Na^+ and Na^+/K^+ compared to un-inoculated plants under salt stress. In addition, the accumulation of Na^+ in leaves was less in bacterially inoculated plants compared to un-inoculated plant leaves. Several reports are available on PGPR mediation of salt tolerance in plants by reducing the transport of Na^+ from the roots to leaves (Yasar et al., 2006; Zhang et al., 2013; Win et al., 2018; Romero-Munar et al., 2019). ROS are induced during salt exposure and lead to oxidative stress in plant cells (Miller et al., 2010; Sharma et al., 2012). Fortunately, plants have antioxidants to scavenge these enhanced ROS. In the present study, POD, PPO, and SOD enzyme activities were evaluated in tomato plants as a salt defense response, and augmented expression with a high number of isoforms of these enzymes was found in BHU-AV3-inoculated plant root. Four POD isoforms, two SOD isoforms,

and four PPO isoforms with different molecular weights were significantly up-regulated in tomato plant roots inoculated with BHU-AV3 compared to un-inoculated plants under salt stress. The expression of multiple isoforms of antioxidant enzymes is involved in reducing the content of ROS and preventing the cell-damage (Kim et al., 2005; Zhang et al., 2013; Vighi et al., 2017; Arora and Bhatla, 2017; Sukweenadhi et al., 2018). In one study, new POD and SOD isoforms were expressed in response to salt stress tolerance in potato plants (Rahnama and Ebrahimzadeh, 2005). BHU-AV3-inoculated plant leaves had lower expression of antioxidant enzyme activities and fewer number of their isoforms compared to un-inoculated plant leaves under salt stress.

The roots of BHU-AV3-inoculated plants accumulated higher proline content compared to non-inoculated plant roots under salt stress, suggesting the role of higher proline in the maintenance of osmotic balance inside the root (Claussen, 2005; Zhu et al., 2019). In addition, increased proline content protects membrane proteins and enzymes from oxidative burst (Szabados and Savouré, 2010). Several studies have confirmed the ability of microbes to mitigate the effects of oxidative bursts by increasing the activity of osmolyte contents and antioxidant enzymes (Khanna et al., 2019; Rajput et al., 2019; Vaishnav et al., 2019; Zahir et al., 2019). Interestingly, BHU-AV3-inoculated plant leaves exhibited less proline content compared to un-inoculated plants. The differences in antioxidant enzyme activities and free proline content between leaves and roots might be due to different metabolisms and functions of tissues (Cavalcanti et al., 2007). A few studies have reported on variability in the salt stress response in separate plant tissues upon bacterial inoculation (Cardinale et al., 2015; He et al., 2018). In another explanation, lower accumulation of proline and antioxidant enzymes indicates that plants are less affected by salt stress (Kohler et al., 2009; Zhang et al., 2013; Latef and Chaoxing, 2014). Negrão et al. (2017) described that root tissues are more prone to salt stress compared to shoot and leaf, as they are directly in contact with soil. Our results suggest that BHU-AV3 reduced osmotic and oxidative stress in plants by inducing proline content and antioxidant enzyme activities in roots, which are basically exposed to the salt stress. This hypothesis is also supported by results of *in situ* detection of ROS, lipid peroxidation, and cell death. BHU-AV3-inoculated plant leaves had less accumulation of ROS content and lower lipid peroxidation and cell death under salt stress compared to un-inoculated plant leaves.

In this study, one of our major focuses was on induced salt stress-responsive proteins in tomato plant roots upon inoculation with BHU-AV3. After a non-targeted protein study, we identified five proteins that were highly expressed under salt stress conditions, namely enolase, ATP synthase, thiamine biosynthesis protein, elongation factor 1 alpha (EF1-alpha), and catalase. The protein expression was further correlated by target-based gene expression analysis of the selected proteins. The gene expression analysis confirmed the up-regulation of all of the tested genes expression in BHU-AV3-inoculated plant roots. The consistency between the protein expression level and transcription level of the five selected protein genes manifests that the expression of these proteins may be controlled at the transcriptional level during bacterial interaction with plants under salt stress (Zhang et al., 2015; Cao et al., 2017).

Induction of energy metabolism under salt stress can be addressed by an increase in the abundance of ATP synthase and enolase proteins in tomato plants upon bacterial inoculation, while un-inoculated plants had lower expression of the same proteins. In a similar way, overexpression of ATP synthase in roots led to greater tolerance to salt stress in plants (Zhang et al., 2006; Li et al., 2011; Agrawal et al., 2016; Cao et al., 2017).

In addition, enolase is an important enzyme of the glycolysis pathway. Studies are available showing that increased ENO gene expression under salt stress generates more energy to cope with stress (Yan et al., 2005; Barkla et al., 2009; Zhang et al., 2018). The glycolysis pathway is the best way to generate energy quickly under normal (non-stress) conditions (Kürsteiner et al., 2003). In the present study, enhancement in the synthesis of ATP synthase and enolase upon bacterial inoculation suggests that these proteins may play important roles in maintaining the energy state and protecting plants against salt stress conditions.

An increase in expression of thiamine protein was observed in BHU-AV3-inoculated plant roots under salt stress. The thiamine synthesis protein supplies thiamine pyrophosphate (TPP) for several metabolic processes in plants (Goyer, 2010). In addition, thiamine is also involved in plant adaptations to different abiotic stresses (Tunc-Ozdemir et al., 2009; Rapala-Kozik et al., 2012). In one study, an exogenous application of thiamine was found to induce salinity tolerance in plants (Sayed and Gadallah, 2002).

EF1-alpha protein is involved in the initiation and elongation stage of mRNA translation and protein synthesis. The higher expression of EF1-alpha protein in BHU-AV3-inoculated plant roots suggests its participation in higher protein synthesis to protect the plant cells against salt toxicity, as previously explained by Shin et al. (2009) and Fercha et al. (2013). In addition, EF1-alpha proteins were also reported to perform a chaperone function by interacting with unfolded proteins, thereby protecting them from aggregation under stress conditions (Ristic et al., 2007; Bukovnik et al., 2009).

Catalase (CAT) is known for its antioxidant nature. It converts hydrogen peroxide (H_2O_2) into water and oxygen. It is highly specific for H_2O_2 and does not require a reductant in activity. A significant induction of CAT removed the ROS produced during salt stress in BHU-AV3-inoculated plants. Our results are in conformity with other findings that report enhanced activities of CAT enzymes in PGPR-inoculated plants under oxidative stress (Chen et al., 2016; Mesa-Marín et al., 2018; Afridi et al., 2019).

CONCLUSION

The current report extends our understanding of the salt tolerance mechanisms in tomato plants following inoculation with a salt-tolerant PGPR strain, BHU-AV3. Our findings clearly demonstrated that inoculation with

BHU-AV3 increased salt tolerance in tomato plants and that the roots were physiologically more responsive to alleviating salt stress. The tomato plant roots showed more severe changes in accumulating Na^+, proline, and antioxidant enzymatic activities compared to the leaves under salt stress with BHU-AV3 inoculation. Enhanced activities of these parameters in roots resulted in a decrease in oxidative stress in tomato plants, as measured in leaves with respect to ROS content, MDA content, and cell death assay. The protein study revealed that carbohydrate and energy metabolism, antioxidative enzymes, and translation-related proteins were up-regulated in BHU-AV3-inoculated plant roots in response to salt stress. These proteins may work cooperatively to enhance salt tolerance and enable them to survive under severe stress. Insights gained from such research increases our understanding of plant–microbe interactions and could aid in engineering plants with improved salt tolerance. In addition, such salt-tolerant PGPRs boost the potential to decrease the use of agrochemicals on cultivated land and perhaps enhance crop productivity on saline soils around the world.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the NCBI GenBank, Accession number MK588751.

AUTHOR CONTRIBUTIONS

AV designed and conceived of the research and drafted the manuscript. JS and PS helped in most of the experimental work. RR helped in the compilation of data and interpretation of the results. HS and BS coordinated the work and edited the manuscript.

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REVIEW
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Plant Growth-Promoting Bacteria: Biological Tools for the Mitigation of Salinity Stress in Plants

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Salinity stress is one of the major abiotic stresses threatening sustainable crop production worldwide. The extent of salinity affected area is expected to cover about 50% of total agricultural land by 2050. Salinity stress produces various detrimental effects on plants' physiological, biochemical, and molecular features and reduces productivity. The poor plant growth under salinity stress is due to reduced nutrient mobilization, hormonal imbalance, and formation of reactive oxygen species (ROS), ionic toxicity, and osmotic stress. Additionally, salinity also modulates physicochemical properties and reduces the microbial diversity of soil and thus decreases soil health. On the other hand, the demand for crop production is expected to increase in coming decades owing to the increasing global population. Conventional agricultural practices and improved salt-tolerant crop varieties will not be sufficient to achieve the yields desired in the near future. Plants harbor diverse microbes in their rhizosphere, and these have the potential to cope with the salinity stress. These salinity-tolerant plant growth-promoting bacteria (PGPB) assist the plants in withstanding saline conditions. These plant-associated microbes produce different compounds such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-acetic acid (IAA), antioxidants, extracellular polymeric substance (EPS), and volatile organic compounds (VOC). Additionally, the naturally associated microbiome of plants has the potential to protect the host through stress avoidance, tolerance, and resistance strategies. Recent developments in microbiome research have shown ways in which novel microbe-assisted technologies can enhance plant salt tolerance and enable higher crop production under saline conditions. This focused review article presents the global scenario of salinity stress and discusses research highlights regarding PGPB and the microbiome as a biological tool for mitigation of salinity stress in plants.

Keywords: microbiome, plant growth-promoting bacteria, salinity stress, salt stress amelioration, sustainable agriculture

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INTRODUCTION

A major challenge for world agriculture is to fulfill the food demand of the increasing global population, which is currently growing at a rate of around 1.05% per year (World Population Prospects, 2019). Plant growth, productivity, yield, and food quality are severely influenced by several biotic and abiotic stresses (Shi-Ying et al., 2018; Singh et al., 2018). The biotic stresses include

damages or infections caused by various pests or pathogens. The abiotic stresses include drought, salinity, temperature, heavy metals, and other organic contaminants. Among all abiotic stresses, soil salinization is the most detrimental (Daliakopoulos et al., 2016) and is considered to be one of the most significant limiting factors of agricultural productivity and food security. Worldwide, about 20% of agricultural land is affected by salinity, and this is continuously increasing (Gupta and Huang, 2014). It is estimated that by 2050, about 50% of agricultural land will be salinity affected. Salinization of agricultural land occurs mostly due to the accumulation of salts in soil (Bharti et al., 2016; Shi-Ying et al., 2018), particularly sodium (Na^+) and chloride (Cl^-) ions. High Na^+ accumulation limits water conductance, soil porosity, and aeration. In addition, soil salinity stress negatively affects the microbial diversity within and around the roots of plants. A plant under salinity stress undergoes several morphological, physiological, and molecular changes, which impede its growth and development (**Figure 1**). Besides, a high salt concentration affects enzyme activities, stomatal conductance, and the rate of photosynthesis (Kumar and Verma, 2018). Salinity stress also causes oxidative stress by enhancing the production of reactive oxygen species (ROS), which damage cell membranes, proteins, lipids, and nucleic acids (DNA, RNA) and may also induce programmed cell death (**Figure 2**). Salinity also leads to hypertonic stress due to the excessive accumulation of Na^+ and Cl^- ions (Shi-Ying et al., 2018).

Many salt-tolerant crops varieties have been developed through transgenic technologies and conventional breeding approaches. However, these two approaches are insufficiently, labour-intensive and time-consuming. In light of upcoming challenges, it has now become necessary to use alternative technologies simultaneously to promote sustainable agriculture, such as the use of plant growth-promoting bacteria (PGPB). The rhizosphere has complex microbial diversity, which may be considered as the natural relations between plants and microbes. Several recent studies have revealed that PGPB act as elicitors of salinity tolerance in plants and promote their growth (Vacheron et al., 2013; Daliakopoulos et al., 2016; Tiwari et al., 2016). Mostly, PGPB reside around the root zone of plants in saline soil. The various PGPB-mediated mechanisms include biofilm formation, extracellular polymeric substance (EPS) production, nitrogen fixation, phytohormone production, and ACC-deaminase activity (Ansari et al., 2019). PGPB also promote nutrient uptake and homeostasis in plants and increase antioxidant activities during salt stress. Plant growth-promoting bacterial endophytes reside in healthy plant tissues without causing them any disease. These endophytic PGPB can also promote salinity stress tolerance and plant growth (Ali et al., 2014).

Kumar et al. (2020) reported that the growth and yield of French bean (*Phaseolus vulgaris*) are optimized by the application of PGPR consortia against salt stress. Siderophore-producing rhizobacteria may represent a promising alternative to chemical fertilizers due to simultaneously tackling salt-stress effects and enhancing available iron in saline soils (Ferreira et al., 2019). In response to higher salinity stress, resistance is achieved by a change in membrane transport properties such as the regulation of Na^+/H^+ antiporters and various ion channels (Kumar et al., 2017). Besides, transcription factors (TFs) play a vital role in providing tolerance against salinity stress through regulation of the expression of stress-related genes. There are several TF families; among these, NAC, AP2/ERFBP, bZIP, MYB, and WRKY have been identified as potential players for improving crop tolerance against salinity stress (Barnawal et al., 2017; Tang et al., 2019). Further, PGPB also control plant pathogens through the production of antibiotics and competition for the ecological niche and nutrients (Kumar and Verma, 2018). Therefore, the interaction between plant and PGPB is a part of adaptation due to the mutagen effect (Nautiyal et al., 2008; Shi-Ying et al., 2018). Thus, to provide a sustainable solution for agriculture and cope up with salinity stress, it is necessary to explore the diversity of the microbes so as to understanding their physiological and functional features and harness their potential.

Salinity Status in India and the World

India has a mainland coastline of 6100 km, which is prone to salinity problems. Apart from that, regions away from the coast also experience salinity issues. In India, about 6.7 million ha of land are affected by salinity (Narayana and Babu, 1983). The affected soils are divided into three major categories: saline soils, alkali soils, and coastal soils. Gujarat has the highest amount (almost 71% of the total saline soils in India) of salt-affected soils (1.2 million ha). The states affected with saline soils are in the following order, Gujarat > Rajasthan > Maharashtra > Haryana > Bihar > Uttar Pradesh > Karnataka (**Figure 3**). The problem of alkali soils is mainly faced by Uttar Pradesh, which accounts for almost 36% of the total alkali soils in India. The states falling next in the list are Gujarat, Maharashtra, Tamil Nadu, Andhra Pradesh, Haryana, Rajasthan, Punjab, Karnataka, Madhya Pradesh, Bihar, and Jammu and Kashmir. Gujarat also has the maximum (0.5 million ha) coastal salt-affected soil in India: about 37% of the total coastal salt-affected soils of India. Internationally, the most salinity-affected regions include the Asia Pacific and Australia. These two continents cover a total agricultural area of 2016.63 million ha, out of which 27% (549.30 million ha) is salinity affected. In Africa, 72.2 million ha of land are salinity affected, which is approximately 6.40% of the total agricultural area. The Americas have a total of 1223.41 million ha of total agricultural area, of which 130.5 million ha of land is saline. In Europe, 17.30% of the land area faces the problem of salinity (**Figure 4**; FAO, 2019; World Population Prospects, 2019).

Effects and Causes of Soil Salinity

The world is saline; this is evident from the fact that, on average, the oceanic waters have a salinity of about 3.5%, which constitutes about 96.5% (roughly 1.3 billion km^3) of the Earth's water (Kırtel et al., 2018). Apart from this, roughly 12.87 million km^3 of saline water is groundwater, and 85400 km^3 of saline water is found in lakes (Eakins and Sharman, 2010; Kırtel et al., 2018). The acceptable limit of electrical conductivity (EC) causing no damage to crops is <0.7 dS/m, while EC over 3.0 dS/m severely affects the crop yield (Ayers and Westcot, 1985). The EC of seawaters is approximately 10–100 dS/m (Wang et al., 2006).

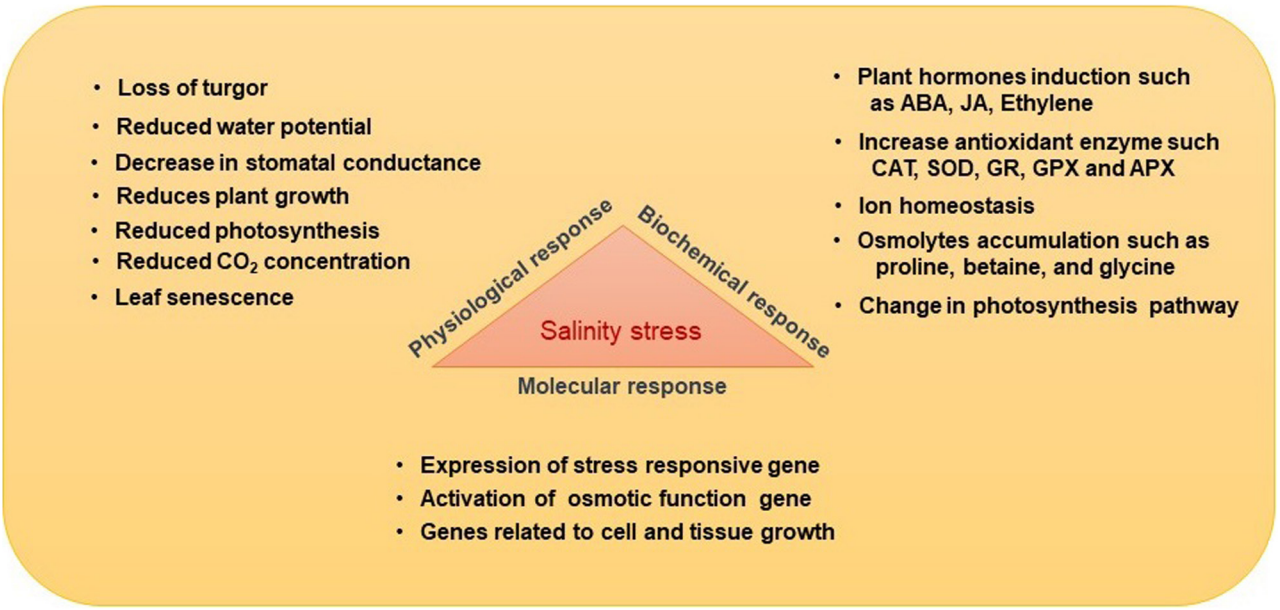

FIGURE 1 | Effects of salinity stress on different plant attributes.

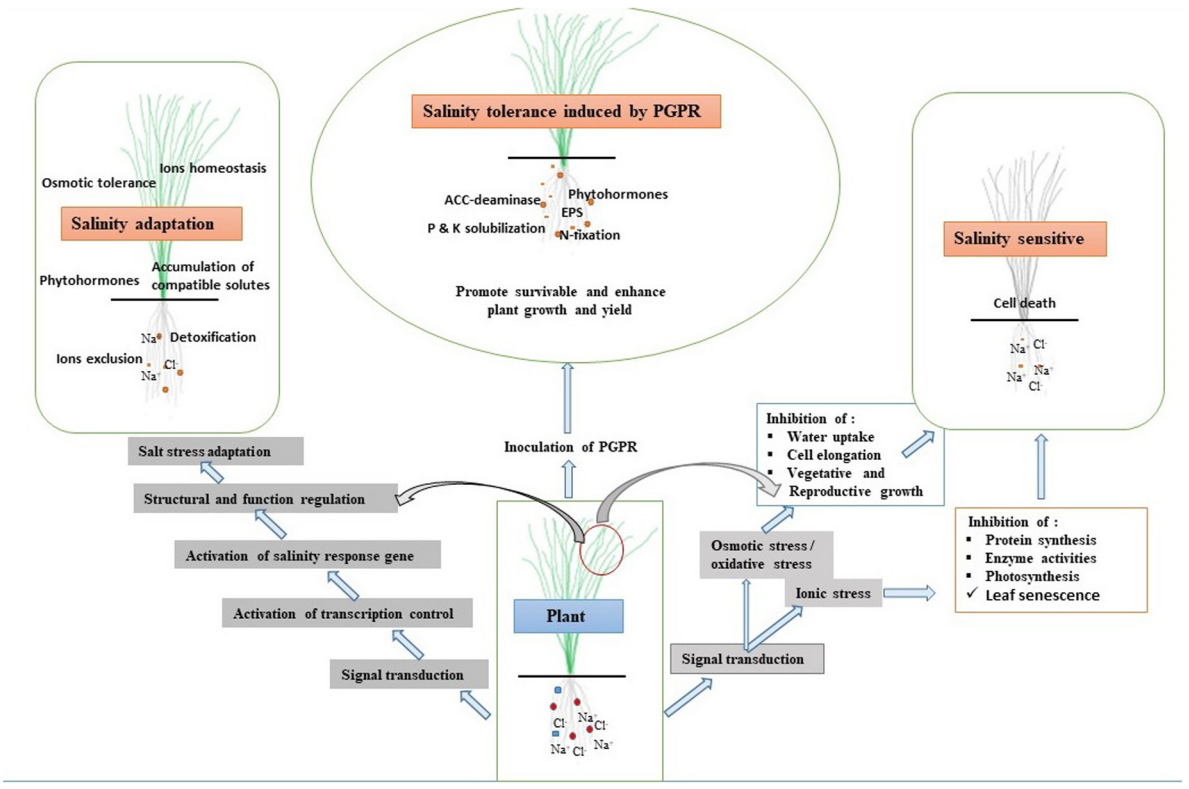

FIGURE 2 | Effects of salinity stress on plant signaling mechanism, adaptation, and PGPR-induced stress tolerance.

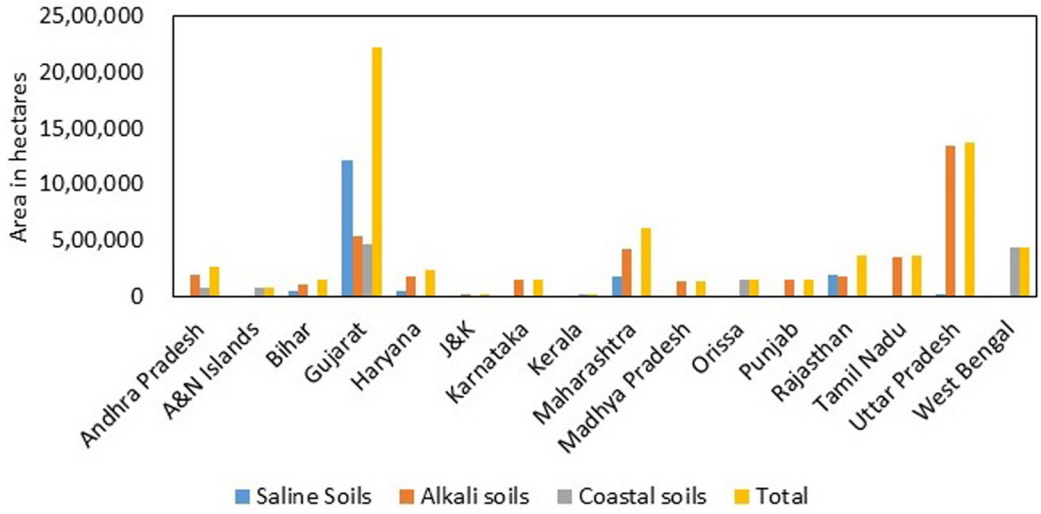

FIGURE 3 | Extent and distribution of salt-affected soils in India (Central Soil Salinity Research Institute [CSSRI], 2019).

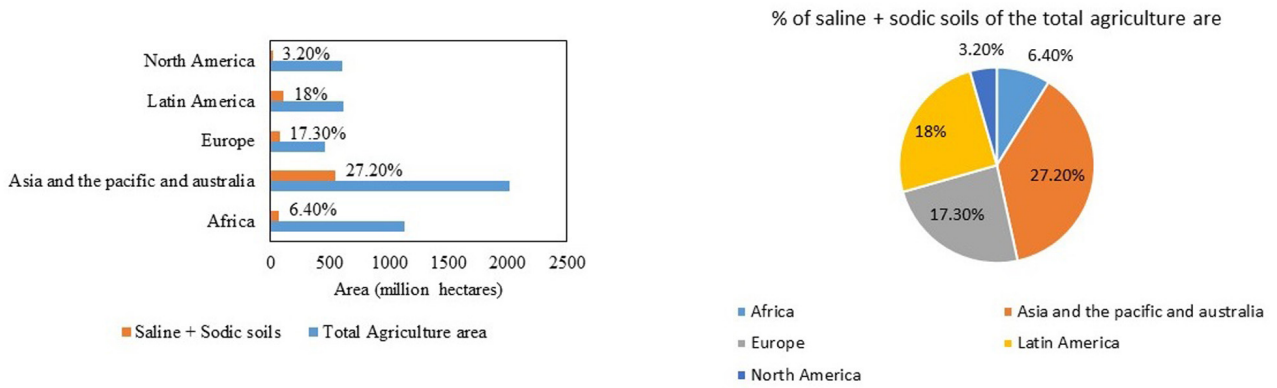

FIGURE 4 | Area of the total agricultural land in different continents that has sodic and saline soil (in millions of hectares of land) (FAO, 2019; Chauhan et al., 2019).

The accumulation of salts in water affects the ability of plants to extract water from the soil. The TDS value considered suitable for irrigation water is <450 mg/l (Ayers and Westcot, 1985), while its value in seawater ranges between 5000 and 70000 mg/l (0.5 and 7%) (Abdel-Aal et al., 2010). Thus, there is a huge problem with saline soil conditions in areas near coasts, which are mostly irrigated with mixed waters from the sea or oceans that have a high concentration of salts.

The salinity of soil can be broadly classified into two categories, natural and anthropogenic. Natural processes such as rainfall and its subsequent evaporation and derivation of the soil from a saline parent material are the most important factors. Some soils also contain natural salt deposits, which lead to saline conditions in the soil. Many times, soils in coastal areas are saline because of the sea sprays received. The salts from salt-affected land can also seep into lowland areas nearby through irrigation water. Ancient natural fossil salt deposits are found in arid and semi-arid regions. Saline material is sometimes found beneath the top layer of soil, which creates a highly concentrated solution when it is dissolved in water. When this reaches the topsoil, either by groundwater pumping or surface streams in lowland areas, it creates saline soil conditions. Water evaporation takes place in the pure state, which leaves behind salts in the soil. As a result of irrigation with salty water and subsequent evapo-transpiration, the salt concentration in soil continues to increase (Carter, 1975). Groundwater irrigation also causes saline conditions when it contains high salt concentrations from natural deposits of high-salt minerals.

In water stress conditions, phreatophytes growing along canals elevate the levels of salts by consuming water and leaving the salts behind in the remaining water used for irrigation. Such plants are found along the canals and drains of irrigated areas (Carter, 1975). The geographical location of a place also plays a role in its salinization. The downstream region of the Indo-Gangetic plain, especially the Bengal flood plain, suffers mainly because of its geographical location. The sedimentation from the Ganga and the Brahmaputra rivers is another way through which saline conditions are created in some parts of Bangladesh

(Mahmuduzzaman et al., 2014). A high level of sedimentation in the region causes waterlogging and flooding with saline water due to blocked rivers and upstream drainage congestion.

Impacts of Salinity on Soil and Plant

Surface crusting is one of the impacts of salinity on the soil. Crusting is the process of formation of hard white layers over the surface of the soil in the early growing seasons, especially when the soil is without the canopy cover of the crops. Surface crusting is driven by many factors. Based on the dispersion method, it is categorized into physical and chemical dispersion. Physical dispersion is caused by the impact of raindrops and irrigation water, which destroys weak soil aggregates, creating a surface seal, also known as crusting (Warrence et al., 2002). Chemical dispersion is caused by the water due to its ESP and EC. Soil crusts consist of two distinct parts, an upper skin (0.1 mm thick) caused by the impact of raindrops and a washed-in layer caused by the accumulation of clay particles. It is found that soils that disperse easily are 1000 times more permeable than the average crust. The surface crust effectively seals off the subsoil hydraulically, creating more runoff potential on the upper crust (Hardy et al., 1983). Farmers quite often use rotary hoes to till the surface crust, which is a short-term solution. Residue cover acts as a shield to prevent the direct impact of raindrops on the soil surface and provides important pathways for water entry into it. Soil salinity reduces the content of nutrients and microbial diversity in the salinity-affected area. The organic matter, nitrogen, dissolved organic carbon, and microbial carbon biomass (MCB) of soil are highly affected by salinity (Xu et al., 2020). Further, microbial activities such as soil respiration and soil enzyme activity are depressed by salinity. Thus, salinization of soil is recognized as a major threat to agricultural activity, human resources, and health (Shrivastava and Kumar, 2015).

Salinity affects almost every aspect of plant morphology, physiology, and biochemistry and thus causes significant loss of crop yield. A higher concentration of salt in soil restricts the uptake of water and essential nutrients by plant roots. The higher concentration of ions (Na^+) in root causes osmotic stress, decreases water potential, and disturbs the nutrient balance. Also, a higher Na^+ concentration outside the plant cell negatively impacts intracellular K^+ influx, which is an essential element required for plant growth. Moreover, excess Na^+ concentration inside the cell causes various physiological disorders, such as reduced seed germination, seedling growth, flowering, and fruiting (Singh et al., 2015). Excess salt also decreases the pigment (chlorophyll) content of the leaves, the leaf area, and photosynthetic efficiency. The inhibition of photosystem II (PSII) activity, which is a major site of the electron transport chain (ETC), occurs during salinity stress (Mehta et al., 2010; Kalaji et al., 2011). Several cellular enzymes are affected by salinity stress, such as RNase, DNase, proteases, and enzymes involved in nitrogen metabolism and synthesis of amino acids (Nathawat et al., 2005; Siddiqui et al., 2008). Salinity stress also indirectly induces the accumulation of ROS, such as singlet oxygen, superoxide radicals, and H_2O_2. The ETC in the chloroplast and mitochondria are major sites of ROS production under salinity stress conditions (Gill and Tuteja, 2010).

SIGNALING MECHANISMS

Plants adopt various mechanisms to survive under salinity stress through modifications at the morphological, physiological, and biochemical levels. These diverse modifications require modulation in different stress-related genes involved in regulatory and signaling pathways (Bharti et al., 2016; Numan et al., 2018). It is fundamental to understand the mechanisms involved in signaling of salinity stress in plants as well as between plant and bacteria (**Figure 5**). In general, plant signal transduction starts with the perception of signals by receptors on the surface, and this is followed by the generation of secondary messengers like inositol phosphates and ROS (Deinlein et al., 2014). The secondary messengers target proteins like CDPK (calcium-dependent protein kinases), MAPK (microtubule-associated protein kinase), and protein phosphatase involved in the control of gene expression through modulation of Ca^+ concentration. There are other genes involved in the regulation of plant hormones and other cellular activities (Miller et al., 2010). Transcription factors play a unique role in salinity stress tolerance in crops because of their unique roles in modulating different stress-responsive genes. These TFs include a large number of families, like AP2/ERF, bZIP, MYB, NAC, and WRKY, which modulate the expression and function of many genes. The modulation of the salinity stress-related genes also depends on

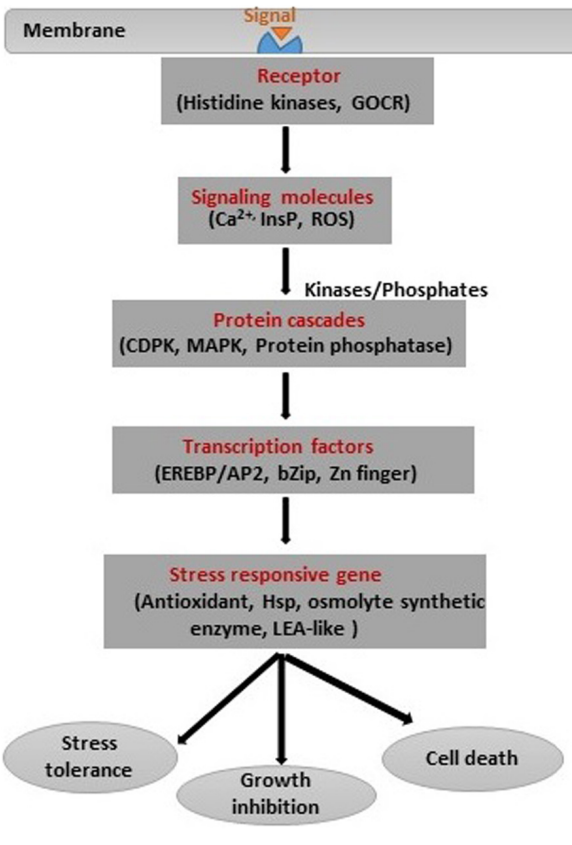

FIGURE 5 | Stress signal transduction and different signaling intermediates.

the post-transcriptional modulation of TFs. The role of TFs in the expression of the various genes and salinity stress tolerance has been extensively studied. The overexpression of *bZIP* gene in *Tamarix hispida* (Wang et al., 2010) and *CkdREB* gene in *Caragana korshinskii* (Wang et al., 2011) gave resistance against salinity stress. PGPB and endophytes also play a significant role in inducing plant signaling under salinity stress conditions. For instance, PGPR such as *Arthrobacter protophormiae* (SA3) and *Dietzia natronolimnaea* (STR1) enhanced salinity stress tolerance in wheat plants by modulating the expression of a regulatory component CTR1 (Constitutive Triple Response1) of the ethylene signaling pathway and DREB2 TF (Barnawal et al., 2017). Kim et al. (2014) found that *Enterobacter* spp. Increased the expression of salt stress-responsive genes such as *DREB2b, RD29A, RD29B*, and *RAB*18 in *Arabidopsis* under salinity stress. In another study, *D. natronolimnaea* was found to induce the expression of *TaMYB* and *TaWRKY* genes in wheat (Bharti et al., 2016). Further, genes related to antioxidants, HSP, and osmolyte synthetic enzymes are activated in response to salinity stress. However, salinity stress signal transduction has remained intriguing owing to its complexity and commonality with drought stress signals (Joshi et al., 2019).

In addition, epigenetic processes such as DNA methylation and post-translational modifications of histones influence the efficiency of stress-induced gene expression under salinity stress (Dietz et al., 2010; Golldack et al., 2011). However, the expression of salinity-related genes and protein translation depends on the type of plant. For instance, expression of TF *bZIP24* induced transcription in the salt-sensitive species of *Arabidopsis* but repressed it in salt-tolerant species (Miller et al., 2010; Golldack et al., 2011). The emerging tools, such as genomics, transcriptomics, and proteomics, will help in understanding plant stress signaling in response to salinity in greater detail in the future.

MICROBIAL RESISTANCE AND RESILIENCE UNDER SALINITY STRESS

There is increasing interest in understanding modifications to rhizospheric microbial diversity and community structure in response to different abiotic and biotic stresses. The ability of diverse soil microbial communities to withstand changes in environmental conditions can be described in terms of resistance and resilience. The term resistance describes the ability of a microbial community to avoid changes in its community structure in the presence of an environmental stressor, while resilience describes the ability of the microbial community to return to its original state when stress is absent and original environmental conditions are re-established (Allison and Martiny, 2008). Salinity stress affects microbial properties, community structure, and functions. However, various processes can continue at the same rate if the community contains a high degree of functional redundancy. One microbial taxon can be replaced by another with the potential to tolerate and survive under the stress; the new bacterium can then continue to perform the same functions. The salinity-resistant microbial community improves the health of salinity-impacted soil, maintains ecological functions, and sustains and promotes the growth of plants (Mbodj et al., 2018; Kumar and Verma, 2019).

Salt-tolerant bacteria can survive in different salt concentrations (30%) and overcome the effect of salt by different mechanisms, such as accumulation of compatible solutes for osmoregulation, production of extracellular proteases, and activation of Na^+/H^+ antiporters. *Staphylococcus epidermidis* (P-30) can survive in high salt concentration (up to 20%) and possesses plant growth-promoting properties (Das et al., 2015). The use of such salt-tolerant bacteria for inoculation or the use of their genes for the development of transgenic plants have been found to be successful in imparting salt stress tolerance in plants. For instance, the *codA* gene of *Arthrobacter globiformis* encoding choline oxidase was expressed in tomato (*Lycopersicon esculentum*). It induced the synthesis of glycine betaine and improved the salinity tolerance of the plants (Goel et al., 2011). In another study, it was found that inoculation of *D. natronolimnaea* STR modulated the transcriptional machinery responsible for salinity tolerance in wheat plants, such as salt overly sensitive (SOS) pathway-related genes (SOS1 and SOS4). Besides, enhanced gene expression of various antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) and higher proline content in PGPR-inoculated wheat plants were also observed (Bharti et al., 2016). Hence, the application of beneficial stress-tolerant microbes not only helps in improving the microbial community structure but also in enhancing plant and soil health under salinity. Further research will be required to reveal the hidden mechanisms of stress-tolerant microbial diversity.

PGPB AS A SALINITY-ALLEVIATING AGENT

Phytohormone Production and ACC-Deaminase Activity

Plant growth-promoting bacteria are known to increase the growth of the plant by the production of hormones such as auxin, cytokinin, and gibberellin and the reduction of ethylene by ACC deaminase. Ethylene is a gaseous hormone that is known to accumulate in plants under abiotic stress conditions. The level of ethylene accumulation in plants varies in different species, genus, organs, and tissues. Ethylene is involved in growth and developmental processes, such as seed germination, root hair development and elongation, fruit ripening, leaf abscission, and organ senescence (Ahmad et al., 2011), through the regulation of several stress-related genes. Therefore, ethylene is essential for plant growth and development. However, the higher concentrations of ethylene accumulation under stress conditions can become detrimental and inhibit plant growth (Erice et al., 2017). PGPB regulate the ethylene level in plants through ACC deaminase, which cleaves ethylene precursor ACC to ammonia and α-ketobutyrate and consequently facilitate plant growth and confer stress tolerance (**Table 1**; Ansari et al., 2019). PGPB with ACC deaminase activity modify the number of root tips and their

TABLE 1 | Mechanisms of salinity stress tolerance in different plants.

Crops	Bacterial Strains	Mechanism/Action	References
Cereals			
Maize (*Zea mays* L.)	*Rhizobium* and *Pseudomonas*	Increase proline synthesis, maintain the water level and selective uptake of ions, and decrease electrolyte leakage	Bano and Fatima (2009)
	Bacillus sp. and *Arthrobacter pascens*	Promote plant growth by phosphate solubilization and siderophore production under salt stress	Ullah and Bano (2015)
Rice (*Oryza sativa* L.)	*Bacillus amyloliquefaciens*-SN13	During salinity stress, increase plant biomass, water content, and proline and decrease reactive oxygen activities	Chauhan et al. (2019)
	Bacillus amyloliquefaciens NBRISN13	Modify gene expression and microbial community in the rhizosphere	Nautiyal et al. (2013)
	Pseudomonas alcaligenes and *P. pseudoalcaligenes*	Alleviate the harmful effects of salinity stress and maintain bacterial diversity in the rhizosphere	Rangarajan et al. (2002)
	Pseudomonas pseudoalcaligenes and *Bacillus pumilus*	Reduce lipid peroxidation and superoxide dismutase activity and promote plant growth and development	Jha and Subramanian (2014)
Barley (*Hordeum vulgare* L.)	*Hartmannibacter diazotrophicus*	Ameliorate salt stress through ACC deaminase activity	Suarez et al. (2015)
Wheat (*Triticum aestivum* L.)	*Pseudomonas pseudoalcaligenes*	A higher concentration of glycine betaine-like quaternary compounds and higher shoot biomass at lower salinity levels	Jha et al. (2011)
	Bacillus amyloliquefaciens NBRISN13	Modulates the gene expression profile of leaf and rhizosphere community	Nautiyal et al. (2013)
	Azotobacter vinellandii (SRI*Az*3)	Higher IAA, gibberellins (GA_3), zeatin (Zt), proline, and malondialdehyde	Sahoo et al. (2014)
	Bacillus pumilus	Limits uptake of toxic ions and increases production of antioxidants	Khan et al. (2016)
	Burkholderia sp. MTCC 12259	Enhances production of ACC deaminase	Sarkar et al. (2018)
	Curtobacterium albidum strain SRV4	Increases nitrogen fixation, exopolysaccharide (EPS), hydrogen cyanide (HCN), and IAA production, and ACC deaminase activity under salinity stress	Vimal et al. (2019)
	Sphingomonas pokkalii sp.	Modulates rice gene and regulates the negative effect of salinity stress	Palaniyandi et al. (2014)
	Pseudomonas pseudoalcaligenes and *Bacillus pumilus*	Reduce lipid peroxidation and superoxide dismutase activity	Jha and Subramanian (2014)
	Bacillus subtilis SU47 and *Arthrobacter* sp. SU18	Increase total soluble sugars, proline content, and dry biomass	Upadhyay et al. (2012)
	Dietzia natronolimnaea STR1	Modulates the expression of stress-responsive genes, involving induction of *TaMYB* and *TaWRKY* expression	Bharti et al. (2016)
	Bacillus pumilus strain FAB10	Biofilm formation on the root surface, enhanced amount of EPS, IAA, ACC deaminase activity, and solubilized phosphate	Ansari et al. (2019)
	Serratia sp. SL- 12	Production of ACC deaminase and promotion of plant growth promotion under salinity stress	Singh and Jha (2016)
	Klebsiella sp. SBP-8	Reduces salinity effects by ACC deaminase activities and induces systemic tolerance	Singh et al. (2015)
	Arthrobacter protophormiae (SA3) and *Dietzia Natronolimnaea* (STR1)	Enhance photosynthesis, level of IAA, reduce abscisic acid (ABA)/ACC content, and modulate the expression of a regulatory component (*CTR1*) of the ethylene signaling pathway	Barnawal et al. (2017)
	Chryseobacterium gleum sp. SUK	ACC deaminase activity, production of IAA, siderophore, ammonia, and hydrogen cyanide, promoting plant growth under salinity stress	Bhise et al. (2017)
	Azospirillum strains	Significantly increases shoot dry weight and grain yield	Nia et al. (2012)
Pulses			
Mung bean (*Vigna radiata* L.)	*Pantoea* sp. and *Enterococcus*	Increase salinity tolerance due to ACC deaminase activity and plant growth promotion	Panwar et al. (2016)
	Pseudomonas syringae; *Pseudomonas fluorescens*; *Pseudomonas fluorescens* and *Rhizobium phaseoli*	Contain ACC deaminase, reduce ethylene production, and promote nodulation under salinity stress condition	Ahmad et al. (2011)
Peanut (*Arachis hypogaea* L.)	*Brachybacterium saurashtrense*, *Brevibacterium casei*, and *Haererohalobacter*	Halotolerant PGPR promote plant length, shoot length, root length, and total biomass under saline conditions	Shukla et al. (2012)

(Continued)

TABLE 1 | Continued

Crops	Bacterial Strains	Mechanism/Action	References
Soybean (*Glycine max* L.)	*Pseudomonas putida* H-2-3	Increases chlorophyll content and length and fresh and dry weight of shoots	Kang et al. (2014b)
Pea (*Pisum sativum* L.)	*Arthrobacter protophormiae*	Improves colonization of diverse bacterial population, ACC deaminase activity, and protection against salinity stress	Barnawal et al. (2014)
Vegetables			
Cucumber (*Cucumis sativus* L.)	*Burkholderia cepacia* SE4, *Promicromonospora* sp. SE188 and *Acinetobacter calcoaceticus* SE370	Reduce concentration of sodium ions, catalase, peroxidase, polyphenol oxidase, and total polyphenol, while potassium and phosphorus are abundantly available. Reduced level of ethylene content in plant under salt stress	Kang et al. (2014a)
Lettuce (*Lactuca sativa*)	*Azospirillum*	Increases ascorbic acid content in response to salinity stress	Fasciglione et al. (2012)
	Pseudomonas	Induction of antioxidant enzyme system	Kohler et al. (2009)
Tomato *(Solanum lycopersicum* L.)	*Streptomyces* sp.	Production of proline and ACC deaminase and promotion of plant growth	Palaniyandi et al. (2014)
	Sphingomonas sp.	Production of EPS and antioxidants	Halo et al. (2015)
	Enterobacter spp.	Increases IAA production, induces expression of salt stress-responsive genes such as *DREB2b, RD29A*, and *RD29B*	Kim et al. (2014)
Beet (*Beta vulgaris* L.)	*Micrococcus yunnanensis*, *Planococcus rifietoensis*, and *Variovorax paradoxus*	Growth-promotion under salinity conditions with the help of nitrogen fixation, production of IAA and siderophores, phosphate solubilization, and ACC deaminase activity	Zhou et al. (2017)
Others			
Cotton (*Gossypium hirsutum* L.)	*Pseudomonas*	Salinity tolerance by the modulation of phytohormone IAA	Egamberdieva et al. (2015)
Peppers (*Capsicum annuum* L.)	*Bacillus*	ACC deaminase activity promotes salinity stress tolerance and reduces ethylene in plant	Wang et al. (2018)
Arabidopsis thaliana	*Bacillus megaterium*	Upregulation and adjustment of jasmonic acid (JA) metabolism	Erice et al. (2017)
	Bacillus amyloliquefaciens FZB42	Promotes salt adaptation through regulation of transcripts associated with phytohormones, photosynthesis, osmoprotectant synthesis, and translocation of Na^+ ions	Liu et al. (2017)
	Klebsiella sp.	Modulates *rbcL* and *WRKY1* genes expression	Sapre et al. (2018)
	Hartmannibacter diazotrophicus E19	Production of EPS and ACC deaminase	Suarez et al. (2015)

surface area. Hence, PGPB promote nutrient acquisition and survival under stress conditions. It is reported that the production of ACC deaminase enzyme and a decrease in the level of ethylene are the main reasons for PGPB-mediated plant growth promotion under salinity stress (Bhise et al., 2017). For example, *Pseudomonas syringae* in moong (Ahmad et al., 2011), *Rhizobium phaseoli* in bean (Ahmad et al., 2011), *Pseudomonas fluorescens* in groundnut (Saravanakumar and Samiyappan, 2007), and *Pseudomonas putida* in canola (Cheng et al., 2007) effectively alleviated salinity stress (**Table 1**).

Auxins are the other major plant hormones that are regulated by PGPB. Auxins are the group of hormones like indole-3-butyric acid (I3B) and indole-3-acetic acid (IAA). Bacteria producing IAA include *Actinobacteria, Nocardia, Frankia, Kitasatospora*, and *Streptomyces*. Cytokinins (CK) are also produced by PGPB. Plant totipotent cells are maintained by CK in their shoot and root apical meristems (Howell et al., 2003). In plants, three receptors are responsible for CK signaling, which are CRE1/AHK4/WOL, AHK2, and AHK3. The higher levels of cytokinin are positively correlated with increased plant growth.

Abscisic acid (ABA) is commonly called the stress hormone, and this is upregulated when there is water deficiency under salinity stress conditions in the root zone. The increase in the level of ABA under salinity helps the plant to cope with the impact of stress. ABA helps in the accumulation of compatible solutes such as sugars and proline in root vacuoles, as well as of Ca^{2+} and K^+, which mitigate the effects of high salinity (Numan et al., 2018). Gibberellins are also produced by bacteria, which helps in the promotion of the growth and yield of plants. Rice roots colonized by *Rhizobium* show higher production of gibberellins and auxins, which leads to increased plant growth and development (Bottini et al., 1989). Naz et al. (2009) reported that when a halotolerant bacterium was inoculated in soybean plant, root and shoot length and biomass were improved through the overproduction of proline, ABA, *trans*-zeatin riboside, GA3, and IAAs. *Rhodococcus* and *Novoshingobium* species have been shown to metabolize ABA *in vitro*, which apparently helps in reducing the levels of ABA in plants. Some of the PGPR help in the production of ABA, while others are shown to metabolize it and produce variable effects under salinity stress (Qin et al., 2016).

Extracellular Polymeric Substance (EPS) Production

A number of biopolymers, such as polysaccharides, polyamides, and polyesters are produced by microorganisms under natural conditions. A wide spectrum of multifunctional polysaccharides are synthesized, including structural, intracellular, and

extracellular or exo-polysaccharides (Haggag, 2010; Verma et al., 2015; Vurukonda et al., 2016; Gupta et al., 2019). EPS-producing PGPB can play a significant role in alleviating salinity stress (Ashraf et al., 2004; Upadhyay et al., 2011) as EPS binds with cations, such as Na^+, and decreases bioavailable ions for plant uptake. The production of EPS is an important criterion for the classification of stress-tolerant microbes. Most of the bacteria survive under stress conditions due to the production of EPS (**Table 1**). EPS promotes bacterial survival due to enhancing water retention capacity and regulating the diffusion of organic carbon sources. Bacteria also contain high molecular weight lipopolysaccharide–protein (LP) (carbohydrate complexes) and polysaccharide–lipid (PL) complexes that are responsible for desiccation tolerance. EPS also helps in the establishment of plant-microbe interactions (Ashraf et al., 2004; Haggag, 2010; Vurukonda et al., 2016) by providing a micro-environment in which microbes can survive under stress conditions. It also helps bacteria to attach to the plant and colonize it in response to root exudates. The composition and concentration of EPS changes dramatically under drought and salinity stress conditions. EPS is secreted in soil by microbes in the form of slime material and binds with soil due to Van der Waals forces, hydrogen bonding, cation bridges, and anion adsorption mechanisms (Naseem and Bano, 2014; Ansari et al., 2019). Thus, slime material forms a protective capsule around soil aggregates, and when plants are inoculated with EPS producing microbes, it displays resistance against salinity. Production of EPS by soil microbes around roots also increases water potential and uptake of nutrients by plants (Ashraf et al., 2004; Naseem and Bano, 2014).

The formation of biofilm is a common property of microbes under salinity stress. Biofilm is an aggregate of microbes in which they adhere to each other and protect themselves from adverse effects. Recent studies suggest that EPS modulates the physical and chemical attributes of microbes under saline conditions (Haggag, 2010). EPS plays an important role in maintaining the structural stability of the biofilms (Zhang et al., 2011; Zheng et al., 2016). The types of substances secreted determine the strength of these biofilms against salinity stress. High salinity can lead to disruption of biofilms produced through effects on the microbial metabolism and physiological processes (Bassin et al., 2011; Li et al., 2018). *Curtobacterium albidum* strain SRV4 alleviated salinity stress in rice plant due to production of EPS in addition to nitrogen (N_2) fixation, IAA production, and ACC deaminase activity (Vimal et al., 2019).

Induction of Synthesis of Plant Osmolytes and Antioxidant Activity

Organic osmolytes produced by bacteria include highly soluble organic compounds, such as sugars, sugar alcohols, glucosyl glycerol, betaines, amino acids, and tetrahydropyrimidine (Ciulla et al., 1997). Organic solutes accumulated in the bacterial cytoplasm may or may not be synthesized by bacteria. Sometimes the organic osmolytes are taken up from the environment (Ciulla et al., 1997). Likewise, in plants, osmolyte accumulation takes place to combat salinity stress. The accumulation of osmolytes in the cytoplasm helps to maintain the osmotic balance of the cell. Major plant osmolytes include di- and oligo-saccharides, sugar alcohols, glycine, betaine, proline, and glutamate (Chen and Jiang, 2010; Suprasanna et al., 2016). Sugars, primarily disaccharides such as sucrose, trehalose, oligosaccharides raffinose, and fructans, act as osmoprotectants and are major drivers in the plant stress tolerance mechanism. Apart from their osmoprotective functions, these organic osmolytes carry out several other vital functions, such as helping the survival of the microbiome inhabiting the plants. Sucrose accumulation is associated with the survival of *Craterostigma plantagineum* under plant tissue dehydration (Norwood et al., 2000). Sugar alcohols such as pinitol, mannitol, myo-inositol ononitol, and sorbitol act as osmoregulators during salinity stress and also act as signaling molecules (Slama et al., 2015; Suprasanna et al., 2016). Amino acids such as proline, betaine, and γ-aminobutyric acid are some of the organic osmolytes. Proline accumulation is observed in plant members of the *Aizoaceae* family and also most of the halophytes. DMSP (Dimethyl sulfoniopropionate) also acts as an osmoprotectant (Suprasanna et al., 2016).

Enzymatic and non-enzymatic antioxidants play a major role in the defense mechanism through regulation of ROS levels. The important enzymatic antioxidants include CAT, guaiacol peroxidase, SOD, APX, monodehydro-ascorbate reductase, dehydrogenase ascorbate, and glutathione reductase. Non-enzymatic antioxidant compounds comprise ascorbate, glutathione, carotenoids, tocopherols, flavonoids, etc. (Sengupta et al., 2016).

Essential Nutrient Uptake

A low level of essential nutrients is one of the major causes of the reduction of plant growth and productivity. Salinity reduces the uptake and accumulation of essential plant nutrients such as nitrogen (N), phosphorus (P), and potassium (K^+) and water due to high osmotic pressure and ion toxicity. Besides, plants need extra nutrients for maintenance of growth under stress conditions (Meena et al., 2014; Abbas et al., 2015; Sharma and Archana, 2016). Crop yield is also known to be affected in saline soils due to effects on the nutrient uptake and translocation (Nautiyal et al., 2008; Shi-Ying et al., 2018). Plant-associated PGPB are known to improved nutrient uptake and accumulation in many ways (Jaiswal et al., 2016).

Nitrogen, an important nutrient element required for plant growth, is limited under salinity stress. The inoculation of PGPB increases N uptake by symbiotic and non-symbiotic association with the plants (Santi et al., 2013). Phosphorus is the second important element after N and is taken up by roots in monobasic ($H_2PO_4^-$) or dibasic (HPO_4^{2-}) soluble forms. In natural conditions, the majority of P is present in inorganic and organic forms in soils and is mostly unavailable to plants. PGPB convert the unavailable P forms into available ones by acidification and chelation (Etesami et al., 2014). Potassium has a significant role in the growth and development of the plant. To achieve maximum yield, K is needed in adequate quantities (50–300 kg ha^{-1}) by all crops. However, most of the K of soil is not directly available for plant uptake. Moreover, K availability to plants decreases due to salinity stress. In this situation, K-solubilizing bacteria (KSB) are

an effective tool to fulfill the K requirement of crops (Mukherjee et al., 2019). It is reported that KSB convert mineral K into available K for plant uptake (Etesami et al., 2017; Vasanthi et al., 2018). The rhizobacterium *Burkholderia* releases K from minerals in soil (Kang et al., 2014a). A study by Mayak et al. (2004) showed increased P and K uptake by tomato plants when inoculated with *Achromobacter piechaudii.* The NPK contents of the wheat leaves increased significantly under salt stress conditions upon inoculation with *Bacillus aquimaris* (Upadhyay and Singh, 2015).

Plant growth-promoting bacteria also release/increase the availability of mineral elements like Cu, Fe, Mn, Zn, etc., to plants by chelation and acidification of soil (Etesami et al., 2014). Siderophore production is a major feature of PGPB. Iron is the second most abundant metal in the Earth's crust. It is essential for certain iron-sulfur complex enzymes and iron-containing proteins and plays a major role in plant growth by participating in the synthesis of chlorophyll. Salinity enhances Fe-related deficiency, i.e., chlorosis in plants. The Fe availability is also reduced in saline conditions because of the inhibition of the proton pumps. Siderophore-producing bacterial strains have a higher affinity toward Fe than phytosiderophores, and thus they can remove Fe from Fe^3-phytosiderophore complex. Studies have suggested that microbial activity plays a major role in Fe accumulation in roots and also its transport to leaves (Masalha et al., 2000). A study by Rungin et al. (2012) showed that the siderophore-producing endophytes *Streptomyces* increased root and shoot biomass due to enhanced supply of Fe. Siderophore producing-PGPB have been shown to impart salt tolerance in several plant models (Kavamura et al., 2013; Ramadoss et al., 2013). The increased root exudates owing to PGPB-induced root growth can also, in turn, increase the availability of nutrients such as P and micronutrients (Kang et al., 2014a).

ROLE OF THE PHYTOMICROBIOME IN SALINITY STRESS TOLERANCE

Endophytic PGPB employ mechanisms similar to those used by rhizosphere microbes in supporting plant growth and in imparting stress tolerance (**Figure 6**). The microbial communities inhabiting inside or on the surface of the plant are called the phytomicrobiome. All of the microbes in a certain phytomicrobiome may not be required at a given time. The phytomicrobiome also plays an important role in the interaction of plants with microbial communities of the rhizoplane, rhizosphere, and phyllosphere (Singh and Trivedi, 2017). The microbial communities associated with the plants may inhabit different plant parts. The microbial communities associated with different parts of plant are known as the root, shoot, leaf, flower, and seed microbiome (Qin et al., 2016). The root microbiome of the plant is crucial in determining the survival of the plant under conditions of stress, including salinity and

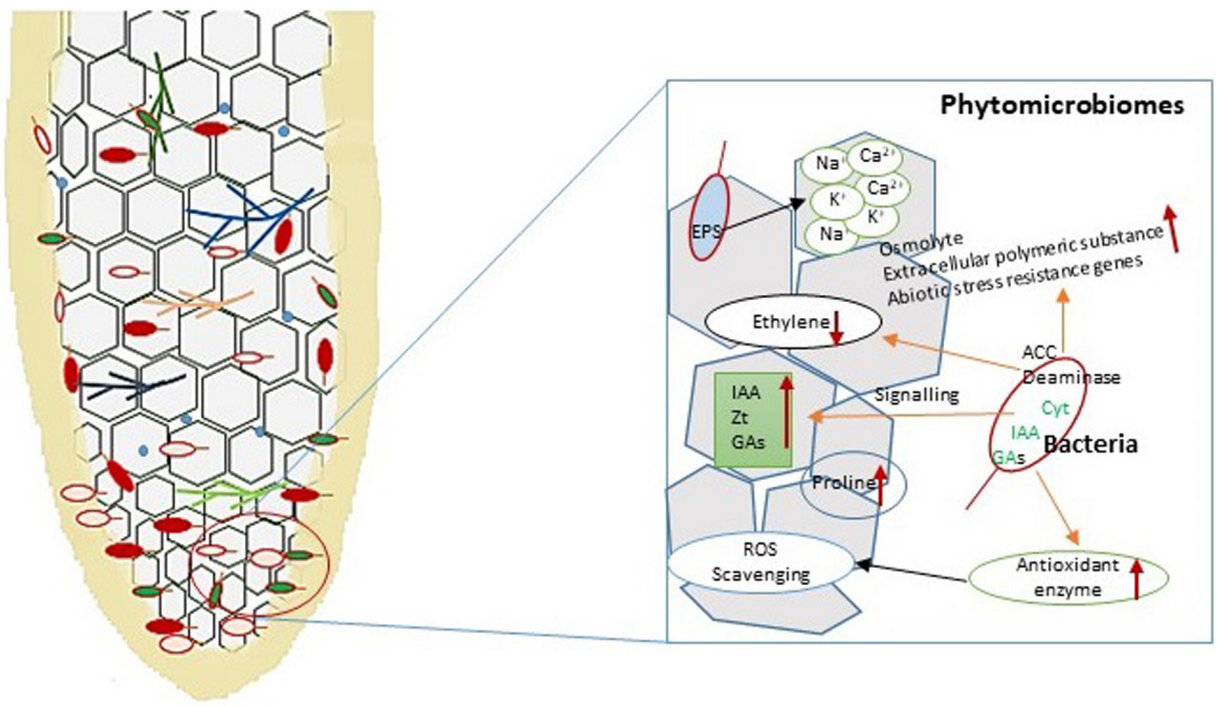

FIGURE 6 | Diagrammatic representations of plant–microbe interaction in the root and their different functions under salinity stress. Bacteria produce signaling molecules that help to support plant growth and development under stress conditions. Under salinity stress, bacterial the enzyme ACC deaminase reduces ethylene synthesis and enhances indole-3-acetic acid (IAA), Zeatin (Zt), and gibberellins (GA) in the plant. Exopolysaccharides (EPS) secreted by bacteria bind with different ions (Ca^{2+}, K^+, and Na^+) to prevent their effects on the plant. Endophytic bacterial strains induce antioxidants for controlling reactive oxygen species (ROS) generation in the plant under abiotic stress conditions.

drought stresses. Bacteria isolated from extreme environmental conditions have been shown to exhibit salinity stress resistance properties. *P. fluorescens* were isolated from rhizosphere soil of the Saharan region and showed a PGPB property in maize under salinity stress (Zerrouk et al., 2016). Several microbes inhabiting plants growing under high salt conditions also show adaptations toward salinity stress and thrive well in such conditions. These are called the halophyte microbiome. A major adaptation of halophilic microbes is that they maintain the protein structure and enzymatic activity for various metabolic processes even at high salt concentrations (Ruppel et al., 2013).

The basic mechanism by which salinity-tolerant microbes thrive in saline habitats is by avoiding high salt concentrations inside the cytoplasm. This is achieved through modifications in the cell wall construction in which specific membrane proteins, lipids, and exopolysaccharides are formed. Another method of avoiding high salt concentration inside the cytoplasm is by pumping ions out of the cell. Na^+/H^+ antiporters such as NhaA, an antiporter of *Escherichia coli* and many enterobacteria (Hunte et al., 2005), help in pumping out excess Na^+ (Ruppel et al., 2013). Other adaptations for survival under high salinity conditions include the development of proteins and enzymes capable of performing metabolic functions. Some microbes also develop organic osmolytes, which accumulate in the cytoplasm to make them resistant against osmotic pressure under high salinity stress. The organic osmolytes are also called compatible solutes as they provide resistance to different molar concentrations of salinity stress by maintaining a suitable molar concentration in the cytoplasm (Kunte, 2006).

FUTURE PERSPECTIVES AND CONCLUSION

Increasing salinity is posing serious threats to agricultural productivity, making it a matter of concern for policymakers and agriculture scientists. Salinity stress is also suppressing beneficial microbes in the soil. Understanding the complexity of plant-soil-microbe interactions under salinity stress offers novel possibilities for employing potential PGPBs as a sustainable tool for achieving high crop productivity. However, despite significant research already having been done in this area, there is a lack of knowledge on some important aspects. Knowledge regarding communication (signaling) between plants and microbes is still limited. Besides, the mechanisms of growth promotion in the presence of PGPB under salinity stress still need to be delineated in further detail up to the gene level. There is also a need to understand the role of epigenetic processes in salinity stress tolerance in plants and to delineate the functional interplay of PGPB. Further research should be focused on the utilization of effective salt-tolerant PGPB in salt-affected agricultural fields to promote the development of bacterial inoculants as commercial biofertilizers for improving salinity tolerance and crop productivity.

The combined use of rhizosphere PGPB and endophytic microbiomes may have a synergistic effect in alleviating salinity stress and for sustainably enhancing agricultural productivity. Overall, the future research should be focused on: (1) exploring the mechanisms of cross-talk between PGPB and plants in salinity stress environments, (2) understanding the mechanisms by which salt tolerance is conferred to plants by PGPB, (3) identifying the genes involved in plant growth promotion under salinity stress in natural environments, (4) transferring the identified genes through biotechnology into crop plants, (5) exploring the combined application of salt-tolerant PGPB and mycorrhizae under natural conditions, and (6) promoting the role of PGPB as a bio-fertilizer for sustainable agricultural production.

AUTHOR CONTRIBUTIONS

AK and SS wrote the manuscript. AG created the table, and reference setting. JV edited the manuscript. All authors contributed to the article and approved the submitted version.

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Mechanistic Insights of the Interaction of Plant Growth-Promoting Rhizobacteria (PGPR) With Plant Roots Toward Enhancing Plant Productivity by Alleviating Salinity Stress

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Agriculture plays an important role in a country's economy. The sector is challenged by many stresses, which led to huge loss in plant productivity worldwide. The ever-increasing population, rapid urbanization with shrinking agricultural lands, dramatic change in climatic conditions, and extensive use of agrochemicals in agricultural practices that caused environmental disturbances confront mankind of escalating problems of food security and sustainability in agriculture. Escalating environmental problems and global hunger have led to the development and adoption of genetic engineering and other conventional plant breeding approaches in developing stress-tolerant varieties of crops. However, these approaches have drawn flaws in their adoption as the process of generating tolerant varieties takes months to years in bringing the technology from the lab to the field. Under such scenario, sustainable and climate-smart agricultural practices that avail bacterial usage open the avenues in fulfilling the incessant demand for food for the global population. Ensuring stability on economic fronts, bacteria minimizes plant salt uptake by trapping ions in their exopolysaccharide matrix besides checking the expression of Na^+/H^+ and high-affinity potassium transporters. Herein we describe information on salinity stress and its effect on plant health as well as strategies adopted by plant growth-promoting rhizobacteria (PGPR) in helping plants to overcome salinity stress and in mitigating loss in overall plant productivity. It is believed that acquisition of advanced knowledge of plant-beneficial PGPR will help in devising strategies for sustainable, environment-friendly, and climate-smart agricultural technologies for adoption in agriculture to overcome the constrained environmental conditions.

Keywords: plant productivity, phytohormones, rhizosphere, PGPR, soil salinity

INTRODUCTION

With rapid urbanization, the reduction in agricultural land left less space to expand the cultivation of plants. Under such circumstances, expansion in plant production relies on increasing the fertility of soils to ensure food for all under the current global food security scenario (Godfray et al., 2010). In this direction, soil quality and water availability play a pivotal role in sustainable agricultural productivity. Any disbalance of salt in soil and water leads not only to decline in plant productivity but also even to their abandonment as it progresses with change in the land pattern from fertile to a marginal one. Although primary salinity is natural in the environment, the contribution by anthropogenic sources such as urbanization and deforestation is worth noting as these result in enhancing loss of the cultivable capacity of soils (land degradation and disturbance in the physical and the biological properties of soil) that affect plant productivity worldwide. Enhancement in salt deposits in an agricultural field hampers the growth of crop plants. In the scenario of decreased availability of fertile land, studies were directed in adopting genetic engineering approaches to complement traditional breeding methods in the development of salt-tolerant crops of food and fiber (Rozema and Flowers, 2008; Zhu et al., 2011; Dodd and Perez-Alfocea, 2012; Joshi et al., 2015). Despite significant efforts, the complexity in understanding the biological aspects of salt-stress-induced changes (morphological, biochemical, and physiological) renders limited success in developing salinity-stress-tolerant plants.

To cope up with the limited success in bringing technology-driven transgenics from the lab to the field, alternative strategies, such as the introduction of salt-tolerant microbes, are explored for adoption in augmenting and, as such, enhancing the growth of crops in salt-affected soils (Dodd and Perez-Alfocea, 2012; Etesami and Beattie, 2017; Etesami, 2018). Among them, plant growth-promoting rhizobacteria (PGPR) constitutes an important class of microorganisms that were found effective in inducing systemic tolerance in plants to tolerate abiotic stresses (Dutta and Khurana, 2015; Etesami and Beattie, 2018). However, PGPR from hypersaline soils (halotolerant PGPR) expressing plant growth-promoting (PGP) traits were found least affected by environmental factors such as climate, soil characteristics, etc., and thus are more efficient in enhancing salt tolerance in plants than PGPR from non-saline habitats (Giongo et al., 2008; Upadhyay et al., 2009; Egamberdieva and Kucharova, 2009; Khan et al., 2016). As part of the plant–bacterial interaction at the rhizospheric plane, plants were found to dictate the growth of microbiota for driving adaptation to changing environmental conditions (Berendsen et al., 2012). While many excellent reviews discussed a range of diverse plant-beneficial traits of microbiota encompassing both bacteria and fungi (Qin et al., 2016; Ilangumaran and Smith, 2017; Etesami and Beattie, 2017, 2018; Backer et al., 2018; Egamberdieva et al., 2019), the present study is aimed at highlighting the importance of plant–bacterial interactions, with comprehensive inputs about the mechanistic insights that operate at the plant level in mitigating salt stress toward improvement in crop yield as part of the climate-smart agricultural practices geared for feeding the ever-increasing global population.

SOIL SALINITY AND PLANT GROWTH

Increase in the concentration of salts, preferably sodium chloride (NaCl; electrical conductance >4 dSm^{-1} or 40 mM), attributed to both natural (salts released by weathering of rocks, salt from seawater influx, air-borne salts from oceans, etc.) and anthropogenic (surface runoff and irrigation-based salt deposition year after year) sources, renders the soil no longer suitable for cultivation (Pitman and Lauchli, 2002; Rengasamy, 2002). Despite suitable soil water columns, excessive salinity raising the concentration in soil solutions deprive plants of using it *via* osmotic reduction. High soil salt concentrations induce its effects right from imbibition of water to seed germination and root elongation that together have a great effect on the yield of crop plants (Katembe et al., 1998; Kaymakanova, 2009). It has been observed that the pre-treatment of seeds with different PGPR promotes seed germination and seedling growth (Poupin et al., 2013; Rahmoune et al., 2017; Bakhshandeh et al., 2020). As part of the mechanism, it is believed that PGPR helps in maintaining the balance of hormones, e.g., auxin to cytokinin levels during germination and the early stages of plant development, thereby playing a critical role in dictating the genetic program that controls post-embryonic roots and shoot growth (Chu et al., 2019; Qessaoui et al., 2019).

At later stages of plant growth, soil salinity interferes with root turgor that led to reduction in water absorption, decrease in the plant water column that progresses through dehydration and osmotic stress, inhibition of the metabolic machinery, disturbance in the transpiration system, and, most importantly, interference with parameters pertaining to photosynthesis (Kaushal and Wani, 2015). Photosynthesis refers to a major attribute in dry matter and, as such, in plant productivity, showing a decrease in saline condition owing to the reduction in leaf turgor and reduced leaf surface area (Qin et al., 2010; Tanveer and Shah, 2017). It occurs either through (1) decreased stomatal opening and CO_2 uptake, which in turn is associated with the reduction in stomatal conductance or (2) operation of a less-efficient Calvin cycle due to limited chlorophyll content (Lycoskoufs et al., 2005; Chaves et al., 2009). Stunted growth (seedling) with reduced biomass and leaf area are observed effects of salinity stress in the growth (vegetative stage) of plants (Takemura et al., 2000; Wang et al., 2003). PGPR employ different mechanisms in encouraging plant growth, prominently being nutrient availability and securing mineral assets such as phosphorus, phytohormone production, production of volatile compounds in controlling seed- and soil-borne phytopathogen, and synergism with other plant-beneficial microorganisms in enhancing resistance against different stresses (Bhattacharyya and Jha, 2012; Bhattacharyya et al., 2015; Bell et al., 2015; Kurepin et al., 2015; Bach et al., 2016; Yuan et al., 2016). Additionally, a limited canopy that prevents water loss by transpiration also constitutes a

plant survival mechanism under high salt concentrations (Savé et al., 1994; Ruiz-Sánchez et al., 2000; Colmer et al., 2005; Cassaniti et al., 2009, 2012).

SALINITY STRESS, PGPR, AND PLANT PRODUCTIVITY: A TRIANGULAR CONJECTURE

Salinity is a stress of global magnitude, having a substantial effect on plant growth, and is accountable for a significant loss in their productivity. Exerting adverse effects on germination, vigor, and yield, it led to drastic reduction in plant productivity, as observed in plants growing in arid and semi-arid areas (Paul and Lade, 2014). With an increase in salt concentration, disturbance in the cellular ion balance led to an enhancement in reactive oxygen species (ROS) production, besides taking a huge toll in exerting ionic toxicity on the accumulation of Na^+ and Cl^- ions (Grover et al., 2011). ROS (free oxygen radicals, superoxide, and hydrogen peroxide) are capable of damaging cellular structures and damage of biomolecules (proteins, lipids, etc.) besides talking a huge toll on chlorophyll degradation and lipid peroxidation that are, in turn, associated with a reduction in photosynthetic activity, damage of cellular membranes, and ultimately proceeding with induction of programmed cell death (Apel and Hirt, 2004). Interfering in cellular enzymatic functions, the accumulation of Na^+ and Cl^- ions produces diverse effects on different physiological fronts and in its effect on the growth and the development of plants (Nunkaew et al., 2015; Acosta-Motos et al., 2017). Photosynthesis capacity is reduced due to the interference of these ions with the opening and the closing of the stomata and in exerting osmotic stress as reflected in plants through reduction in leaf area and chlorophyll content (Munns, 1992; Kang et al., 2014a). Suppression of plant growth, a phenomenon of disturbed metabolic activities as a result of nutritional and hormonal imbalance together with abscission and senescence, is observed once the intensity of salinity stress, together with temperature, crosses the limit (Glick, 2014; Paul and Lade, 2014; Hashem et al., 2015). The accumulation of Cl^- ion leads to inhibition of nitrate reductase activity in the photosynthetic pathway (Azza Mazher et al., 2007; Nadeem et al., 2014). Elevation in ethylene (C_2H_4) levels progresses with drastic effects on plant health such as defoliation, senescence, etc. (Barnawal et al., 2014; Glick, 2014). Upon overcoming the storage capacity of cells, the accumulation of salts progresses to dehydration of cells, ultimately leading to plant death (Kang et al., 2014a).

Constituting an excellent environment for them to flourish, plant-beneficial microorganisms play an important role in achieving sustainability in plant productivity under the current paradigm of climatic change. As part of the climate-smart agricultural practices, microorganisms improve nutrient availability to plants and, in return, get nutrients as root exudates from these plants (Patel et al., 2015; Hamilton et al., 2016; Singh and Strong, 2016). Halotolerant PGPR employs a wide range of strategies as adaption for survival under saline conditions and, in turn, executes a number of plant-beneficial mechanisms for improving the growth of crop plants growing under salinity stress (**Figure 1**). These include (1) making nutrients available to plants *via* solubilization of phosphorus and potassium, siderophore production for iron uptake, and fixation of atmospheric nitrogen (Etesami and Beattie, 2017; Etesami, 2018), (2) maintenance of water balance by changing the architecture of roots for hydraulic conductance (Arora et al., 2012), (3) selective uptake of K^+ to Na^+ ions for maintaining a high K^+/Na^+ ratio that indirectly reduces the intercellular accumulation of K^+ to Na^+ ions (Islam et al., 2016; Etesami, 2018), (4) exopolysaccharide (EPS)-mediated alleviation of salt stress by decreasing Na^+ accumulation in roots and, as such, preventing their translocation to the leaves (Nunkaew et al., 2015; Qin et al., 2016; Etesami and Beattie, 2017), (5) production of volatile compounds and osmoprotectants that enhance the plants' survival under salt stress (Creus et al., 2004; Timmusk et al., 2014), (6) protecting plants from oxidative stress by upregulating the activity of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase as part of the antioxidant defense system (Islam et al., 2016), (7) maintenance of hormonal level for alleviation of salt stress (Etesami et al., 2014; Singh et al., 2015; Etesami and Beattie, 2017), (8) modulation in the expression of stress-responsive genes (Gond et al., 2015; Qin et al., 2016; Kaushal and Wani, 2016; Etesami and Beattie, 2017), and (9) production of extracellular enzymes that impart protection against phytopathogens competing with beneficial bacterial species for nutrients (Hariprasad et al., 2011; Dubey et al., 2014; Etesami, 2018).

PGPR IN THE ALLEVIATION OF SALT STRESS

Salinity stress adversely affects plant morphological, physiological, and biochemical functioning that, in turn, proves detrimental to plant health. Salt tolerance—a parameter quantified over given time—is survival, growth (vegetative), and biomass (harvestable) of the plant growing under salt stress to non-saline habitats (Munns, 2002b). The plants adopt either by inheriting genetic traits that impart salinity tolerance or by adopting a selectable mechanism of salt exclusion from the roots, thereby delaying salinity stress (Munns, 2002b; Zhu, 2007). A few (in particular, halophytes) conduct movement of accumulated salts *via* the xylem for precipitation at the leaf surface, while others have developed specialized structures (salt glands) in shoots, whereby salt is excreted on the surface for removal by wind or water (Ilangumaran and Smith, 2017). Additionally, plants undergo valuable interactions with bacterial species residing in the rhizospheric region, with an interaction pattern ranging from mutualism to antagonism. Colonization and successful establishment in the rhizospheric region are considered as a prerequisite for their interaction at the root surface. Traits that promote colonization of PGPR at the root surface include the availability of sufficient nutrients besides the property of being motile and capable of adherence (*via* pilli, surface-localized proteins, etc.) to plant roots (Jan et al., 2011). On one side where root exudates (organic acids, phenolics, sugars, amino acids, etc.) help microbes to flourish, it prompts

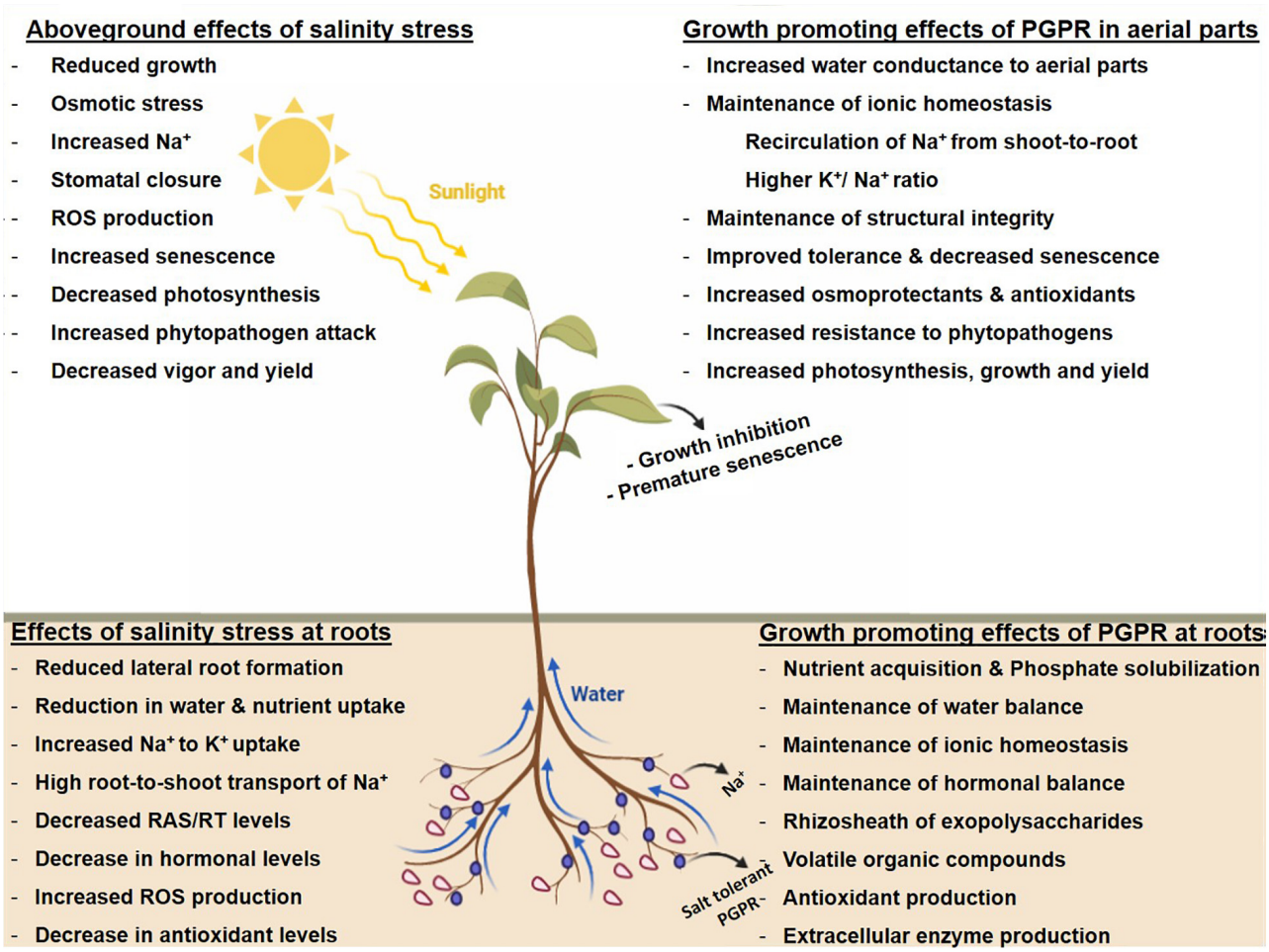

FIGURE 1 | Salinity stress and tolerance mechanisms induced by plant growth-promoting rhizobacteria.

changes (both physical and chemical) in plants related to defense, nutrient deficiency, and tolerance against heavy metals besides being important in eliciting strong responses against different abiotic stresses such as salinity as a mechanism of promoting plant growth (Jan et al., 2011; Nadeem et al., 2014; Rashid et al., 2016). **Table 1** a detailed account of the growth-promoting attributes of PGPR in agroecosystems is given in the following discussion.

Maintenance of Water Balance and Nutrient Acquisition

The hydration of cells, having a greater impact on physiological and metabolic processes, determines behavioral growth in plants. Hydraulic gradients in the xylem regulate water conductance from the roots to the leaves against an imbalance between the rate of transpiration and the available water absorbed from the soil (Passioura, 2010; Chavarria and dos Santos, 2012). The sustained transpiration of water from the leaf surface without any replenishment causes a reduction in xylem water potential that progressively leads to leaf dehydration, depending on the environmental conditions, stage of the growth of plant, canopy characteristics, and water quality as part of irrigation. The accumulation of salts at the root surface causes a transition in the root architecture (supresses lateral root formation) over time that influences the availability and uptake of soil nutrients. Salinity-induced osmotic stress proceeds with a decrease in diffusion and, as such, mass flow of nutrients as they are carried to the roots of plants by water (Zhu, 2001; Munns, 2002a; Ashraf, 2004; Sánchez-Blanco et al., 2004; Meloni et al., 2008; Franco et al., 2011; Chavarria and dos Santos, 2012). Under osmotic stress conditions, the aboveground plant parts undergo little photosynthetic activity and switch to the use of photo-assimilates, which causes a reduction in plant growth. All these events lead to a subsequent reduction in plant productivity (Chartzoulakis et al., 2002; Giri et al., 2003; Katerji et al., 2005; Bhatnagar-Mathur et al., 2007; Álvarez et al., 2012; Gómez-Bellot et al., 2013).

The inoculation of bacterial isolates to the roots of pepper plants resulted in an enhanced roots system, thereby increasing the ability of plants to uptake water from the surroundings (Marasco et al., 2013). The expression of aquaporins (water-conducting proteins) present in plasma and intracellular

TABLE 1 | Plant growth-promoting rhizobacteria (PGPR)–plant interactions under salinity stress and plant beneficial effects recorded thereof.

Sample number	Plant species	PGPR species inoculation	Effects observed	References
(1)	Maize (*Zea mays*)	*Achromobacter xylosoxidans*	Improved maize growth and productivity under drought stress	Danish et al., 2020
		B. licheniformis FMCH001	Enhances plant water use efficiency *via* growth stimulation in both normal as well as in drought conditions	Akhtar et al., 2020
		Bacillus sps.	Induces plant response for defense enzymes, chlorophyll, proline, and soluble sugar under salt stress	Misra and Chauhan, 2020
		Bacillus sp. NBRI YN4.4	Improves photosynthetic pigments and soluble sugar content and decreases proline level under stress conditions; also enhances soil enzymes dehydrogenase, alkaline phosphatase, and betaglucosidase, which help in improving soil health	Dixit et al., 2020
		Ochrobactrum sp. NBRISH6	Helps in maintaining homeostasis through various mechanisms under deficit water stress condition	Mishra et al., 2020
		A. brasilense	Induced the development of a more extensive root system, regardless of growth medium nitrate concentration	Pii et al., 2019
		Burkholderia cenocepacia CR318	Helps in the health and the growth of crop including phosphate and potassium solubilization and antimicrobial activity	You et al., 2020
		P. aeruginosa strain FB2 and *B. subtilis* strain RMB5	Shows effectivity against a range of fungal phytopathogens	Ali et al., 2020
		Serratia liquefaciens KM4	Maintenance of water balance, enhanced antioxidant enzyme activities, increased nutrient uptake	El-Esawi et al., 2018b
		Pseudomonas sp., *Arthrobacter* sp., *Bacillus* sp., and members of other bacterial groups	Enhanced phosphate solubilization, IAA and ACC deaminase activity	Aslam and Ali, 2018
		A. brasilense Ab-V5 and Ab-V6, *Rhizobium tropici* CIAT 899	Enhanced antioxidant enzyme activities	Fukami et al., 2018
		Bacillus aquimaris DY-3	Maintenance of water balance, development of pigment system, enhanced antioxidant enzyme activities	Li and Jiang, 2017
		Bacillus amyloliquefaciens SQR9	Enhanced solute accumulation, enhanced antioxidant enzyme activities, increased expression of salinity stress response genes	Chen et al., 2016
		Staphylococcus sciuri	Enhanced antioxidant enzyme activities	Akram et al., 2016
		Bacillus spp., *Arthrobacter pascens*	Phosphate solubilization, maintenance of water balance, increased antioxidant enzyme activities	Ullah and Bano, 2015
		Pantoea agglomerans	Increased expression of aquaporin genes	Gond et al., 2015
		P. syringae, *P. fluorescens*	Enhanced ACC deaminase activity	Zafar-ul-Hye et al., 2014
		Proteus penneri, *P. aeruginosa*, *A. faecalis*	Enhanced exopolysaccharide production	Naseem and Bano, 2014
		Azotobacter chroococcum	Enhanced growth, increased phosphate solubilization and K^+/Na^+ ratio	Rojas-Tapias et al., 2012
		Bacillus megaterium	Improved expression of ZmPIP isoforms	Marulanda et al., 2010
		Rhizobium, *Pseudomonas* spp.	Osmotic regulation	Bano and Fatima, 2009
		Pseudomonas spp., *Enterobacter* spp.	ACC deaminase activity	Nadeem et al., 2009
		Pseudomonas syringae, *Enterobacter aerogenes*, *P. fluorescens*	ACC deaminase activity	Nadeem et al., 2007
		Azospirillum brasilense	Maintenance of ion homeostasis, decreased nitrogenase activity	Hamdia et al., 2004
(2)	Rice (*Oryza sativa*)	*Bacillus aryabhattai*, *Achromobacter denitrificans*, and *Ochrobactrum intermedium*	Helps to accumulate under salt stress and exhibits greater resistance to heavy metals	Sultana et al., 2020
		Klebsiella sp. PD3	Degrades phenanthrene; also shows ACC deaminase activity and phosphate solubilization	Li X. et al., 2020
		Bacillus amyloliquefaciens SN13	Induces metabolic and physiological parameters *via* different enzymes to reduce the impact of stress	Bisht et al., 2019
		Bacillus sp. JBS-28	Promotes grain yields; also decreases arsenic accumulation in arsenic-contaminated soil and paddy fields	Aw et al., 2019

(Continued)

TABLE 1 | Continued

Sample number	Plant species	PGPR species inoculation	Effects observed	References
		Bacillus aryabhattai MS3	Phosphate solubilization, enhanced siderophore and IAA production	Sultana et al., 2018
		Halobacillus dabanensis SB-26, *Halobacillus* sp. GSP 34	Nitrogen fixation and IAA production	Rima et al., 2018
		Enterobacter sp. P23	Growth promotion, phosphate solubilization, increased siderophore, and IAA production, reduction in ethylene production, enhanced antioxidant enzyme activities	Sarkar et al., 2018
		B. stratosphericus (NBRI 5Q and NBRI 7A)	Increased growth and biomass production, Phosphate solubilization, IAA production, enhanced ACC deaminase activity	Misra et al., 2017
		Thalassobacillus denorans (NCCP-58), *Oceanobacillus kapialis* (NCCP-76)	Increased germination and growth of root and shoot, developed pigment system, reduced Na^+ ion accumulation	Shah et al., 2017
		Bacillus pumilus	Growth promotion, enhanced antioxidant enzyme production, reduced Na^+ ion accumulation	Khan et al., 2016
		Bacillus and *Citrobacter*	Growth promotion, phosphate solubilization, IAA production	Habib et al., 2016
		Pseudomonas PF1 and TDK1	Enhanced antioxidant enzyme production	Sen and Chandrasekhar, 2015
		Serratia sp., *Pseudomonas* sp.	Growth promotion, phosphate solubilization, IAA production	Nakbanpote et al., 2014
		Alcaligens sp., *Bacillus* sp., *Ochrobactrum* sp.	ACC deaminase activity	Bal et al., 2013
		P. pseudoalcaligenes, B. pumilus	Reduction in ROS production, delay of senescence	Jha and Subramanian, 2013
		B. amyloliquefaciens NBRISN13 (SN13)	Solute accumulation, enhanced expression of SOS1, EREBP, SERK1, and NADP-Me2	Nautiyal et al., 2013
(3)	Wheat (*Triticum aestivum*)	*Variovorax paradoxus* RAA3; *Pseudomonas* spp. DPC12, DPB13, DPB15, DPB16; *Achromobacter* spp. PSA7, PSB8; *Ochrobactrum anthropi* DPC9	ACC deaminase activity; improves the growth of plants in water-stressed rain-fed environments	Chandra et al., 2019
		Planomicrobium chinense and Bacillus cereus with salicylic acid	Reduces moisture stress in plants	Khan and Bano, 2019
		Bacillus siamensis, Bacillus sp., and *Bacillus methylotrophicus*	ACC deaminase activity	Amna et al., 2019
		Bacillus subtilis	Induction of systemic resistance	Lastochkina et al., 2017
		Dietzia natronolimnaea	Enhanced expression of SOS-related genes, increased tissue-specific expression of ion transporters, modulation of ABA signaling cascade	Bharti et al., 2016
		Serratia marcescens CDP-13	ACC deaminase activity, minimizes the salinity-induced oxidative damages to the plants	Singh and Jha, 2016
		Arthrobacter spp. SU18, *B. aquimaris* SU44, *B. aquimaris* SU8	Root dry weight and shoot biomass	Upadhyay and Singh, 2015
		Azosprillium lipoferum, *Pseudomonas fluorescens* 169	Development of pigment system	Saghafi et al., 2013
		Azospirillum	Development of pigment system, enhanced solute accumulation, increased seedling growth and plant yield	Nia et al., 2012
		Piriformo sporaindica, Azospirillum	Development of pigment system, enhanced solute accumulation, increased seedling growth	Zarea et al., 2012
		Azospirillum lipoferum	Growth and biomass accumulation	Bacilio et al., 2004
(4)	Soybean (*Glycine max*)	*Bradyrhizobium diazoefficiens* USDA110, *Bacillus velezensis* S141	Enhanced nodulation and N2-fixing efficiency by producing larger nodules	Sibponkrung et al., 2020
		Bradyrhizobium	Improves plant development and increases nodulation	Zeffa et al., 2020
		P. fluorescens LBUM677	Enhances plant biomass, oil content, and lipid composition	Jiménez et al., 2020
		A. woluwensis, M. oxydans, A. aurescens, B. megaterium, and *B. aryabhattai*	Maintains osmotic balance and regulates salt tolerance	Khan et al., 2019

(Continued)

TABLE 1 | Continued

Sample number	Plant species	PGPR species inoculation	Effects observed	References
		L. adecarcoxylata LSE-1, *Bradyrhizobium* sp. LSBR-3	Promotes plant growth with increased plant productivity	Kumawat et al., 2019
		Bacillus firmus SW5	Development of root system, enhanced antioxidant enzyme levels	El-Esawi et al., 2018a
		Bradyrhizobium japonicum USDA 110, *P. putida* TSAU1	Development of root system with nodule formation, increased phosphate acquisition	Egamberdieva et al., 2017
		Pseudomonas simiae AU	Increased chlorophyll content, phosphate solubilization, IAA and siderophore production; decrease in Na^+ accumulation at root surface	Vaishnav et al., 2016a
		Bacillus thuriengenesis NEB17	Increased PEPCO and RuBisCo expression, enhanced production of pyruvate kinase, proteins of photosystems I and II, isocitrate lyase, and antioxidant glutathione-S-transferase	Subramanian et al., 2016
		P. putida H-2-3	Enhanced production of ABA, salicylic acid, and gibberellins	Kang et al., 2014b
		P. fluorescens	Enhanced cytokinin production	Bhattacharyya and Jha, 2012
		Bradyrhizobium japonicum, *Bacillus subtilis* SU-12, *Serratia proteamaculans*	Exopolysaccharide production, antioxidant activity	Han and Lee, 2005
(5)	Tomato (*Solanum lycopersicum*)	*Bacillus subtilis* Rhizo SF 48	ACC deaminase activity; protects against oxidative damage and enhances plant growth against drought stress	Gowtham et al., 2020
		Funneliformis mosseae, *Enterobacter* sp. EG16, and *Enterobacter ludwigii* DJ3	Enhances plant growth and tolerance to Cd in Cd-contaminated soil	Li Y. et al., 2020
		Leclercia adecarboxylata MO1	IAA- and ACC-deaminase-producing abilities; improves plant tolerance to salinity stress	Kang et al., 2019
		Pseudomonas putida UW4 (ACC deaminase)	Increased shoot growth and expression of Toc GTPase	Yan et al., 2014
		Pseudomonas aeruginosa T15, *Pseudomonas fluorescens* NT1, *Pseudomonas stutzeri* C4	Decreased ethylene levels, increased root and shoot length	Tank and Saraf, 2010
		Achromobacter piechaudii ARV8	Enhanced induced systemic tolerance, enhanced ACC deaminase activity	Mayak et al., 2004
(6)	Common bean (*Phaseolus vulgaris*)	*Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp.	ACC deaminase activity	Gupta and Pandey, 2019
		Mycorrhizae, *Bacillus subtilis*, and *Pseudomonas fluorescence*	Controls the infection of *Sclerotium rolfsii*; also acts as biofertilizers	Mohamed et al., 2019
		Rhizobium	Increased nutrient content and dry weight	Yanni et al., 2016
		Pseudomonas chlororaphis TSAU13, *Pseudomonas extremorientalis* TSAU20	Increased dry weight and root length	Egamberdieva, 2011
		Azospirillum brasilense, *Rhizobium* spp.	Enhanced root branching, increased secretion of flavonoids	Dardanelli et al., 2008
(7)	Radish (*Raphanus sativus*)	*Bacillus* sp. CIK-516	Improves plant growth and enhances Ni phytoextraction	Akhtar et al., 2018
		Lactobacillus sp., *P. putida* and *Azotobacter chroococcum*	Helps to mitigate salinity stress at the time of germination	Hussein and Joo, 2018
		Arthrobacter scleromae SYE-3	Increased shoot length	Hong and Lee, 2017
		Staphylococcus kloosii, *Kocuria erythromyxa*	Increased chlorophyll content, increased shoot and root fresh and dry weight	Yildirim et al., 2008a
		Bacillus spp.	Induction of plant growth	Yildirim et al., 2008b
(8)	Barley (*Hordeum vulgare*)	*Hartmannibacter diazotrophicus*	Growth induction, enhanced ACC deaminase activity, increased root and shoot dry weight	Suarez et al., 2015
		Curtobacterium flaccumfaciens	Promotes plant growth	Cardinale et al., 2015

membrane determines the hydraulic conductance (*L*) at the root surface and, as such, the uptake of water from salinized soil for a plant (Moshelion et al., 2015; Qin et al., 2016). Plasma membrane intrinsic proteins (*PIP*s) constitute important aquaporins for a plant, which helps in its adaptation to changing environmental conditions (Marulanda et al., 2010; Moshelion et al., 2015). An expressional analysis of *Zea mays* roots inoculated with *Bacillus megaterium* and *Pantoea agglomerans* showed up-regulated *PIP2* and *ZmPIP1-1* genes that contribute to the increase in the *L*-values under salinity stress conditions (Gond et al., 2015). These studies reveal that PGP bacteria determine the resistance of plants to water stress irrespective of the nature of interaction in determining the specificity for growth-promoting activity. Plant−bacterial interactions at the root surface assist plants in maintaining the availability of water and helps in the acquisition of nutrients through nitrogen fixation, phosphate solubilizations, and siderophore production as part of their mechanism in fulfilling the nutritional requirements of plants (Beattie, 2015; Pii et al., 2015). Nitrogen−an essential nutrient that limits plant productivity−is often applied exogenously. However, inorganic fertilizers that compensate nitrogen deficiency often lead to a change in soil structure and, as such, composition of soil microflora (Rueda-Puente et al., 2003). Studies were performed on exploring the naturally occurring nitrogen fixers which have the potential for exploration toward plant growth promotion. Of the different interactions, the nitrogen-fixing assembly of rhizobia in the roots of legumes is an extensively studied relationship between plants and bacteria. In this symbiotic relationship, the rhizomes provide the legumes with nitrogen and, in return, get reduced carbon as nutrient and suitable environment for nitrogenase activity (Backer et al., 2018). Being a sensitive process, all stages of nitrogen fixation in leguminous plants were found to be prone to salinity effects, which result in a decrease in the nitrogen content of leguminous plants (de la Peña and Pueyo, 2012; Bruning and Rozema, 2013). In this regard, the commercial preparation of halotolerant free-living diazotrophs such as *Azotobacter* sp., *Azospirillium* sp., etc., proved beneficial than rhizobia in nitrogen fixation in a variety of crops worldwide, thereby found effective in increasing the yield of various cereal crops (Vessey, 2003; Bashan and de-Bashan, 2015; Sharma et al., 2016).

Phosphorus is a major essential macronutrient that constitutes another limiting nutrient for plants after nitrogen. The abundance of insoluble forms and the intensive agricultural practices in both saline and fertile soils deplete plants of this essential nutrient. On the second line, phosphate-solubilizing microorganisms (PSMs) convert and as such make non-soluble forms of phosphate to easily available soluble forms for efficient utilization by the plants (Backer et al., 2018). Compared to complementation with NPK fertilizers, the employment of phosphate-solubilizing bacteria was found effective in enhancing phosphate availability to plants without exacerbating the soil salinity levels (Etesami, 2018; Etesami and Beattie, 2018). The liberation of reactive forms of phosphate from organic compounds on utilizing enzyme phytase of PSMs constitutes another mode of phosphate availability to plants. Additionally, the production of hydrogen cyanide (HCN), which was earlier thought as a plant-protective mechanism, was found to be associated with an enhancement in phosphate availability to plants (Rijavec and Lapanje, 2016). Siderophore (iron-binding ligands) production is associated with the deprivation of pathogenic microorganisms of iron (a micronutrient) and making it available for use in respiration, photosynthesis, and nitrogen fixation by plants (Ahmed and Holmstrom, 2014; Saha et al., 2016).

Maintenance of Ionic Homeostasis

Alleviating the nutritional imbalance caused by a high influx of salt ions regulates the exchange of nutrients (both macro and micro) to minerals. Microbes increase nutrient availability to plants through the increased production of siderophores (metal chelation) and bringing changes in pH at the surface of rhizospheres (Dodd and Perez-Alfocea, 2012; Lugtenberg et al., 2013). Disturbance in ionic homeostasis is observed in crops that are poor excluders of Na^+ (rice, beans, etc.) and sensitive to Cl^- ions (citrus, soybean, etc.) grown in soils with high salt levels (Munns, 2002a; Tester and Davenport, 2003). Under salinity stress, the influx of Na^+ into the roots undergoes translocation to the aerial parts *via* the xylem, with the final accumulation taking place at the leaf surface rather than at the roots (Tester and Davenport, 2003). As such, excluding Na^+ from plants becomes difficult as only a small proportion of it undergoes recirculation to the roots *via* the phloem, thereby restricting it to the aerial parts, thus causing toxicity in plants. An increase in the concentration of Na^+ disturbs the Na^+/K^+ ratio that progresses with the inhibition of cytosolic activities besides interfering with the activities of enzymes involved in respiration and photosynthesis (Baral et al., 2015; Jacoby et al., 2016). Considering the importance of Na^+ homeostasis to the growth of plants, the regulatory network of Na^+/H^+ antiporter and high-affinity K^+ transporters (HKT) is put to work for the efflux of Na^+ ions from the cells throughout the plants (Tester and Davenport, 2003; Davenport et al., 2005). With localization on the plasma membrane, the Na^+/H^+ antiporter (also referred to as SOS1, salt overlay sensitive channel) efflux Na^+ in response to its increasing cytosolic levels (Qiu et al., 2002). Also, the increase in plant Na^+ level interferes with the uptake of K^+ at the root surface *via* the low-affinity K^+ uptake system. To increase salinity tolerance, plants activate high-affinity K^+ transporters, thereby increasing the uptake of K^+ over Na^+ ions in plants (Rodríguez-Navarro and Rubio, 2006). Additionally, the activation of membrane-bound Ca^{2+} channels in response to a depolarization event generates a Ca^{2+} signal that indicates the occurrence of salt stress in plants. The Ca^{2+} signal is sensed by calcineurin B-like protein (CBL4; also referred to as SOS3) which undergoes complex formation with CBL-interacting protein kinase; CIPK24 (also referred to as SOS2) enables the phosphorylation of SOS1 for its activation, an event important in maintaining the Na^+/K^+ ratio by sustaining K^+ transporters (Epstein, 1998; Halfter et al., 2000; Zhu, 2002).

Microbes minimize the accumulation of ions by increasing Na^+ exclusion at the roots besides boosting the working affinity of K^+ transporters that indirectly reduce their build-up in aerial parts, thereby contributing to the maintenance of ion

homeostasis in plants. Besides promoting biofilm formation at the root surface that prevents the influx of Na^+ into the roots, EPS production by PGPR strains traps cations in their matrix, thereby make it unavailable for uptake by the plants (Dodd and Perez-Alfocea, 2012). The inoculation of *Aeromonas hydrophila* and *Bacillus* sp. capable of producing EPS to the roots of wheat traps Na^+ ions, thereby making it unavailable for accumulation at the leaf surface (Ashraf et al., 2004). The inoculation of *B. subtilis* GB03 to the roots of *Arabidopsis thaliana* results in the down-regulation of *HKT1*, thereby reducing the uptake of Na^+ (Zhang et al., 2008; Qin et al., 2016). Restricting the uptake of Na^+ at the root surface leads to induction in the expression of *HKT1* in shoots for facilitating the recirculation of Na^+ from the shoot toward the roots, which helps in maintaining a high K^+/Na^+ ratio in plants (Zhang et al., 2008; Qin et al., 2016; Ali et al., 2019). With the RNA interference-mediated mutation of Ca^{2+}-dependent protein kinase, CPK12 increases the sensitivity of *Arabidopsis thaliana* to salt stress (Zhang et al., 2018). The inoculation of *Azotobacter* strains C5 and C9 increases the exclusion of Na^+ and, in anticipation, enhances K^+ uptake, which subsequently led to an increase in proline, polyphenol, and chlorophyll content in maize leaves grown under salt stress (Rojas-Tapias et al., 2012). While studying the short- and the long-term effects of salt stress on *A. thaliana*, the inoculation of *Burkholderia phytofirmans* PsJN was found to attribute tolerance to a high amount of salts *via* alteration in the expression of ion homeostasis-associated genes (HKT1, KT1, SOS1, and Na^+/H^+ exchanger NHX2) (Pinedo et al., 2015). Similarly, the inoculation of *B. subtilis* GB03 to *Puccinella tenuiflora* showed an upregulation in the expression of Pt*SOS1* and Pt*HKT1* with less Na^+ accumulation under a high salt concentration (Niu et al., 2016).

Exopolysaccharide Production

Exopolysaccharide are homo- or hetero-polysaccharides produced by rhizobacteria that enable their survival under inhospitable conditions. Though polysaccharides vary in composition, glucose, galactose, and mannose are abundant monomers that, in association with other sub-unit components such as amino sugars, uronic acids, etc., form a capsule-like protective biofilm on the surface of cells (Upadhyay et al., 2011; Rossi and De Philippis, 2015). Formed under adverse conditions, the adsorption of EPS on soil *via* cation bridges and Van der waals forces stabilizes soil structure and aggregation (Sandhya et al., 2009). Binding soil particles to aggregates, EPS form an enclosed matrix that increases root-adhering soil per root tissue (RAS/RT), conferring protection against environmental fluctuations. The protective EPS capsule possesses strong water-holding capacity that helps in the nutrient uptake by plants besides maintaining a higher water potential around the plant roots, protecting the plant from desiccation and ensuring plant growth and survival under salinity stress (Upadhyay et al., 2011; Selvakumar et al., 2012; Balsanelli et al., 2014). In addition to its role in nodule formation in legume–rhizobia associations, it forms a protective biofilm around the roots, thereby imparting protection to plant against salinity stress (Stoodley et al., 2002; Skorupska et al., 2006). Additionally, EPS rhizosheaths around the plant roots get hold of Na^+ ions, thereby make these unavailable to plants. The inoculation of *Halomonas variabilis* (HT1) and *P. rifietoensis* (RT4) under salinity stress stabilizes soil structures and aggregation, thereby increasing the growth of chickpea (*Cicer arietinum* var. CM-98) (Qurashi and Sabri, 2012). Exerting a capability to fight salt stress, the inoculation of *Bacillus subtilis* to *Helianthus annus* was found to downregulate the expression of $HKT1/K^+$ transporter (Zhang et al., 2008). *Pseudomonas aeruginosa* inoculation reduces salt stress and promoted growth that led to an enhancement in yield in *Helianthus annus* (Tewari and Arora, 2014). EPS are also used as seed priming agents that promote seed germination and, as such, crop yield under salinity stress conditions (Tewari and Arora, 2014). The seed inoculation of *Enterobacter* sp. MN17 and *Bacillus* sp. MN54 of *Chenopodium quinoa* results in improved plant water relation following growth under a high salt (400mM NaCl) concentration (Yang et al., 2016). The inoculation of *B. subtilis* subsp. *inaquosorum* and *Marinobacter lipolyticus* SM19 significantly reduces the adverse effects of salinity stress in wheat (Atouei et al., 2019). Additionally, the inoculation of halotolerant *Pseudomonas* PS01 strain was found to be associated with the regulation of the expression of genes related to salt stress in *A. thaliana* (Chu et al., 2019).

Production of Volatile Organic Compounds

Volatile organic compounds (VOCs; lipophilic in nature) are low molecular weight compounds that serve as signals for development and systemic response within the same or neighboring plants (Choudhary et al., 2008; Niinemets, 2010). The PGPR-mediated production of VOCs induces a range of physiological changes in plants that stimulate its growth (increasing shoot biomass) besides inducing systemic resistance to disease and controlling the plant pathogens (Lee et al., 2012; Park et al., 2015; Tahir et al., 2017). VOCs promote the biosynthesis of osmo-protectants such as glycine betaine whose accumulation imparts protection to PS-II besides maintaining the enzymatic activity and the membrane integrity of cells under osmotic stress conditions (Mäkelä et al., 2000; Jagendorf and Takabe, 2001). The VOCs of *B. subtilis* reduce salt stress through an enhancement in the tissue-specific expression of the $HKT1/K^+$ transporter that enhances nutrient uptake at the root surface while minimizing the influx of Na^+ to the roots (Zhang et al., 2008). *P. chlororaphis* O6 production of 2R, 3R-butanediol prevents water loss by inducing stomatal closures in *A. thaliana*, thereby imparting tolerance to *A. thaliana* (Cho et al., 2008). The process is mediated by Aba-1 and OST-1 kinases of jasmonic acid, ethylene, and salicylic acid pathways in plants. An increase in the VOC level on priming wheat plants with *B. thuringiensis* AZP2 imparts self-protection to the plants that enhances survival (fivefold higher) with higher photosynthesis, resulting in increased biomass under salt stress conditions (Timmusk et al., 2014). VOCs produced by *P. simiae* up-regulates γ-glutamyl hydrolase, vegetative storage (regulating Na^+ homeostasis), and RUBISCO large-chain (associated with an increase in chlorophyll content and, as such, photosynthesis)

proteins that are considered important in eliciting induced systemic resistance in soybean (*Glycine max*) (Vaishnav et al., 2015). Butanoic acid released by *Alcaligens faecalis* strain JBCS1294 attribute salt tolerance to plants *via* reprogramming of auxin and gibberellin pathways (Bhattacharyya et al., 2015). A blend of 7-hexanol, 3-methylbutanol, and 2-undecanone was found effective in mimicking VOCs in attributing plant growth effects on inoculation with different bacterial species (Ledger et al., 2016).

Antioxidant Production

Reactive oxygen species (ROS; including superoxide $O_2^{-\cdot}$, hydroxyl radical $OH^{\cdot}$, hydrogen peroxide H_2O_2, etc.), generated as a metabolic by-product in plants, functions primarily as a signaling molecule. Abnormality in the cellular metabolic process of plants growing under stress conditions enhances the production of ROS, which results in DNA damage, changes in redox state, abnormality in protein formation, denaturation of membranous proteins, lipid peroxidation, reduction in membrane fluidity, interference with enzymatic activity, and overall homeostasis of cell that progresses to cell damage and even to plant cell death (Miller et al., 2010; Halo et al., 2015). Under such conditions, both enzymatic (SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase, etc.) and non-enzymatic antioxidants (GSH, glutathione; tocopherols; ascorbic acid, etc.) play a vital role in neutralizing the ROS and, as such, protect plant cells against oxidative stress (Kim et al., 2014; Kaushal and Wani, 2015). In this regard, PGPRs extend their antioxidant enzyme machinery as protection to plants against oxidative stress. Salt stress induction triggers adaptive response mechanisms, including the accumulation of compatible compounds (organic and inorganic) that decrease the hydraulic conductivity of membranes for reducing cellular osmotic stress (Hasegawa et al., 2000; Munns, 2002a; Abdul-Jaleel et al., 2007). The inoculation of *Pseudomonas* sp. to basil plants (*Ocimum basilicum* L.) grown under stress conditions results in increasing the CAT activity, while the application of a microbial consortia (*Pseudomonas* sp., *B. lentus*, and *A. brasilense*) results in enhancement in APX and GPX (Heidari and Golpayegani, 2011). Similarly, tomato seedlings inoculated with *Enterobacter* spp. showed an increase in APX activity (Sandhya et al., 2010), while the inoculation of PGPR to gladiolus showed an enhancement in SOD and CAT activities (Damodaran et al., 2013). The inoculation of PGPR to *Solanum tuberosum* grown under stress conditions results in an enhancement in the activity of APX, SOD, CAT, and glutathione reductase (Gururani et al., 2013). The inoculation of *B. amyloliquefaciens* NBRISN13 (SN13) of rice grown under salinity stress results in an enhancement in chlorophyll content and plant biomass besides increasing proline content and the expression of antioxidant enzymes such as CAT (Nautiyal et al., 2013). An up-regulation in stress-responsive genes associated with proline biosynthesis was observed on treating *A. thaliana* with *Enterobacter* sp. (Kim et al., 2014).

The inoculation of microbial consortia (*A. nitroguajacolicus* strain YB3 and YB5, *P. jessenii* R62, and *P. synxantha* R81) to IR-64 variety of rice grown under stress conditions induces and, as such, enhances SOD, peroxidase (POD), CAT, and APX levels (Gusain et al., 2015). A significant increase in the transcription of stress-responsive genes, *AtRSA1* (associated with ROS detoxification) and *AtWRKY8* (associated with maintenance of ion homeostasis), while reducing the expression of *AtVQ9* (negative regulator of *AtWRKY8*), was observed on inoculating *Paenibacillus youginensis*-to *A. thaliana* seedlings (Sukweenadhi et al., 2015). The inoculation of maize seedling with *B. amyloliquefaciens* SQR9 improved the glutathione, POD, and CAT levels besides showing an enhancement in soluble sugar and chlorophyll content (Chen et al., 2016). The physiological effects of the treatment were assessed as enhancement in *RBCL* (related to photosynthesis), *HKT1*, and *NHX-1*, *-2*, and *-3* genes. Modulation in the expression of complete gene families associated with abscisic acid (ABA) signaling, ion transport, SOS pathway, and antioxidants was observed on inoculating wheat with salt-tolerant *Dietzia natronolimnaea* (Bharti et al., 2016). The inoculation of soybean by *P. simiae* strain AU results in the enhancement of pyrroline-5-carboxylase synthase, associated with the synthesis of proline as part of tolerance to stress conditions (Vaishnav and Choudhary, 2019). The study goes well with previous reports regarding the enhancement in proline content during stress conditions (Ghosh et al., 2018; Patel et al., 2018). The inoculation of *Azospirillum lipoferum* FK1 of chickpea exhibited enhanced antioxidant enzyme levels besides demonstrating an increase in nutrient uptake and, as such, improvement in its growth and development (El-Esawi et al., 2019). The bacterial consortium of *P. fluorescens* S3, *B. mojavensis* S1, and *B. pumilis* mitigates salt-induced growth inhibition of barley through an enhancement in the water conductance and the nutrient uptake of plants. The inoculation of rice with *Trichoderma asperellum* and *P. fluorescens* results in an enhancement in the activity of POD, APX, SOD, and CAT that contributes to the alleviation of salt stress (Singh et al., 2020).

Enzymes and Metabolites of Bacterial Origin

Plant diseases are considered as a major constraint to crop yield. It has been observed that salinity stress contributes to an increase in the susceptibility of plants to attacks by different pathogens (Besri, 1993). As the usage of chemicals in the control of plant pathogens imparts deleterious effects, PGPR emerged as a potential substitute as a biological control strategy in the management of pathogen-associated diseases in plants (Compant et al., 2010; Etesami and Alikhani, 2018). PGPR-based mechanisms employed in the biological control of pathogens include the following:

(1) Synthesis of cell-wall-degrading enzymes: The production of hydrolytic enzymes such as cellulases, glucanases, chitinases, protease, etc., hydrolyzing polymeric compounds such as cellulose, hemicellulose, chitin, cell wall proteins, etc., was found capable of inhibiting a variety of plant pathogens (Pal and Gardener, 2006; Mabood et al., 2014; Husson et al., 2017; Vaddepalli et al., 2017). Similarly, protease produced by different PGPR agents was found effective in reducing the infections of *Fusarium* sp. and *M. phaseolina* (Dunne et al., 1997;

Gohel et al., 2004). The biocontrol potential of chitinase produced by *Paenibacillus illinoissensis* spp. provides protection against blight and damping off diseases in pepper (*Capsicum annuum*) caused by *Phytophthora capsica* and *Rhizoctonia solani* (Jung et al., 2003, 2005). Chitinase produced by *B. suly* reduces the infection severity of *Fusarium* sp. under greenhouse conditions (Hariprasad et al., 2011). The production of chitinases together with β-1,3-glucanases by PGPR such as *B. subtilis* BSK17 for utilizing them as a source of carbon is of prime importance as it forms a major enzyme group capable of degrading the chitin and laminarin components of fungal cell walls (Kumar et al., 2012; Dubey et al., 2014).

(2) Synthesis of antimicrobial metabolites: With maximum reports from *Bacillus* and *Pseudomonas* genera, the production of a wide range of metabolites was found to restrict the growth of pathogens (Couillerot et al., 2009; Olanrewaju et al., 2017).

(3) HCN production by *Pseudomonas* sp., *Bacillus* sp., *Rhizobium*, etc., was found capable of inhibiting cytochrome C oxidase along with other metalloenzymes (Nandi et al., 2017).

(4) The synthesis of siderophores by different PGPR strains possessing a high affinity for Fe^{3+} ions chelates it and, as such, deprives pathogens of this essential mineral (Shen et al., 2013; Olanrewaju et al., 2017).

(5) Prime plants for induction of induced systemic resistance that imparts a faster and stronger response to attacks by different pathogens (Olanrewaju et al., 2017).

Maintenance of Hormonal Balance

Phytohormones regulating plant growth and developmental processes attributes plants protection by imparting tolerance to cope up with diverse changes in the environment (Ryu and Cho, 2015). The exogenous application of phytohormones supplementing the internal hormonal pool was found effective in counteracting the deleterious effects of salt stress (Zahir et al., 2010). The exogenous application of indole-3-acetic acid (IAA) was found effective in stimulating the growth of roots and leaves, thereby alleviating salinity-induced reduction in plant productivity (Albacete et al., 2008; Dodd and Perez-Alfocea, 2012). Diminishing the endogenous hormonal level, metabolites, hormones, and enzymes produced by salt-tolerant (ST) PGPR complements the hormonal status of plants and, as such, contributes to the enhancement of salt tolerance in plants grown under salt stress (Egamberdieva and Kucharova, 2009; Ilangumaran and Smith, 2017). A common trait of PGPR, production of IAA, was found to increase the fitness of plants grown under salinity stress (Dodd et al., 2010; Tiwari et al., 2011). Tryptophan in root exudates is utilized by rhizobacteria for its conversion through multiple routes to IAA for it to be readily absorbed by plant roots (Spaepen and Vanderleyden, 2011; Ilangumaran and Smith, 2017). Complementing the endogenous IAA pool of plants, its function in plants depends on the internal IAA levels (ranging in function from promotion to inhibition of plant growth). Required for cell division and elongation in plants, the inoculation of ST-PGPR *P. putida* modulated internal IAA pools that resulted in an increase in the growth parameters in cotton plants grown under salinity stress (Yao et al., 2010; Egamberdieva et al., 2017). The inoculation of *P. stutzeri*, *P. putida*, and *Stenotrophomonas maltophilia* to *Coleus* plants was found to lead the production of IAA, cytokinin, and gibberellic acid (Patel and Saraf, 2017). The short-term treatment of *Enterobacter* sp. EJ01 increased the expression of salt stress-responsive genes such as late embryogenesis abundant (*RAB18*), DRE-binding protein (*DREB2b*), stress-inducible priming process (*MPK3* and *MPK6*), etc., genes in *Arabidopsis thaliana*, while increasing the ROS scavenging activity of *Solanum lycopersicum* grown under salinity stress (Ilangumaran and Smith, 2017). The inoculation of halotolerants was found to be associated with an increase in the secretion of salicylic acid that leads to an enhancement in the growth of sunflower plant (Tewari and Arora, 2018). The inoculation of *Leclerciaa decarboxylata* MO1 in *Solanum lycopersicum* showed an improvement in chlorophyll fluorescence besides increasing sugar synthesis and the production of organic acids (Kang et al., 2019).

Cytokinin (CK) is another important class of phytohormones that assists plants in growth and development and in attributing resistance to different stresses (O'Brien and Benková, 2013). Though a common trait of PGPRs, they suffice plants of CK by either synthesizing it or altering its homeostasis in plants (Dodd et al., 2010; Pallai et al., 2012; Kapoor and Kaur, 2016). The inoculation of *Pseudomonas* sp. (*P. aurantiaca* and *P. extremorientalis* TSAU6 and TSAU20) results in alleviating the salinity-induced dormancy of wheat seeds besides enhancing their growth under salinity stress conditions (Egamberdieva, 2009). The inoculation of *B. subtilis* strain in *Platycladus orientalis* and lettuce plant showed an enhanced root-to-shoot signaling of CK, thereby improving plant growth under stress conditions (Arkhipova et al., 2007; Liu et al., 2013). The ability of PGPRs to synthesize CK highlights their importance in stimulating plant growth.

Gibberellins (GA) constitute another important class of phytohormones that play an important role in regulating cell division and elongation and in regulating meristematic activity at the roots and the leaves as part of its role in the developmental and physiological processes of plants (Wang et al., 2015; Guo et al., 2015; Martínez et al., 2016). Bottini et al. reported the production of gibberellin by PGPR strains of *B. licheniformis*, *B. pumilis*, and *Azospirillium* spp. (Bottini et al., 2004). Being a key factor associated with the inhibition of plant growth under stress conditions, PGPRs were found to enhance its levels in plants, thereby attributing a tolerance mechanism to plants for growth under salinity stress (Kang et al., 2014a; Martínez et al., 2016; Shahzad et al., 2016). Kang et al. (2014a) reported enhancement in the internal GA pools on inoculating plants with *B. cereus* MJ-1 and *Promicromospora* sp. SE188. A similar effect of regulating plant growth and development was observed on inoculating plants with *B. aryabhattai* SRB02 (Park et al., 2017). The inoculation of *P. aeruginosa* PM389 and ZNP1 together with *B. endophyticus* J13 and *B. tequilensis* J12 results in the alleviation of the stress-induced effects in *A. thaliana* (Ghosh et al., 2019).

Abscisic acid is a stress hormone primarily known for its role in the abscission of leaves and growth of plants. Synthesized under water deficit conditions, it triggers an adaptive response *via* the activation of a set of genes responsible for stress resistance as part of its survival strategy for the plants (Pliego et al., 2011; Sah et al., 2016). Its synthesis in the roots that occurs in response to low water potential triggers the growth of roots and the emergence of lateral roots, contributing to the enhancement in the uptake of water at the root surface (Vaishnav et al., 2016b). Simultaneously, its translocation from roots to leaves progresses with the control of the stomatal closure events toward regulation of water loss by reducing transpiration at the leaf surface (Yamaguchi-Shinozaki and Shinozaki, 1994; Dodd and Perez-Alfocea, 2012; Kaushal and Wani, 2015). PGPRs capable of producing ABA play an important role in plant–PGPR interactions (Dodd, 2003; Naz et al., 2009; Dodd et al., 2010). They either modulate the biosynthesis of ABA or regulate ABA-mediated signaling pathways in plants, thereby contributing to the growth and survival of plants under salinity stress. The inoculation of PGPRs often mitigate the sensitivity of plants to water scarcity by decreasing its accumulation at the roots and significantly altering its long-distance signaling, i.e., shoot-to-root or *vice versa* flow through the phloem and the xylem, respectively (Dodd and Perez-Alfocea, 2012; Jiang et al., 2012; Belimov et al., 2014). The inoculation of *Phyllobacterium brassicacearum* STM196 results in an enhancement of the ABA levels that reduces transpiration at the leaf surface and, as such, enhances salt stress tolerance in *A. thaliana* (Bresson et al., 2013). A few species of PGPR (*Rhodococcus* sp. and *Novosphingobium* sp.) inhabiting rhizospheric regions capable of metabolizing ABA under *in vitro* conditions represent another stress-relieving mechanism for plants (Belimov et al., 2014). The inoculation of plants with ABA-producing PGPRs (*P. fluorescence* Rt6M10, *A. brasilense* SP245, and *B. licheniformis* Rt4M10) results in enhancement in internal ABA pools, thereby increasing plant growth under salinity stress conditions (Salomon et al., 2014; Cohen et al., 2015). A study reported that PGPR stimulated the production of endogenous ABA in plants, relieving them of the effects of being grown under salinity stress (Forni et al., 2017). Both ABA synthesizing and metabolizing PGPRs are capable of modulating the internal ABA status of plants and, as such, are capable of relieving plants to show normal growth even under salinity stress conditions.

Apart from ABA, the synthesis of another stress hormone, ethylene, was found to improve tolerance or expedite senescence (Morgan and Drew, 1997). Ethylene, a gaseous hormone, significantly enhances the response of plants to stress conditions. Acting as a negative regulator of plant growth, ethylene induces its effects by reducing the growth of roots and modulating the nitrogen-fixing capability of plants (Ma et al., 2002; Mahajan and Tuteja, 2005; Gamalero and Glick, 2015). As ethylene-mediated inhibition of the auxin response factor constraints the growth of plants, secretion of 1-aminocyclopropane-1-carboxylase (ACC) deaminase by PGPR hampers its synthesis in plants (Glick et al., 2007). ACC deaminase secretion by PGPR metabolizes ACC (precursor of ethylene in plants) into α-ketoglutarate and ammonia besides altering the expression of genes encoding ACC synthase and ACC oxidase, which are involved in the synthesis of ethylene (Etesami and Beattie, 2017). ACC deaminase-producing strains of *P. fluorescens* and *Enterobacter* spp. produced a significant effect in increasing the yield of maize grown under salt stress conditions (Nadeem et al., 2009; Panwar et al., 2016). The inoculation of *Pantoea dispera* PSB3 to chickpea results in an enhancement in IAA and ACC deaminase production, which led to an improvement in pod size, seed weight, seed number, and altogether plant biomass (Panwar et al., 2016). The plants were also observed to have a higher K^+/Na^+ ratio, owing to a reduction in electrolyte leakage and a decreased uptake of Na^+ besides leading to an increase in leaf water and chlorophyll content and enhancement in K^+ uptake.

CONCLUSION AND FUTURE PERSPECTIVES

Though much progress has been made in understanding the different attributes of plant–microbe interactions and in formulating methodologies for crops grown under salinity stress, we still lag behind in achieving sustainability in plant productivity. With rising emphasis on environmental protection and sustainability in agriculture for food security, the timely mitigation of the adverse effects of different stresses, in a cost-effective manner, is required. For this to be realized, it becomes imperative to explore novel aspects of the plant-beneficial soil microbiota in relieving plants of stressful conditions. Microbiota from diversified environments needs characterization and exploration in terms of their acclimatization, in-depth knowledge of their ameliorative strategies for growth under stress conditions, and in acquiring knowledge of the intriguing mechanisms commonly employed in attributing plants with a potential to thrive in harsh edaphic conditions. As the efficiency of the microbiota depends on soil characteristics and plant species, a better understanding of plant–microbial interactions in the context of manipulation of stress-responsive genes in plants need further elucidation in terms of revealing their functionalities toward boosting plant defense and attaining enhancement in overall productivity. As the soil microbiota provides beneficial attributes to plants in withstanding salinity stress, newer prospects of understanding in the operational module of regulatory network-mediated plant defense in achieving tolerance against different stresses need to be undertaken in a timely manner. The same goes in terms of prospects of developing novel bioinoculants that could enhance the stability of crops grown under stress conditions and, as such, increase their productivity when grown in nutritionally poor agroecosystems. In addition to the screening and the optimization of PGPR strains for plant-beneficial characteristics under changing environmental conditions, the CRISPR/Cas approach in editing interactive networks of stress-responsive genes needs to be undertaken for their profound effect (metabolic, regulatory, and signaling) in

overcoming stress and inducing tolerance in plants and their interacting partners toward attaining sustainability in agriculture production.

AUTHOR CONTRIBUTIONS

AJ and SR conceived the idea. All authors contributed equally in generating the draft of the different sections and in the repeated editing of the contents present in the finalized version of the manuscript.

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frontiers in Microbiology

REVIEW
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Insights Into Microbially Induced Salt Tolerance and Endurance Mechanisms (STEM) in Plants

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Salt stress threatens the achievement of sustainable global food security goals by inducing secondary stresses, such as osmotic, ionic, and oxidative stress, that are detrimental to plant growth and productivity. Various studies have reported the beneficial roles of microbes in ameliorating salt stress in plants. This review emphasizes salt tolerance and endurance mechanisms (STEM) in microbially inoculated (MI) plants that ensure plant growth and survival. Well-established STEM have been documented in MI plants and include conglomeration of osmolytes, antioxidant barricading, recuperating nutritional status, and ionic homeostasis. This is achieved via involvement of P solubilization, siderophore production, nitrogen fixation, selective ion absorption, volatile organic compound production, exopolysaccharide production, modifications to plant physiological processes (photosynthesis, transpiration, and stomatal conductance), and molecular alterations to alter various biochemical and physiological processes. Salt tolerance and endurance mechanism in MI plants ensures plant growth by improving nutrient uptake and maintaining ionic homeostasis, promoting superior water use efficiency and osmoprotection, enhancing photosynthetic efficiency, preserving cell ultrastructure, and reinforcing antioxidant metabolism. Molecular research in MI plants under salt stress conditions has found variations in the expression profiles of genes such as *HKT1*, *NHX*, and *SOS1* (ion transporters), *PIPs* and *TIPs* (aquaporins), *RBCS, RBCL* (RuBisCo subunits), *Lipoxygenase2* [jasmonic acid (JA) signaling], ABA (abscisic acid)-responsive gene, and *APX, CAT*, and *POD* (involved in antioxidant defense). Proteomic analysis in arbuscular mycorrhizal fungi-inoculated plants revealed upregulated expression of signal transduction proteins, including Ca^{2+} transporter ATPase, calcium-dependent protein kinase, calmodulin, and energy-related proteins (NADH dehydrogenase, iron-sulfur protein NADH dehydrogenase, cytochrome C oxidase, and ATP synthase). Future research should focus on the role of stress hormones, such as JA, salicylic acid, and brassinosteroids, in salt-stressed MI plants and how MI affects the cell wall, secondary metabolism, and signal transduction in host plants.

Keywords: salt stress, microbes, ion transporters, signal transduction, aquaporins, photosynthesis

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INTRODUCTION

Salinity or salt stress is a major threat to agricultural productivity and global food security. It can affect plant growth and development and thus reduce the biomass productivity of plants in arid and semiarid regions. Salt stress is detrimental to plant growth because it induces osmotic and ionic stress in plants, leading to reduced water uptake, transpiration, photosynthesis, and disrupted ionic homeostasis. Moreover, increased levels of reactive oxygen species (ROS) cause oxidative stress, which damages DNA, proteins, and membranes (Liu et al., 2016). Recent studies have confirmed that microbes can induce salt tolerance and endurance mechanisms (STEM) in plants (**Table 1**) to enable growth and development under harsh stress conditions (Porcel et al., 2016; Barnawal et al., 2017; Chen et al., 2017; Sapre et al., 2018; Yasin et al., 2018; Jia et al., 2019). The various functions of STEM mediating this process can be summarized as follows: (i) conglomeration of osmolytes to abate osmotic stress (Hajiboland et al., 2010; Talaat and Shawky, 2011; Evelin and Kapoor, 2014; Elhindi et al., 2017; Wu et al., 2017; Garg and Bharti, 2018; Hashem et al., 2018); (ii) antioxidant barricading to block oxidative stress (Bharti et al., 2016; Qin et al., 2016; Chang et al., 2018; Chu et al., 2019; Ye et al., 2019); (iii) recuperating nutritional status and ionic homeostasis through P solubilization, siderophore production, nitrogen fixation, ion transporter activity, and exopolysaccharide (EPS) production (Porcel et al., 2016; Elhindi et al., 2017; Zhou et al., 2017; Chang et al., 2018); (iv) physiological modifications in the plant (Barnawal et al., 2017; Chen et al., 2017; Elhindi et al., 2017; Hashem et al., 2018; Ren et al., 2018); and (v) molecular modification of stress-responsive gene expression (Barnawal et al., 2017; Yasin et al., 2018; El-Esawi et al., 2019; Jia et al., 2019). Plant growth-promoting rhizobacteria (PGPR) have been reported to have mitigative effects on the growth of pepper (Yasin et al., 2018), wheat (Bharti et al., 2016; Barnawal et al., 2017), soybean (Khan et al., 2019), oat (Sapre et al., 2018), Panax (Sukweenadhi et al., 2018), and maize (Chen et al., 2016) under salt stress conditions. Similarly, colonization by arbuscular mycorrhizal fungi (AMF) also ameliorated the effects of salt stress in wheat (Fileccia et al., 2017), rice (Porcel et al., 2016), watermelon (Ye et al., 2019), and cucumber (Hashem et al., 2018). The STEM exhibited by PGPR and AMF are illustrated in **Figure 1**.

CONGLOMERATION OF OSMOLYTES AND WATER HOMEOSTASIS TO ABATE OSMOTIC STRESS

In its initial phase, salt stress can be referred to as physiological drought because elevated ion levels during salt stress change the soil texture to reduce soil porosity and decrease water uptake. Osmolyte conglomeration is a major STEM that improves water uptake in microbially inoculated (MI) plants. This reduces the water potential by accumulating osmolytes, such as amino acids (proline), amines (e.g., glycinebetaine, polyamines), sugars, and organic acids (e.g., oxalate, malate). In addition to osmotic adjustment, these osmolytes are responsible for conserving membrane integrity, protein stability, and ROS scavenging to ultimately promote their positive effects on plant physiological functions, such as growth, photosynthesis, and crop yield, during salt stress (Zou et al., 2013). Proline and glycinebetaine enhance protein and membrane stabilization to impart osmoprotection to salt-stressed plants. However, contrasting results regarding proline production have been reported in MI and non-inoculated (NI) plants. Increased proline content in NI plants compared to AMF-inoculated (AI) plants can indicate higher stress conditions. AI plants show decreased proline content because microbial colonization helps the plant mitigate the stress. Some studies have suggested that proline accumulation is a salinity stress indicator rather than a consequence of mycorrhizal colonization (Sheng et al., 2011; Echeverria et al., 2013; Evelin et al., 2013; Evelin and Kapoor, 2014); however, other studies have found higher proline accumulations caused by AM colonization (Hajiboland et al., 2010; Talaat and Shawky, 2011; Garg and Baher, 2013; Elhindi et al., 2017; Hashem et al., 2018). Higher proline levels have been observed in PGPR-inoculated maize (Ullah and Bano, 2015), *Gladiolus* (Damodaran et al., 2014), *Mentha* (Bharti et al., 2014), *Chrysanthemum* (Wang et al., 2018), and *Panax* (Sukweenadhi et al., 2018). Increased proline levels in MI plants can be due to (i) upregulated expression P5CS, a gene involved in proline synthesis; (ii) increased efficiency of the enzymes P5CS and glutamate dehydrogenase (involved in glutamate synthesis) given that proline is synthesized from glutamate; and (iii) arrest of proline dehydrogenase (responsible for proline degradation) (Abo-Doma et al., 2016). In the nodules of AI-inoculated pigeon pea plants, reduced activity of trehalase (trehalose degrading enzyme) and increased activity of trehalose-6-P synthase and trehalose-6-phosphatase (enzymes involved in the biosynthesis of trehalose) led to higher trehalose levels (Garg and Pandey, 2016). Higher concentrations of acetic, malic, citric, oxalic, and fumaric acids were observed in AI maize plants compared to NI plants and led to enhanced salinity tolerance (Sheng et al., 2011). Arbuscular mycorrhizal fungi treatment alters polyamine levels in plants to impart stress tolerance (Evelin et al., 2013; Talaat and Shawky, 2013). Evelin et al. (2013) observed increased spermidine and spermine (Spd + Spm)/putrescine (Put) ratios in AI fenugreek plants compared to NI plants. Salinity tolerance in MI plants is advantageous and can correlate with their ability to join DNA, proteins, and phospholipids. Polyamine levels were modulated in response to mycorrhizal colonization in two cultivars of wheat (Sids 1 and Giza 168) under saline conditions. AM colonization led to higher putrescine but lower spermidine and spermine levels in Giza 168; however, in Sid 1, a reduction in putrescine and an increase in spermidine and spermine levels were reported (Talaat and Shawky, 2013). The accumulation of total soluble sugars (TSS), such as glucose, sucrose, and maltose, during salt stress in MI plants is another mode of STEM via osmotic adjustment. Conversion of starch into dextrins and maltose is accompanied by a- and b-amylases, respectively. Researchers have confirmed that enhanced salt stress tolerance in MI plants is due to higher TSS accumulation (Talaat and Shawky, 2011; Liu et al., 2016; Garg and Bharti, 2018; Zhu et al., 2018). Arbuscular mycorrhizal fungi inoculation modifies

TABLE 1 | STEM in various plant species under salt stress.

Microbial species	Plant	STEM in host plants	References
Enterobacter spp. EJ01	*Arabidopsis thaliana*	IAA, increased expression of APX, salt stress-responsive genes such as *DREB2b*, *RD29A*, *RD29B*, and *RAB18* in *Arabidopsis*	Kim et al., 2014
Claroideoglomus etunicatum	*Oryza sativa*	Increased net photosynthetic rate, stomatal conductance, and transpiration rate	Porcel et al., 2015
Bacillus spp. and *Arthrobacter pascens*	*Zea mays* L.	P solubilization, siderophore production, osmolyte accumulation, and higher antioxidant enzyme activity	Ullah and Bano, 2015
Klebsiella, Pseudomonas, Agrobacterium, and *Ochrobactrum*	Groundnut	IAA production, N_2 fixation, phosphate solubilization, ACC deaminase activity, and HCN production	Sharma et al., 2016
Dietzia natronolimnaea	*Triticum aestivum*	Altered ABA signaling cascade upregulated *TaABARE* and *TaOPR1*, which upregulated and increased expression of *TaST (*a salt stress-induced gene) and proline content	Bharti et al., 2016
Variovorax spp.	*Pisum sativum*	Improved plant water relations, ion homeostasis, and photosynthesis	Wang et al., 2016
Pantoea dispersa PSB3	*Cicer arietinum*	Decreased Na^+ uptake and elevated chlorophyll and K^+ uptake as well as relative leaf water levels	Panwar et al., 2016
Bacillus amyloliquefaciens SQR9	*Zea mays*	Higher chlorophyll and antioxidant production, Na^+ exclusion from roots; increased expression of *RBCS*, *RBCL* (RuBisCo subunits), ion transporters (*HKT1*, *NHX1*, and *NHX2*), and *H(C)-Ppase* (encoding HC pumping pyrophosphatase)	Chen et al., 2016
Enterobacter spp. UPMR18	*Abelmoschus esculentus*	Increase antioxidant enzyme activities and upregulation of antioxidant pathway genes (CAT, APX, and GR)	Habib et al., 2016
Bacillus, Marinobacterium, Enterobacter, Pantoea, Pseudomonas, Acinetobacter, Rhizobium, and *Sinorhizobium*	*Triticum aestivum* L.	IAA and siderophore production	Sorty et al., 2016
Claroideoglomus etunicatum	*Oryza sativa*	Reduced Na^+ root-to-shoot distribution, upregulation of *OsNHX3, OsSOS1, OsHKT2;1*, and *OsHKT1*;5 genes	Porcel et al., 2016
Funneliformis Mosseae	*Cicer arietinum*	Improved nutrient uptake, reduced chlorophyll pigment damage, and higher *RUBISCO* activity	Garg and Bhandari, 2016b
Bacillus subtilis NUU4 and *Mesorhizobium ciceri* IC53	*Cicer arietinum* L.	Increased proline content and P solubilization and improved nutrient acquisition and symbiotic performance of rhizobia	Egamberdieva et al., 2017
Bacillus amyloliquefaciens	*Oryza sativa* L.	Reduced ABA and higher SA, upregulated production of glutamic acid and proline	Shahzad et al., 2017
F. mosseae and *R. irregularis*	*Cajanus cajan*	Higher GR, APX, and SOD activity	Pandey and Garg, 2017
Micrococcus yunnanensis, Planococcus rifietoensi, and *Variovorax paradoxus*	*Beta vulgaris* L.	N_2 fixation, IAA and siderophore production, P solubilization, and ACCd activity	Zhou et al., 2017
Microbacterium oleivorans KNUC7074, *Brevibacterium iodinum* KNUC7183 and *Rhizobium massiliae* KNUC7586	*Capsicum annum* L.	High total soluble sugar, proline contents, Chl contents, and activity of several antioxidant enzymes	Hahm et al., 2017

(Continued)

TABLE 1 | Continued

Microbial species	Plant	STEM in host plants	References
Rhizophagus Irregularis and *Funneliformis mosseae*	*Triticum durum* Desf.	Improved nutrient use efficiency	Fileccia et al., 2017
Bacillus amyloliquefaciens FZB42	*Arabidopsis thaliana*	Upregulated expression of genes correlated to photosynthesis, ROS scavenging, auxin, Na^+ translocation, and JA signaling	Liu et al., 2017
Rhizophagus irregularis	*Robinia pseudoacacia* L.	Improved photosynthesis due to higher expression of three chloroplast genes (*RppsbA*, *RppsbD*, and *RprbcL*) in leaves upregulated expression of three genes (*RpSOS1*, *RpHKT1*, and *RpSKOR*) encoding membrane transport proteins involved in K^+/Na^+ homeostasis in roots	Chen et al., 2017
P. fluorescens SA8 with kinetin (10 mM)	Black gram	Improvement in water use efficiency, gas exchange, and photosynthetic content	Yasin et al., 2018
Funneliformis mosseae and *Diversispora versiformis*	*Chrysanthemum morifolium*	Enhanced uptake root N	Wang et al., 2018
Arthrobacter nitroguajacolicus	*Triticum aestivum* L.	Higher expression of genes such as Cytochrome P450s, APX, Oligopeptide transporters (OPTs), ATP binding cassette (ABC) transporters, Sugar/inositol transporter, ATPase, and ion transporter	Safdarian et al., 2019
C. etunicatum, Rhizoglomus intraradices, and *G. mosseae*	*Cucumis sativus*	Elevated K, Ca, Mg, Fe, Zn, Mn, and Cu content Reduced Na content, higher total phenol as well as activities of SOD, CAT, APX, and GR	Hashem et al., 2018
Paenibacillus yonginensis DCY84T	*Panax ginseng*	Higher nutrient availability and expression of salt-defense-related genes viz. ABA synthesis genes, ROS scavenging genes, and ion-pump-related genes	Sukweenadhi et al., 2018
Klebsiella spp.	*Avena sativa*	*rbcL* and *WRKY1* altered expression levels	Sapre et al., 2018
Rhizophagus irregularis	*Elaeagnus angustifolia* L.	Higher activities of SOD, CAT, and APX, increased uptake of K^+, Ca^{2+}, and Mg^{2+}	Chang et al., 2018
Glomus tortuosum	*Zea mays*	Increased Chl content, RuBisCO activity, and net photosynthetic rate	Xu et al., 2018
Funneliformis mosseae	*Citrullus lanatus* L.	Reduced expression level of PPH (chlorophyll degradation), higher net photosynthesis rate and increased expression of antioxidant response-related genes Cu-Zn SOD, CAT, APX, and GR	Ye et al., 2019
Azospirillum lipoferum FK1	*Cicer arietinum* L.	Improved nutrient acquisition, photosynthetic pigment synthesis, and osmolyte content, and higher antioxidant defense	El-Esawi et al., 2019
Pseudomonas spp.	*Arabidopsis thaliana*	Upregulation in *LOX2*	Chu et al., 2019
Rhizophagus intraradices and *Funneliformis mosseae*	*Arundo donax* L.	Improved nutrient use efficiency	Romero-Munar et al., 2019
Rhizophagus irregularis	*E. angustifolia*	Improved efficiency of photosystem II and enhanced expression of proteins involved in secondary metabolism, antioxidant defense, and signal transduction	Jia et al., 2019

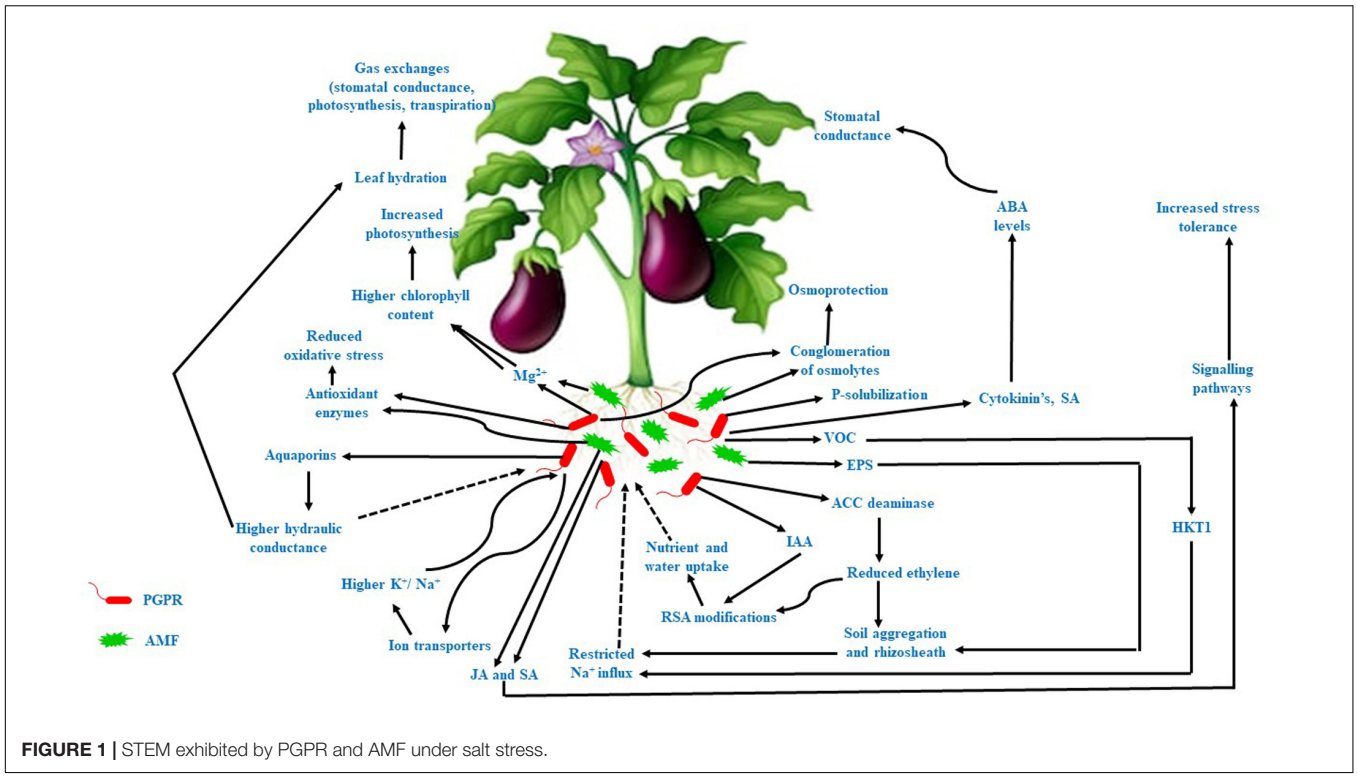

FIGURE 1 | STEM exhibited by PGPR and AMF under salt stress.

leaf sucrose and proline metabolism by regulating the enzymatic activities responsible for sucrose and proline metabolism to enhance osmotic tolerance in the host plant (Wu et al., 2017). Elevated TSS content can be caused by increased photosynthesis, amylase activity, and increased organic acid levels (Yu et al., 2015; Garg and Bharti, 2018; Zhu et al., 2018). Studies in MI chickpea plants have demonstrated that increased salt tolerance can be achieved by the synthesis of proline, glycinebetaine, and increasing TSS (Qurashi and Sabri, 2012; Upadhyay and Singh, 2015). Elevated glycinebetaine levels enhance salinity tolerance in rhizobacterially primed rice plants (Jha et al., 2011), AI wheat (Talaat and Shawky, 2011), and maize (Sheng et al., 2011). According to Rangel (2011), bacteria grown under glucose concentration have low cAMP levels, but when grown under carbon starvation, bacteria show higher cAMP levels. However, the converse is true for eukaryotes. This aspect needs to be addressed in relation to plant microbial crosstalk under salt stress conditions. Future research should focus on unraveling the molecular mechanisms underlying the role of microbes in promoting osmotic adjustment during salt stress.

ANTIOXIDANT BARRICADING TO CAULK THE OXIDATIVE STRESS

The hyperosmotic and hyperionic conditions present during salt stress disrupt cellular redox homeostasis by disrupting the equilibrium between the generation and elimination of ROS, leading to oxidative stress as a secondary stress. Reactive oxygen species target various biomolecules, including nucleic acids, proteins and fatty acids, to alter cellular function, cause DNA damage, reduce membrane fluidity, cause lipid peroxidation, and affect enzymatic activity. It is evident that ROS create oxidative stress; however, they are also involved in ethylene accumulation, auxin biosynthesis, and many signaling events (Kaushal, 2019). Thus, it is essential that an equilibrium is maintained between ROS generation and ROS scavenging systems to balance oxidative damage while managing endogenous signaling events. Plants are equipped with a robust antioxidant system consisting of enzymatic [superoxide dismutase (*SOD*), catalase (*CAT*), ascorbate peroxidase (*APX*), monodehydroascorbate reductase (*MDHAR*), and glutathione reductase (*GR*)] and non-enzymatic (cysteine, carotenoids, glutathione, tocopherols, and ascorbate) constituents. The induction of the antioxidative defense system has been shown to be another STEM activated in MI plants to abate salt stress (Talaat and Shawky, 2011; Li et al., 2012; Bharti et al., 2016; Chang et al., 2018). Higher antioxidant activities have been observed in AI tomato (Hajiboland et al., 2010; Latef and Chaoxing, 2011), *Sesbania* (Abd Allah et al., 2015), pigeon pea (Pandey and Garg, 2017), and *Cucumis* (Hashem et al., 2018) plants during salt stress. Wu et al. (2016), while investigating the impact of AMF colonization and salt stress on male and female *Populus cathayana* seedlings, observed significant increases in the activities of *SOD* and *CAT* in the roots of AI-colonized plants compared to those of NI plants; however, *CAT* activity was similar in the leaves of AI and NI plants. Three-way ANOVA revealed that the activities of *SOD*, *POD*, and *CAT* in roots were influenced by AMF × salt × sex, salt × sex, and AMF × sex, AMF × sex, and AMF × sex × salt, respectively. This demonstrated that

the activities of different antioxidant enzymes were variably affected by the interactions between salt stress, gender, and AMF. A significant increase in the expression levels of genes related to the antioxidant response, such as Cu-Zn *SOD*, *CAT*, *APX*, and *GR*, was reported during salinity alkalinity stress and was further enhanced by AMF inoculation, thereby enabling host watermelon plants to cope with the stress (Ye et al., 2019). Higher root, stem, and leaf biomass was observed in AI seedlings of *Elaeagnus angustifolia* L. during salt stress, which was attributed to the increased activities of *SOD*, *CAT*, and *APX* in the leaves relative to those of NI plants (Chang et al., 2018). In addition to increased activity and expression levels of *CAT*, *GPOX*, *APX*, *SOD*, *MDHAR*, *DHAR*, and *GR*, AI *Cicer arietinum* plants also demonstrated enhanced levels of GSG, GSSH, and total glutathione (Garg and Bhandari, 2016a). Similar observations of STEM involving higher antioxidant barricading have been reported in rhizobacterially inoculated (RI) plants (Gururani et al., 2013; Kim et al., 2014; Tewari and Arora, 2014; Ullah and Bano, 2015; Bharti et al., 2016; Kaushal and Wani, 2016a; Singh and Jha, 2017). A significant increase in the specific activities of *APX* (1.4 times), *SOD* (2.4 times), and *CAT* (1.8 times) was observed in RI *Solanum* plants under salt stress, and antioxidant enzyme activity was positively correlated with the mRNA expression levels of the corresponding genes encoding these enzymes (Gururani et al., 2013). In a similar study, higher activities of *APX* and *CAT* and upregulation of antioxidant pathway genes (*CAT*, *APX*, and *GR*) were observed in *Enterobacter* spp. UPMR18-colonized okra plants, improving the physiological performance and salt tolerance of the plants (Habib et al., 2016). In *Arabidopsis thaliana* roots colonized with *Burkholderia phytofirmans* PsJNA, genes involved in ROS quenching (*APX2*) were significantly more transcribed, helping the plant to abate oxidative stress (Pinedo et al., 2015). In PGPR *Dietzia natronolimnaea* STR1-colonized wheat plants, the gene expression of certain antioxidant enzymes (*APX*, *MnSOD*, *CAT*, *POD*, *GPX*, and *GR*) was enhanced to alleviate salt stress (Bharti et al., 2016). Higher activities of antioxidant enzymes in MI plants can correlate with improved nutritional status (Cu, Mn, and Fe) because these enzymes are in fact metalloenzymes, and their activities are therefore governed by the presence of these nutrients (Kohler et al., 2009). Moreover, the activity of these enzymes also depends on the plant species, microbes, and stress timing. AI plants were able to abate the effect of salt stress by enhancing the activity of antioxidant enzymes, including *SOD*, *CAT*, and *APX*, and increasing ascorbic acid levels, which correlates with lower lipid peroxidation and electrolyte leakage. Zn, Cu, Mn, and Fe serve as co-factors for *SOD* isozymes, and their increased uptake in AI plants was able to boost *SOD* activity (Hashem et al., 2018).

SALINITY TOLERANCE THROUGH RECUPERATION OF NUTRITIONAL STATUS AND IONIC HOMEOSTASIS

Salinity can lead to altered nutritional status and ionic homeostasis in plants, hampering the plant's productivity. Microbial colonization during stress improves the physiological performance of plants with an additional STEM involving enhanced nutrient uptake and selective ion absorption and translocation (Evelin et al., 2012; Bharti et al., 2014; Kang et al., 2014b; Tewari and Arora, 2014; Porcel et al., 2016; Hashem et al., 2018). The availability of nutrients such as P, N, Mn, and Fe is restricted in saline soils. Microbial inoculation simplifies the process of acquiring these nutrients for plants under salt stress conditions to promote plant health and productivity. Plant growth-promoting rhizobacteria strains act as phosphate-solubilizing rhizobacteria to increase the uptake and availability of P to plants (Prasad et al., 2015; Etesami, 2018). Salt tolerance was enhanced in PGPR-colonized *Mentha* (Bharti et al., 2014), wheat (Upadhyay and Singh, 2015), *Chrysanthemum* (Zhou et al., 2017), and groundnut (Shukla et al., 2012) plants due to enhanced phosphate nutrition. Improved phosphorus absorption was found in AI plants under mycorrhizal inoculation, even under salt stress conditions (Sharifi et al., 2007; Al-Khaliel, 2010; Bowles et al., 2016). It is postulated that phosphate is absorbed and converted to polyphosphate by the extraradical mycelium. Recent studies have demonstrated the involvement of AM aquaporins in the translocation of polyphosphate via mycorrhizal hyphae. Thus, adequate P uptake in AI plants helps selective ion absorption, limits toxic ions in vacuoles, and preserves membrane integrity (Evelin et al., 2012) to reverse the effects of salt stress. However, it has been suggested that enhanced growth in MI plants was caused by improved photosynthesis and WUE (water use efficiency) rather than by the increased mineral uptake (Ruiz-Lozano et al., 1996; Garg and Bhandari, 2016b; Chen et al., 2017). Moreover, Feng et al. (2002) reported that salt tolerance in AMF plants was conferred by increased soluble sugar accumulation rather than P levels. Rhizobacterial strains often secrete siderophores to cope with iron deficiency in plants surrounded by saline soil. Siderophores are high affinity low molecular weight Fe (III) chelators that scavenge Fe^{3+} to form an iron–siderophore complex that can be readily solubilized to increase iron availability to plants. Recent studies have reported enhanced salinity tolerance in rhizobacterially colonized plants resulting from siderophore production (Shukla et al., 2012; Sorty et al., 2016; Navarro-Torre et al., 2017; Zhou et al., 2017). Rhizobacterial strains that produce EPSs improve plant growth under salt stress through rhizosheath development around the plant roots, which limits the Na^+ influx inside the stele (Ashraf et al., 2004). In wheat plants, EPS production by PGPR ameliorated salt stress via fusion of Na^+ ions to EPS, leading to enhanced plant nutrition and growth. EPS adheres to soil particles to build macro aggregates that stabilize soil structure and ultimately improve its hydraulic water holding and cation exchange capacity (Upadhyay et al., 2011a,b).

Salt stress impedes plant growth through elevated levels of Na^+ and a lower K^+/Na^+ ratio. *Bacillus*-colonized *Gladiolus* plants displayed increased K^+ uptake relative to Na^+, reducing the Na^+/K^+ ratio under saline conditions (Damodaran et al., 2014). Rhizobacterially inoculated maize plants improved ionic balance by enhancing root K^+ uptake and Na^+ exclusion to confer salt tolerance (Rojas-Tapias et al., 2012). In an interesting study by Pinedo et al. (2015), PGPR *B. phytofirmans* PsJN-primed

Arabidopsis plants were found to sustain salt stress conditions, and this correlated with altered expression of genes involved in ionic equilibrium, such as *Arabidopsis* K^+ Transporter1 (*KT1*), High-Affinity K^+ Transporter1 (*HKT1*), Sodium Hydrogen Exchanger2 (*NHX2*), and *Arabidopsis* Salt Overly Sensitive 1 (*SOS1*). *Bacillus subtilis* GB03-colonized *Puccinellia tenuiflora* displayed increased expression of *PtHKT1* and *PtSOS1* and downregulated expression of *PtHKT2* genes in plant roots. Thus, limiting Na^+ ion uptake in roots and their subsequent translocation reduced Na^+ ion accumulation (Niu et al., 2016). Higher Na^+ concentrations in rhizospheres provide strong competition against K^+ ions, elevating the Na^+/K^+ ratio and causing higher stress by disrupting metabolic and physiological processes. Higher K^+/Na^+ ratios in AI plants is caused by the controlled translocation of Na^+ ions to aboveground tissues of host plants and their accumulation in vacuoles. Salinity caused an escalation in the Na^+ shoot to root ratio levels; however, this ratio was reduced in AMF seedlings (Evelin et al., 2012; Porcel et al., 2016). Additionally, it has been confirmed that enhanced K^+ absorption and reduced Na^+ transportation to shoot tissues lead to higher K^+/Na^+ ratios in AI plants during salt stress (Sharifi et al., 2007; Talaat and Shawky, 2011; Estrada et al., 2013a), which serve to preserve enzymatic processes and protein synthesis.

AMF acts as a primary barrier for absorption of ions during fungal nutrient uptake from soil or their transportation to host plants. This is attributed to the capability of AMF to retain these minerals in intraradical mycelium and vesicles via ionic accumulation in vacuoles (Mardukhi et al., 2011). This type of selective ion absorption (higher K^+, Mg^{2+}, and Ca^{2+} uptake; reduced Na^+ uptake) leads to higher K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ ratios in *Rhizophagus intraradices*. In addition, AI plants have increased Na^+ content, up to a certain limit, which is then reduced at higher salinity levels, suggesting an AMF-induced buffering effect on Na^+ uptake (Hammer and Rillig, 2011). AI-colonized wheat plants showed a significant increase in yield at various salinity levels that correlated to higher levels of N, P, and K, and reduced levels of Na^+ in the leaves (Talaat and Shawky, 2013). Arbuscular mycorrhizal fungi improved K^+ ion retention in maize plant tissues following upregulated expression of *ZmAKT2* and *ZmSKOR* (Estrada et al., 2013b). A significant increase in K^+ levels and decrease in Na^+ levels were observed in AMF plants, suggesting selective uptake of K^+ but not Na^+ into the xylem of plant roots, which thus increases the K^+/Na^+ ratio under salt stress conditions to improve plant growth (Elhindi et al., 2017). Nitrogen assimilation in AI host plants is more efficient due to nitrate assimilation and higher enzyme production in the extraradical mycelia (Evelin et al., 2009; Kapoor et al., 2013). Enzymatic activities and protein synthesis were preserved in AI plants by increased nitrate reductase activity (caused by the elevated nitrate assimilation), increased K^+ accumulation, and an improved K^+/Na^+ ratio (Talaat and Shawky, 2014). Wang et al. (2018) reported enhanced N uptake by plant roots in AI plants, which increased root length and root and shoot weight, to be the major mechanism underlying enhanced salt tolerance in *Diversispora versiformis*-colonized *Chrysanthemum morifolium*. Increased absorption of nutrients including Fe, K, Ca, Fe, Zn, and Mg, but restricted Na and Cl uptake in AI-colonized plants has been reported as a STEM to maintain ionic equilibrium and mitigate the effects of salt stress (Evelin et al., 2012; Kapoor et al., 2013). During salt stress, increased rhizospheric Na^+ levels obstruct Ca^{2+} absorption and therefore disrupt the Ca^{2+}:Na^+ ratio of host plants, ultimately decreasing their hydraulic conductivity and disturbing Ca^{2+} signaling. Improving nutritional status is essential for conserving membrane integrity in AI plants. Mycorrhizal association increased Ca^{2+} and Mg^{2+} absorption by plant roots, even under soil salinity (Giri and Mukerji, 2004; Sharifi et al., 2007). Ca^{2+} levels were increased in *Piriformospora indica* -colonized barley plants, leading to the activation of signal transduction pathways to enhance stress tolerance in host plants (Alikhani et al., 2013). Given that Mg^{2+} is centrally located in the chlorophyll molecule, deceases in its uptake can reduce chlorophyll content and photosynthesis and eventually hamper plant growth. In AI host plants, an increase in Mg^{2+} uptake increases chlorophyll concentration to boost photosynthesis and plant performance while under stress conditions (Abdel Latef and Chaoxing, 2014). Enhanced salt tolerance in *Rhizophagus irregularis*-colonized *E. angustifolia* seedlings was correlated with increased K^+, Ca^{2+}, and Mg^{2+} uptake. Additionally, AM symbioses altered root architecture, and extraradical mycelia improved mineral uptake. K^+ accumulation was increased in the roots and leaves of AI seedlings, leading to an enhanced K^+/Na^+ ratio in the plants and suggesting a STEM in plants (Chang et al., 2018).

MODIFICATIONS IN PLANT PHYSIOLOGICAL STATUS

Microbially induced STEM includes phytohormonal modifications and alterations in other physiological processes, such as gas exchange, photosynthesis, and nutrient and water uptake. Various studies have established the role of microbes in alleviating the negative effects induced by salt stress on plant physiological performance (Porcel et al., 2015; Chen et al., 2017).

Phytohormonal Modulations

Microbes can promote plant growth during salt stress by altering the hormonal status of NI plants. Various phytohormones, including auxins, gibberellins, cytokinin, ethylene, ABA, JA, and SA, are involved in signaling events during plant–microbe interactions that can rescue plants under stress conditions. Numerous studies have reported roles for bacteria in phytohormonal modulations in response to salt stress, but there are relatively few studies related to AMF-mediated phytohormonal salt stress tolerance in host plants. Production of auxins, such as indole acetic acid (IAA), by PGPR strains has been well documented in RI plants and ensures plant survival during salt stress (Egamberdieva, 2009; Jha et al., 2012; Sharma et al., 2016; Sorty et al., 2016; Navarro-Torre et al., 2017). Rhizobacterially inoculated wheat plants showed elevated IAA levels in their rhizospheres compared to NI plants, which led to improved plant growth and survival under stress conditions (Tiwari et al., 2011). Higher root growth was reported in *Pseudomonas chlororaphis* TSAU13-primed wheat

seedlings, tomato, and cucumber plants. IAA production by PGPR *P. chlororaphis* TSAU13 altered phytohormonal levels in plants and consequently enhanced stress tolerance compared to NI plants (Egamberdieva and Kucharova, 2009; Egamberdieva, 2012). Higher IAA levels in wheat plants inoculated with PGPR strains *Arthrobacter protophormiae* and *D. natronolimnaea* enabled host plants to survive salt stress (Barnawal et al., 2017). Chickpea plants co-inoculated with IAA-synthesizing rhizobacterial strains (*B. subtilis* NUU4 and *Mesorhizobium ciceri* IC53) exhibited increased root and shoot biomass, along with enhanced nodule formation relative to untreated plants and plants treated solely with *M. ciceri* IC53 (Egamberdieva et al., 2017). Similar observations of increased IAA levels have been reported in PGPR inoculated peanut (Sharma et al., 2016), barley (Cardinale et al., 2015), and wheat (Singh and Jha, 2016; Sorty et al., 2016). Plant growth-promoting rhizobacteria can modulate GA levels in RI plants (Kang et al., 2014a,b). Increased endogenous levels of gibberellins in *Pseudomonas putida* H-2-3 primed soybean plants (Kang et al., 2014b) and improved plant growth in salt stress conditions. Although cytokinin production is common in microbial strains and imparts stress tolerance to inoculated plants (Liu et al., 2013), very few studies have reported the role of cytokinin in MI plants. Higher proline content, in addition to increased shoot and root biomass, has been reported during salt stress in soybean plants inoculated with the cytokinin synthesizing rhizobacterial strains *Arthrobacter*, *Azospirillum*, and *Bacillus* (Naz et al., 2009). Accelerated ethylene synthesis above threshold values while under stress conditions restricts plant growth by negatively affecting root development and seed germination. However, rhizobacterial strains possessing *ACCd* can limit ethylene levels by cleaving the ethylene precursor, 1-aminocyclopropane-1-carboxylate (ACC), to produce ammonia and a-ketobutyrate. Various studies have reported plant growth promoted by *ACCd*-producing PGPR strains that also alleviates salt stress (Ali et al., 2014; Bharti et al., 2014). Nadeem et al. (2009) reported that *ACCd*-producing PGPR strains (*Pseudomonas fluorescens*, and *Enterobacter* spp.) improved the mineral status of maize plants, thereby helping them to counteract salt stress. Priming chickpea plants with *ACCd*-producing *Pantoea dispersa* PSB3 led to decreased Na^+ uptake and elevated chlorophyll levels and relative leaf water levels, resulting in increased pod number and weight, biomass, and seed weight during salt stress (Panwar et al., 2016). Pea plants primed with *ACCd*-producing *Variovorax paradoxus* 5C-2 displayed root to shoot K^+ ionic flow and Na^+ ion root deposition, which led to elevated K^+/Na^+ ratios in the shoots. Moreover, a higher photosynthesis rate and decreased stomatal resistance caused the plant biomass to increase, thus enhancing stress tolerance (Wang et al., 2016). *Enterobacter* spp. UPMR18-treated okra plants displayed higher antioxidant enzyme activities and increased transcription of ROS pathway genes (Habib et al., 2016). Abscisic acid is a major stress phytohormone capable of alleviating abiotic stress by mediating the important physiological processes of stomatal opening and photosynthesis. Enhanced root and shoot growth were observed in rice plants treated with ABA-producing endophytic bacteria (Shahzad et al., 2017). *D. natronolimnaea* STR1-treated wheat plants showed enhanced salt tolerance via alteration of the ABA signaling cascade, which was confirmed by upregulation of *TaABARE* (ABA-responsive gene) and *TaOPR1* (12-oxophytodienoate reductase 1) genes (Bharti et al., 2016). Treatment of maize plants with *Bacillus amyloliquefaciens* SQR9 conferred salt stress tolerance; treated plants counteracted increased ABA levels and exhibited enhanced chlorophyll levels, glutathione content, and K^+/Na^+ ratios (Chen et al., 2016). A significant increase in JA content and decrease in SA were recorded during salt stress in soybean plants (Kang et al., 2014b). Increased nutrient acquisition and salt stress tolerance were observed in maize plants upon inoculation with SA-synthesizing *Serratia marcescens* (Lavania and Nautiyal, 2013). Rice plants treated with *B. amyloliquefaciens* RWL-1 have been shown to have elevated endogenous SA levels and lower endogenous JA and ABA levels compared to plants treated with GA3 and water (Shahzad et al., 2016). Treatment with *B. megaterium* highlighted the role of *JA-Ile* turnover in the recovery of *Arabidopsis* plants from salt stress (Erice et al., 2017). Elevation of photosynthetic pigments and shoot biomass was reported in soybean plants under salt stress conditions in response to gibberellins produced by *Aspergillus fumigatus* (Khan et al., 2011). Decreased ABA production was found to regulate transpiration rate in cucumber plants colonized by AMF. However, JA and SA synthesis was increased upon AMF inoculation, which decreased oxidative damage to enhance salt stress tolerance (Hashem et al., 2018). In another study, higher levels of JA and its precursor, OPDA, were found in *Digitaria eriantha* colonized by *R. irregularis* under salt stress conditions, demonstrating the key role of JA in conferring salt tolerance to plants (Pedranzani et al., 2016). Strigolactones are the latest class of phytohormones found to be involved in adventitious root formation, reproductive development, and stress responses. A positive correlation was found between ABA and strigolactones in AMF-colonized *Sesbania*. Raised ABA levels caused higher H_2O_2 production, which led to increased SA synthesis and subsequently protected mycorrhizal plants from salt stress (Ren et al., 2018). Aroca et al. (2013) reported that strigolactone production was induced in AM-colonized lettuce plants under salt stress conditions and was correlated to ABA. More research studies targeting the role of AMF in altering phytohormonal levels in plants during salt stress are needed.

Improved Photosynthesis and Other Physiological Changes

Arbuscular mycorrhizal fungi-inoculated plants sustain higher chlorophyll and carotenoid levels through enhanced Mg^{2+} ion uptake (Evelin et al., 2012; Hashem et al., 2015), which is otherwise restricted by salt stress. Physiological changes of AI and RI plants also included a higher quantum yield of PSII and increased net photosynthetic rate (Talaat and Shawky, 2014; Chen et al., 2016, 2017; Hidri et al., 2016; Wang et al., 2016; Yasin et al., 2018) relative to NI or control plants. Glycinebetaine preserves the activities of RuBisCO and rubisco activase involved in CO_2 fixation and secures PSII pigment-protein complexes (Talaat and Shawky, 2014) to confer salt stress tolerance to plants (Porcel et al., 2016; Hu et al., 2017). Higher RuBisCO activity has been reported in AMF plants (Garg and Bhandari, 2016b;

Chen et al., 2017) and is correlated with increased *RprbcL* gene expression (Chen et al., 2017). AM symbiosis alleviates the physiological drought effects on photosynthesis by improving the water status of colonized plants through increases in their leaf area and stomatal conductance (Chen et al., 2017). Arbuscular mycorrhizal fungi can protect the photosystems by reducing non-photochemical quenching in inoculated plants relative to uninoculated ones and thus enhance their photosynthetic efficiency under salt stress conditions (Hu et al., 2017). Higher stomatal conductance and strong photosystem efficiency in AMF-colonized plants reduced photorespiration and lowered ROS production, which subsequently conferred salt stress tolerance (Ruiz-Sánchez et al., 2010). In AM rice plants under salt stress conditions, the enhanced quantum yield of photosystem II and reduced non-photochemical quenching-maintained photosynthesis, transpiration, and stomatal conductance led to increased biomass (Porcel et al., 2015). Ye et al. (2019) reported that AMF alleviated the decrease in the maximum photochemical efficiency of Photosystem II (Fv/Fm), photochemical quenching and increase in non-photochemical quenching (*NPQ*) observed in salt-stressed watermelon plants. Numerous studies have reported higher WUE, stomatal conductance, transpiration rate, and photosynthesis in AI plants exposed to salt stress compared to non-colonized plants (Sheng et al., 2008; Evelin et al., 2012; Elhindi et al., 2017). Wu et al. (2015) investigated the role of AMF in different sexes of *P. cathayana* during salt stress. Fv/Fm was elevated in males compared to females. Moreover, the improved efficiency of photosystem II and the antioxidant machinery in mycorrhizal seedlings alleviated the effects of salt stress. Increases in Fv/Fm, *NPQ*, and *ETR* were observed in the leaves of AI *E. angustifolia* compared to non-mycorrhizal plants under salt stress (Jia et al., 2019). Increased electrical conductivity was noticed in AMF-colonized plants and is caused by the reduced electrolyte permeability of root plasma membranes relative to non-mycorrhizal plant roots (Garg and Manchanda, 2008). Increased hydraulic conductance (Aroca et al., 2007), root system modifications (Campanelli et al., 2013; Alqarawi et al., 2014), and an improved water status were observed in AI plants under salt stress (Chen et al., 2017). Improved water status in AMF plants correlates with the expression of aquaporin genes (*RpPIP1*;*1*, *RpPIP1*;*3*, *RpPIP2*;*1*, *RpTIP1*;*1*, *RpTIP1*;*3*, *RpTIP2*;*1*) in leaves and roots of plants under salt stress; however, expression levels vary according to plant species, salinity levels, and location or tissue of expression (Chen et al., 2017). Higher WUE was observed in sweet basil plants colonized with *Glomus deserticola* compared to control plants under salt stress (Elhindi et al., 2017).

Significant increases in fresh and dry weight, plant height, and chlorophyll content were observed in PGPR-inoculated pepper plants relative to NI plants (Hahm et al., 2017). An increase in chlorophyll levels in PGPR-inoculated plants (Shukla et al., 2012; Bharti et al., 2014) and higher WUE of inoculated capsicum plants enabled them to survive under salt stress conditions (Yasin et al., 2018). Higher chlorophyll production, Na^+ exclusion from roots, and antioxidant production enhanced salt stress tolerance in maize plants inoculated with *B. amyloliquefaciens* (Chen et al., 2016). Increased photosynthetic rates and decreased stomatal resistance enhanced the plant biomass of PGPR-inoculated pea plants (Wang et al., 2016). An interesting study by Chatterjee et al. (2018) found that treatment of rice plants with ACC deaminase-containing *Brevibacterium linens* RS16B decreased volatile organic compound (VOC) emissions and enhanced photosynthesis by reducing the availability of ACC and ACC oxidase activity, suggesting that research on volatile emissions during salt stress can reveal new insights related to stress severity and the initiation of secondary metabolism with stress progression. Ansari et al. (2019) demonstrated that PGPR inoculation increased root length, chlorophyll pigments, leaf number, relative water content (RWC), stomatal conductance (gs), and photosynthesis rate (Pn) in alfalfa plants under salt stress. *Pseudomonas* inoculation improved leaf number, chlorophyll pigments, nodule number, Na^+ levels, and K^+/Na^+ ratios; however, *Hartmannibacter* inoculation improved carotenoid content, RWC, and K^+ levels. Higher gs and chlorophyll pigments enhanced Pn in RI alfalfa plants. Inoculation with *Bacillus megaterium* strain A12 ameliorated salt stress in tomato plants by restoring redox homeostasis and photosynthesis to improve plant growth. Higher expression levels of the *PBGD* gene (encodes the enzyme needed for chlorophyll biosynthesis) enhanced chlorophyll content in tomato plants. In addition, reduction of ROS levels upregulated expression of the *PsbA* gene (encodes D1 protein that repairs stress damaged photosystem). Increased cytokinin production diminished the degradation of photosynthetic proteins and elevated the expression levels of genes related to photosystems under stress conditions (Akram et al., 2019).

MOLECULAR ALTERATIONS

Multi-omics approaches, such as metagenomics, metatranscriptomics, and metaproteomics, can be utilized to enhance our understanding of plant behavior under stress conditions. However, it remains a challenge to integrate data from various "omics" tools, although this would be a step toward understanding the complex crosstalk between plants and microbes (Meena et al., 2017). STEM affecting various physiological and biochemical processes under salt stress involve variations in expression levels of genes, including ion transporters, aquaporins, and Δ1-pyrroline-5-carboxylate synthetase (P5CS), and variable levels of antioxidant defense enzymes, late embryogenesis abundant protein photosynthesis, antioxidant defense ionic homeostasis, and other signaling events. However, molecular alterations are categorized as a separate STEM to provide a better understanding, at transcriptional and proteomic levels, of how the plant response to salt stress is influenced.

Transcriptional Studies

Upregulation of *SOS1*, *NADP-Me2* (NADP-malic enzyme), *EREBP* (ethylene-responsive element binding proteins), and SERK1 (somatic embryogenesis receptor-like kinase) and downregulation of GIG (glucose-insensitive growth) and

SNF1 (serinethreonine protein kinase *SAPK4*) expression were observed in *B. amyloliquefaciens* SN13-inoculated rice plants exposed to salt stress. Modulated gene expression mitigated osmotic and ionic stress in plants to improve plant performance under stress conditions (Nautiyal et al., 2013). A significant increase in the transcriptional levels of *AtRSA1* and *AtWRKY8* and decrease in the expression of *AtVQ9* (*AtWRKY8* antagonist) in PGPR-treated *Arabidopsis* plants suggested enhanced plant performance under stress conditions resulting from microbial colonization. *AtRSA1* forms a complex with the *AtRITF1* transcription factor to regulate Na^+ ion homeostasis and ROS detoxification during salt stress; *AtWRKY8* and *AtVQ9* are also involved in preserving ion homeostasis at reduced Na^+/K^+ ratios in the cytosol (Sukweenadhi et al., 2015). Upregulated expression of genes involved in stress responses [e.g., *RAB18* (*LEA*), *RD29B* regulons of ABA-responsive elements], proline biosynthesis (e.g., *P5CS1* and *P5CS2*), and *MPK3* and *MPK6* stress responses have also been reported (Kim et al., 2014). Upregulated expression of the *TaCTR1* (Serine/Threonine protein kinase–ethylene responsive) and *TaDREB2* (encodes a transcription factor enhancing abiotic stress tolerance in plants) genes was reported in wheat plants inoculated with PGPR strains (Kaushal and Wani, 2016b; Barnawal et al., 2017). *B. subtilis* GB03-inoculated *P. tenuiflora* plants showed less Na^+ accumulation in response to upregulated expression of *PtHKT1*;*5* (involved in the acquisition of Na^+ from xylem) and *SOS1* (role in Na^+ efflux) and downregulation of *PtHKT2*;*1* (involved in Na^+ absorption in roots) to ameliorate salt stress in host plants (Niu et al., 2016). Improved physiological performance led to salt tolerance in *B. amyloliquefaciens* SQR9-treated maize plants and correlated to significant increases in the expression of *RBCS* and *RBCL* (RuBisCo subunits), ion transporters (*HKT1*, *NHX1*, and *NHX2*), and *H(C)-Ppase* (encoding HC pumping pyrophosphatase). However, the expression of *NCED* (encoding 9-cisepoxycarotenoid dioxygenase) was downregulated in RI seedlings (Chen et al., 2016). Increased expression of ionic transporters, such as *TaNHX* and *TaHKT1*, and the salt-induced stress gene *TaST* (reduces intracellular Na^+ level, raises K^+ content) was observed in PGPR-primed plants relative to NI plants. In addition, upregulated expression of the ABA-responsive gene (*TaABARE*), 12-oxophytodienoate reductase, or *TaOPR1* (enhances antioxidant response) induced expression of *TaMYB* and *TaWRKY*, which led to expression of stress-related genes, including *TaST*. Plant tolerance to salt stress correlated with higher gene expression levels of *CAT*, *APX*, *MnSOD*, *POD*, *GPX*, and *GR*, which together modulated the antioxidant defense system to ultimately confer salt tolerance on inoculated host plants (Bharti et al., 2016). Volatile organic compounds generated by *B. subtilis* reduced gene expression of the high-affinity K^+ transporter (*HKT1*) in the roots of inoculated *Arabidopsis* plants to limit Na^+ uptake by the roots (Zhang et al., 2008). Higher transcriptional levels of genes involved in ABA signaling (*RD29A* and *RD29B*), ROS quenching (*APX2*), and detoxification (*GLYI7*) were reported in *Burkholderia phytofirmans* PsJN-colonized *Arabidopsis* plants. Additionally, expression of (*LOX2*) or *Lipoxygenase2* (involved in JA biosynthesis) was downregulated; however, expression patterns of ion transport genes were varied between roots and shoots. *Arabidopsis* K^+ Transporter 1 or *AKT1* (plasma membrane transporter responsible for K^+ uptake in roots) expression levels decreased in roots and rosettes after 24 h of salt stress. Sodium Hydrogen Exchanger 2 or *NHX2* (a vacuolar antiporter engaged in ion compartmentalization) was upregulated in roots at all time points, but its expression varied at different points depending on salt stress and bacterial inoculation status. Salt Overly Sensitive 1 or *SOS1* is another plasma membrane Na^+/H^+ antiporter (involved in Na^+ removal from the cytoplasm). Bacterial inoculation caused upregulation of *SOS1* expression in roots after 2 h, and expression was then downregulated after 24 and 72 h. However, in rosettes, increased expression levels were detected after 2 and 24 h. Bacterial inoculation downregulated the expression of High-Affinity K^+ Transporter 1 or *HKT 1* (sodium transporter) in roots under salt stress. Moreover, expression of *HKT 1* was upregulated in the rosettes of non-stressed and colonized plants at 24 h but was downregulated at 24 and 72 h regardless of bacterial inoculation status (Pinedo et al., 2015). *Arthrobacter woluwensis* AK1-treated soybean plants exhibited upregulated expression of various genes, including *GmLAX1* (auxin resistant 1), *GmAKT2* (potassium channel), *GmST1* (salt tolerance 1), and *GmSALT3* (salt tolerance-related gene on chromosome 3) whereas downregulated expression of the ion transporter genes *GmNHX1* (chloride channel gene) and *GmCLC1* (Na^+/H^+ antiporter) was observed (Khan et al., 2019). A significant increase in the expression of stress related genes, such as *CAPIP2* (aquaporin), stress related *CaKR1*, *CaOSM1* (osmotin), and *CAChi2* (Class II chitinase), was observed in PGPR inoculated capsicum plants, resulting in the modulation of various biochemical and physiological mechanisms to alleviate salt stress in plants (Yasin et al., 2018). In MI wheat plantlets, transcriptional studies found upregulated expression of P450s genes (*CYP98A1*, *CYP734A5*, *CYP72A15*, and *CYP710A1*) involved in redox reactions and stress responses, *APX*, and Nicotianamine synthase (*NAS*), which is responsible for iron absorption. Moreover, higher expression of oligopeptide transporters (membrane proteins able to transport different substrates), ATP binding cassette (ABC) transporters (proteins mediating energy-driven transport of various substrates), and *HKT* and *NHX* antiporters conferred salt stress resistance on inoculated plants (Safdarian et al., 2019). Increased expression of *PIP* genes was reported in AMF plants compared to non-AMF plants, which improved root water permeability and ameliorated the effects of salt stress conditions (Aroca et al., 2007; Jahromi et al., 2008). Mycorrhizal colonization boosted the expression levels of three chloroplast genes (*RppsbA*, *RppsbD*, and *RprbcL*) (encoding the larger subunit of rubisco) in leaves and genes involved in ion homeostasis (*RpSOS1*, *RpHKT1*, and *RpSKOR*). Higher expression of *RpSOS1* and *RpHKT1* decreased Na^+ accumulation, while increased *RpSKOR* expression improved K^+ accumulation in the leaves of mycorrhizal plants, leading to higher K^+/Na^+ ratios. Additionally, upregulated expression of *RpPIP1*;*1* and *RpPIP1*;*3* was observed in both leaves and roots; however, *RpPIP2*;*1*, and *RpTIP1*;*1* were found to be more highly expressed in the roots of mycorrhizal plants under salt stress (Chen et al., 2017). Higher nitrogen uptake in salt-stressed

mycorrhizal colonized wheat plants correlated with increased expression of *NRT1.1* (involved in nitrate uptake in roots); however, the expression of ammonium transporters (*AMT1.1* and *AMT1.2*) was unaffected. In addition, expression levels of genes related to drought stress (*AQP1*, *AQP4*, *PIP1*, *DREB5*, and *DHN15.3*) were lower compared to non-mycorrhizal wheat plants (Fileccia et al., 2017). Mycorrhizal colonization alleviated the reduced expression levels of *RBCL* (involved in photosynthesis) induced by salt stress; however, salinity raised expression levels of *PPH* (responsible for chlorophyll degradation), which was significantly increased after AMF colonization. Furthermore, genes involved in antioxidant defense responses (*APX*, *Cu-Zn SOD*, *CAT*, and *GR*) showed increased expression levels that were further enhanced by mycorrhizal treatment (Ye et al., 2019).

Proteomics Studies

Microbial colonization under salt stress leads to up- and downregulated expression of various proteins. Proteomic analysis can reveal the protein profile of inoculated plants to decode the STEM. Detected proteins can be utilized for genetic transformation to boost salt tolerance in crops. Molecular studies performed by Alikhani et al. (2013) revealed that proteome trends in *P. indica*-inoculated barley plants were different from those of NI plants under salt stress. The abundance of the protein peroxiredoxins-2E-2 (component of antioxidant defense system) was increased in both PGPR-colonized and NI plants in the presence of 300 mM NaCl; however, the increase in protein level was greater in *P. indica*-primed plants. Higher expression levels of *RBCS* (small chain of RUBISCO) were reported in *P. indica*-colonized plants compared to NI counterparts. Increased expression levels of xyloglucan endotransglycosylase or *XET* (involved in cell wall biosynthesis) and tubulin-folding cofactor A (involved in cell wall division) were observed in inoculated plants; however, there was no change in expression levels in inoculated plants (with 300 mM NaCl). Furthermore, the expression of papain-like cysteine proteases (cell signaling pathways) was reduced in NI plants, whereas its expression levels were increased in inoculated plants, enhancing salt stress tolerance. Proteomic analysis in AI *E. angustifolia* seedlings showed interesting results. Increased expression of a core protein of PS II (D1 precursor processing PSB27) involved in stabilizing the PSII reaction center was found in chloroplasts, and mitochondria showed increased levels of various energy-related proteins, such as NADH dehydrogenase, iron-sulfur protein NADH dehydrogenase, cytochrome C oxidase, and ATP synthase, to provide energy for cellular activities. Phosphoribosyl transferase or *APT* (enzyme in tryptophan synthesis) was also upregulated in response to AMF colonization. Upregulation of four peptidyl prolyl cis-trans isomerases (peptidyl-prolyl cis-trans isomerase (*FKBP12*), peptidyl-prolyl cis-trans isomerase (*CYP18-1*), *FKBP*-type peptidyl-prolyl cis-trans isomerase 5 isoform 1, and peptidyl-prolyl cis-trans isomerase (*FKBP62*), along with four molecular chaperones, prefoldin subunit 1, prefoldin subunit 2, heat shock 70 kDa, and partial and small hsp 17.3 kDa, was observed in mycorrhizal seedlings to enable correct protein folding under salt stress. In this study, upregulated expression of proteins involved in signal transduction, such as G proteins, plasma membrane Ca^{2+} transporter ATPase (*PMCA*), calcium-dependent protein kinases (*CDPKs*), and calmodulin (*CaM*), enhanced Ca^{2+} signaling. Thus, AMF colonization enhanced expression of proteins involved in secondary metabolism, antioxidant defense, and signal transduction to lead to increased salt tolerance in *E. augustifolia* seedlings (Jia et al., 2019).

CONCLUSION AND RECOMMENDATIONS

Microbially inoculated plants induce STEM to counteract salt stress and enhance plant productivity. STEM can promote nutrient uptake, enhanced WUE and photosynthesis, preservation of ionic homeostasis and osmoprotection, and efficient antioxidant metabolism. In recent years, various studies have reported that plant–microbe interactions can develop STEM in host plants; however, some aspects of this phenomenon remain poorly understood. Future studies should investigate the role of phytohormonal crosstalk (e.g., BR, JA, and strigolactones) in MI plants to understand their role in eliciting stress signals during salt stress. Metabolomic studies should aim to understand the STEM underlying the secondary metabolism in salt-stressed MI plants. There is also a need for studies investigating the nutritional uptake of sulfur in MI plants under salt stress, given its involvement with glutathione and cysteine (involved in ABA synthesis). Moreover, as the cell wall is the first line of defense during salt stress, subsequent studies should target biochemical and molecular alterations relating to the cell wall of MI plants. Insights into the plant immune system triggered in response to microbial partners while under stress conditions should also be addressed to harness plant microbial interactions for agricultural benefits.

AUTHOR CONTRIBUTIONS

MK reviewed, wrote, and revised the manuscript.

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Mitigation of Salinity Stress in Wheat Seedlings Due to the Application of Phytohormone-Rich Culture Filtrate Extract of Methylotrophic Actinobacterium *Nocardioides* sp. NIMMe6

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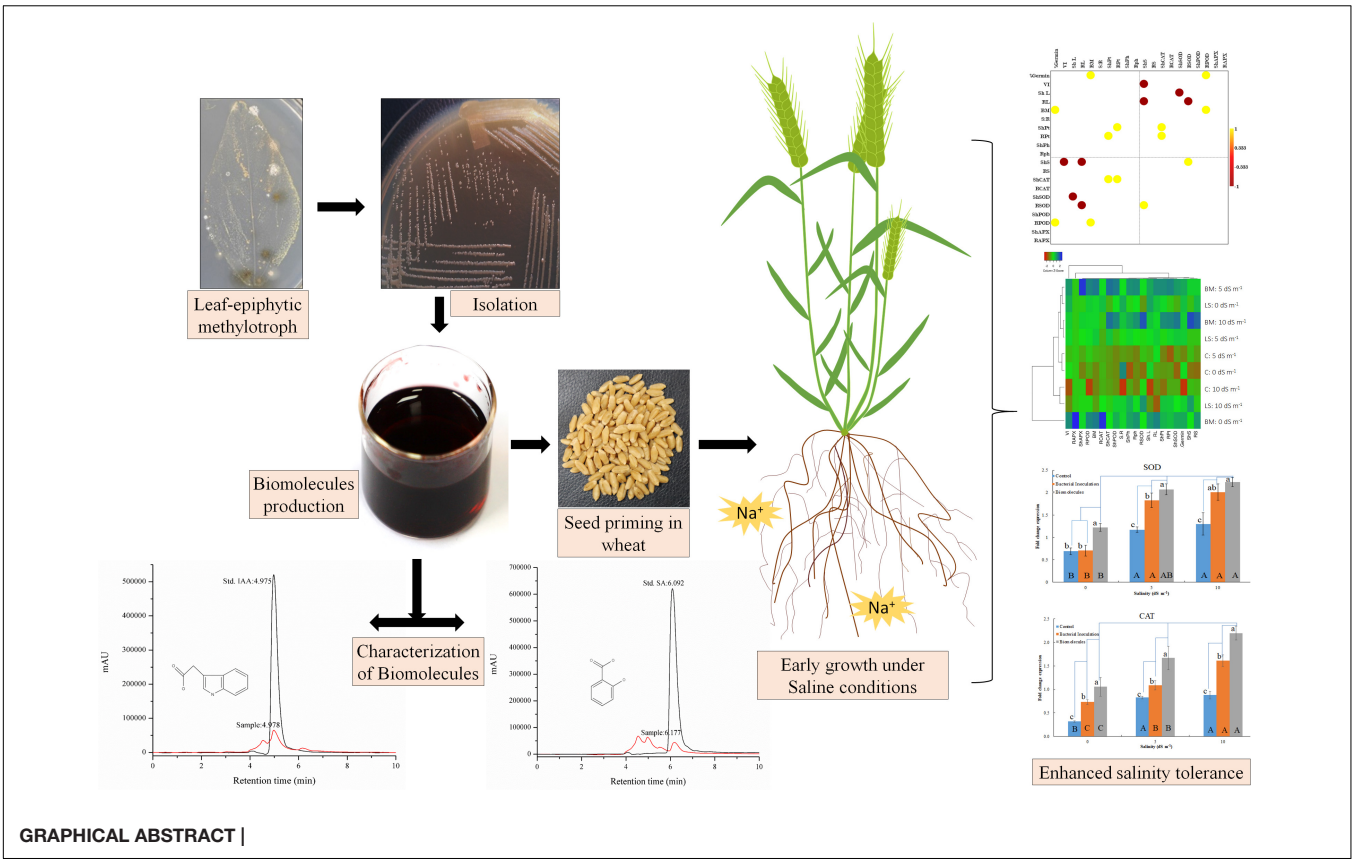
Salinity stress is an important plant growth limiting factor influencing crop productivity negatively. Microbial interventions for salinity stress mitigation have invited significant attention due to the promising impacts of interactive associations on the intrinsic mechanisms of plants. We report the impact of microbial inoculation of a halotolerant methylotrophic actinobacterium (*Nocardioides* sp. NIMMe6; LC140963) and seed coating of its phytohormone-rich bacterial culture filtrate extract (BCFE) on wheat seedlings grown under saline conditions. Different plant-growth-promoting (PGP) attributes of the bacterium in terms of its growth in N-limiting media and siderophore and phytohormone [indole-3-acetic acid (IAA) and salicylic acid] production influenced plant growth positively. Microbial inoculation and priming with BCFE resulted in improved germination (92% in primed seeds at 10 dS m^{-1}), growth, and biochemical accumulation (total protein 42.01 and 28.75 mg g^{-1} in shoot and root tissues at 10 dS m^{-1} in BCFE-primed seeds) and enhanced the activity level of antioxidant enzymes (superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase) to confer stress mitigation. Biopriming with BCFE proved impactful. The BCFE application has further influenced the overexpression of defense-related genes in the seedlings grown under salinity stress condition. Liquid chromatography–mass spectrometry-based characterization of the biomolecules in the BCFE revealed quantification of salicylate and indole-3-acetate (Rt 4.978 min, *m/z* 138.1 and 6.177 min, 129.1), respectively. The high tolerance limit of the bacterium to 10% NaCl in the culture media suggested its possible survival and growth under high soil salinity condition as microbial inoculant. The production of a high quantity of IAA (45.6 μg ml^{-1} of culture filtrate) by the bacterium reflected its capability to not only support plant growth under salinity condition but also mitigate stress due to the impact of phytohormone as defense mitigators. The study suggested that although microbial inoculation offers stress mitigation in plants, the phytohormone-rich BCFE from *Nocardioides* sp. NIMMe6 has potential implications for defense against salinity stress in wheat.

Keywords: *Nocardioides* sp., IAA, salicylic acid, seed-priming, wheat, salinity



GRAPHICAL ABSTRACT |

INTRODUCTION

Salinity, among the most agonizing abiotic stresses, is responsible for significantly declining agricultural productivity (Allakhverdiev et al., 2000). Saline conditions develop predominantly due to natural processes or frequent irrigation with saline water (Mishra et al., 2016). The growth stages of crop plants right from seed germination and plant development up to seed production are highly susceptible to saline conditions (Kumar et al., 2015; Sorty et al., 2016). Exposure of plants to salinity during the early developmental stages negatively influences the overall process of water and nutrient uptake and thus worsen crop growth and productivity (James et al., 2002; Dodd and Pérez-Alfocea, 2012). High salt concentration limits root development by impairing physiological and metabolic balance and significantly reducing the rate of seed germination (Munns et al., 2006). Excessive accumulation of sodium ions in surviving plants interfere with the photosynthetic mechanism in the leaves and generate oxidative stress due to the accumulation of reactive oxygen species (ROS) (Acosta-Motos et al., 2017). A high concentration of ROS damages cellular compartmentalization by causing peroxidation of membrane lipids and leads to the development of early leaf senescence forcefully (James et al., 2002).

Plants employ a range of intrinsic mechanisms at the cellular level to combat stress conditions (Meena et al., 2017). Under associative interaction with the plants, microbial species play an important role in inducing stress-responsive mechanisms which go in parallel to the plant's own responses (Kumar and Verma, 2018). Microbial associations directly influence the expression of several stress-responsive genes, activation of antioxidant enzymes, and accumulation of proteins, compatible solutes, and bioactive compounds that cumulatively add to enhance the stress tolerance capability of plants (Kaushal and Wani, 2016). Currently available strategy to harness the efficiency of microbial inoculation for crop plants grown under abiotic stress condition is mainly based on inoculating either individual microbial species or their consortia as live inoculants (Mazzoli et al., 2017; Sorty et al., 2018; Bakka and Challabathula, 2020). It has been established that the exogenous application of metabolites and phytohormones enhances plant-inherent defense systems that subsequently help plants in stress mitigation (An et al., 2014; Sharma et al., 2019). The biomolecules may act as signaling molecules that regulate cross-talk during plant–environment interactions (Bitla et al., 2017; Ku et al., 2018; Ahmad et al., 2019). The phenomenon of the subsequent protection against stresses due to the application of organic, inorganic, or biostimulants is known as molecular priming (Kerchev et al., 2020). If explored, the potentiality of biomolecules of microbial origin can find a significant role in stress mitigation and growth promotion strategy in plants. Such surge of interest could offer strategic dimensions for wide-scale implications of microbial biomolecules in crops grown in saline areas or exposed to drought (Bulgari et al., 2019).

Seed priming is an important approach that is being widely used in raising crop plants for improving germination, seedling vigor, growth, development, yield, and stress tolerance (Zheng et al., 2016; Hussain et al., 2018). Priming of seeds with phytohormones (hormopriming), osmotic agents (osmopriming), and hydration (hydropriming) has remained beneficial in crop production strategy under drought and saline conditions (Paparella et al., 2015; Sorty et al., 2016). There remains a dearth of knowledge on the potential of microbial products as seed priming agents for mitigating abiotic stress in crop plants (O'Callaghan, 2016; Egamberdieva et al., 2017). We have isolated methylotrophic halotolerant actinobacterium *Nocardioides* sp. NIMMe6 from soybean leaf surface. The bacterium possesses plant-growth-promoting (PGP) and phytohormone-producing capabilities. It has shown its survival at 10% NaCl concentration in culture media and subsequently produced culture filtrate rich in phytohormones, thereby reflecting that it may be employed as an inoculant in saline conditions. Wheat, cultivated in many parts of India, is highly vulnerable to saline conditions (Hampson and Simpson, 1990). We have shown that the application of the bacterium on wheat as an inoculant and through seed priming of the phytohormone-rich bacterial culture filtrate extract (BCFE) resulted in the activation of plant-intrinsic responses against salinity stress. This study describes specific biochemical and molecular mechanisms involved in offering mitigation of salinity stress in wheat seedlings after priming with BCFE and microbial inoculation.

MATERIALS AND METHODS

Identification and Functional Characterization of the Bacterium

Fresh leaves of soybean crop grown at an experimental farm of the ICAR-National Institute of Abiotic Stress Management campus (Baramati, India; N18.1388, E74.5063) were collected for the isolation of the bacterium. The impressions of the dorsal and the ventral sides of healthy, juvenile soybean leaves were taken on the ammonium mineral salt agar medium (Omer et al., 2004) amended with 5.0% NaCl (Knief et al., 2008). After incubation at 30 ± 2°C for 72 h, colonies were purified and pure stock was maintained in 50% (v/v) glycerol at −80°C.

The genomic DNA was isolated using Ultraclean Microbial DNA isolation kit (MoBio, Carlsbad, United States). Polymerase chain reaction (PCR) amplification of 16S rRNA gene was carried out (Sorty et al., 2016), and the PCR product was purified using QIAquick gel extraction kit (Qiagen), followed by sequencing in ABI 3130*xl* automated genetic analyzer (Applied Biosystems, United Kingdom). Sequence analysis was performed using MEGA7 (Kumar et al., 2016). BLAST was performed using National Center for Biotechnology Information – Basic Local Alignment Search Tool[1]. Sequence alignment with the datasets of related organisms was done with the help of ClustalW. A phylogenetic tree was constructed using neighbor joining method (Tamura et al., 2004). The sequence was finally submitted to DNA Data Bank of Japan.

Biolog-based substrate utilization assay was used for the metabolic characterization of the isolate using Biolog GEN III (Biolog Inc., United States) protocol. The bacterial culture (100 μl) was inoculated in a 96-well Biolog GEN III plate having a specific carbon source per well and incubated at 30 ± 2°C. Growth and substrate metabolism was recorded at 12-h intervals using Biolog MicroStation. The culture was grown with different carbon sources including amino acids, carboxylic acids, sugars, and organic acids. Metabolic intensity was tracked colorimetrically using tetrazolium redox dye, which developed a purple color.

Bacterial Growth on N-Deficient Media

The ability of the isolate to grow in nitrogen-deficient media was detected using Ashby's N-free mannitol agar (Sen and Sen, 1965). A 10-μl aliquot of freshly grown culture was inoculated on the agar media plates and incubated at 30 ± 2°C for 48 h. Development of visible growth on the plates was noticed.

Phosphate Solubilization

Phosphate solubilization by the isolate was detected on Pikovskaya's agar (Nautiyal, 1999). A freshly grown culture was spot-inoculated (10 μl) on three separate plates, and the inverted plates were incubated at 30 ± 2°C for 48 h. P solubilization was monitored in terms of the development of a clear halo zone surrounding the microbial growth.

Siderophore Production

Production of siderophore was detected using chrome azurol sulfonate (CAS) agar (Schwyn and Neilands, 1987). Briefly, 10 μl of log culture of the bacterium was spot-inoculated on freshly prepared CAS agar plates and incubated at 30 ± 2°C for 48 h to observe qualitative siderophore production.

Bacterial Salt Tolerance and Growth Curve Analysis

The salt tolerance ability of the strain was detected using stepped gradient concentration of NaCl from 0 to 30% in lysogeny agar medium (Sorty et al., 2016). Freshly grown bacterial culture (10 μl) was spot-inoculated on agar plates amended with NaCl concentration ranging from 1 to 30% and incubated at 30 ± 2°C for 7 days. Appearance of visible growth on the agar medium was recorded. The maximum NaCl concentration showing a visible growth of the isolate was selected as upper end for growth curve analysis.

Growth curve was analyzed in lysogeny broth (LB) medium amended with 1% gradual salt (NaCl) concentration. Freshly grown bacterial cells (100 μl) were inoculated in 100 ml of the medium and incubated at 30 ± 2°C at 150 rpm in a shaker incubator. Three flasks were maintained for each salt concentration. The growth of the bacterium was monitored in terms of change in OD_{600nm} and plotted against time to obtain NaCl-dependent growth curve.

[1]http://www.blast.ncbi.nlm.nih.gov/Blast.cgi

Exopolysaccharide Production

The exopolysaccharide (EPS) production ability of the bacterium was detected on tryptic soy agar (TSA). Freshly grown bacterial culture (10 μl) was inoculated on three separate TSA plates and incubated at 30 ± 2°C for 48 h for qualitative estimation of EPS (Vardharajula et al., 2011).

Indole Acetic Acid Production

The bacterium was grown at 30°C in LB medium supplemented with 120 mM methanol for 48 h at 150 rpm. Spectrophotometric quantification of indole-3-acetic acid (IAA) was done by using Salkowski reagent (Ehmann, 1977). In brief, 0.5 ml of culture filtrate, obtained by pelleting the cells at 7,000 rpm for 5 min, was mixed with 2 ml of Salkowski reagent (2% of 0.5 M $FeCl_3$ in 35% $HClO_4$). To this mixture, 200 μl of orthophosphoric acid was added with mixing, and the tubes were kept in the dark for 30 min at room temperature. Absorbance was recorded at 530 nm, and calibration was obtained against pure IAA (Fisher Scientific, United States).

Preparation of Bacterial Culture Filtrate Extract

The bacterium was grown in LB, a nutritionally rich culture medium most commonly used for growing bacteria for molecular biological (Miller, 1972) and secondary metabolite including phytohormone production studies (Cox et al., 2018). After attaining culture growth at 30 ± 2°C for 36 h at 150 rpm, bacterial cells were separated by centrifugation at 7,830 rpm (Eppendorf 5430R) to obtain a cell-free culture filtrate. The culture filtrate was then mixed with preconditioned xad-16 resin (10% w/v) (Sigma Aldrich, United States) for increasing titer of the extraction of metabolites due to its high adsorption capacity (González-Menéndez et al., 2019). A slurry of the culture filtrate and the xad-16 resin was prepared at 150 rpm for 2 h at room temperature and then poured into a chromatographic glass column (10 mm diameter × 300 mm length) having a built-in sintered disk at the bottom. The column was washed thrice with ethyl acetate and methanol (1:1 v/v). The solvent was evaporated at room temperature, and the BCFE was stored at 4°C for further analysis. A separate set of slurry using xad-16 resin was also prepared with the same LB medium that was used for growing bacterial cells and was subjected to column separation exactly as detailed above. The medium extract thus obtained served as reference blank to ensure that the identified metabolite constituents are produced and excreted into the culture filtrate by the bacterial cells only.

Analysis of Phytohormones Using HPLC and LC–MS

Quantitative estimation of phytohormone in the BCFE was performed using high-performance liquid chromatography (HPLC). An aliquot (10 μl) of BCFE dissolved in methanol was injected onto the RP C-18 column (4.6 mm i.d. × 250 mm length; particle size, 3 μm) (Spincotech, India) using the auto-sampler (SIL 30AC) fitted with the HPLC system (Nexera X2; Shimadzu Corp., Japan) running with the mobile phase [methanol (A); 0.1% formic acid in water (B); final composition A:B, 80:20 v/v]. The system was equipped with a photodiode array detector (SPD M20A) and a column oven (CTO 20AC) maintained at 30°C throughout the analysis. The mixture was eluted under isocratic mode at 0.7 ml min^{-1}. Compounds were characterized on the basis of their retention time (Rt) and co-elution with the standard compounds. The medium extract was also analyzed under a similar set of conditions to exclude traces of IAA or salicylic acid, if any, coming from the medium constituents.

The identity of the compounds was authenticated with the help of mass spectrometry (MS) (Agilent 1200 quadrupole LC–MS system), in which the sample was injected in absolute methanol at a flow rate of 0.5 ml min^{-1}. The compounds were identified on the basis of their *m/z* values.

Gnotobiotic Experiment Under Saline Conditions

The impact of microbial inoculation and BCFE priming was investigated on wheat grown in gnotobiotic condition under a saline environment. Sterile petri dishes having different levels of salinity [electrical conductivity (EC) 0, 5, and 10 dS m^{-1}, maintained using NaCl; pH 7.0 ± 0.2] were prepared with agar-water (0.7%). Wheat seeds (var. Netravati NIAW1415 – recommended for rainfed and restricted irrigation regimes having normal soil conditions) were surface-sterilized using 5% (v/v) sodium hypochlorite solution (Sauer and Burroughs, 1986). Residual hypochlorite was removed by washing three times with sterile Milli Q water. Bacterial cell suspension (~10^9 cfu ml^{-1}) in 1% aqueous carboxymethyl cellulose (CMC) as binding agent was given for microbial inoculation on the wheat seeds. Seed priming of wheat was performed by soaking the seeds with a solution of BCFE prepared in 1% CMC in water (at 1 mg extract per gram of seeds). The seeds were dried in laminar hood on a sterile polythene sheet, sown on the Petri dishes in four replicates ($n = 4$), and incubated in the dark for 72 h at 20°C followed by 12-h dark and light cycle until 7 days. Uninoculated or non-primed seeds served as control.

Germination percentage, length of shoot and root, seedling vigor index, total biomass, and shoot–root ratio were determined from freshly harvested seedlings.

Biochemical Analysis of Wheat Seedlings

Total Protein Content

Total protein content in shoot and root tissue was measured according to Bradford (1976). The tissue extracts prepared for enzyme estimation were mixed with Bradford reagent and allowed to react in the dark for 15 min, and the absorbance was recorded at 595 nm. Total content of protein was quantified using bovine serum albumin as standard.

Estimation of Total Sugar

The total sugar content of shoot and root tissue was estimated using anthrone reagent (Yemm and Willis, 1954). A crushed sample (50 mg) was treated with 2.5 M HCl in boiling water bath for 3 h. The acid was neutralized with an excess of sodium

carbonate, and the total content was diluted to 50 ml. The clear supernatant (1 ml) was mixed with 4 ml of freshly prepared, ice-cold anthrone reagent (0.2% anthrone in 95% H_2SO_4). After thorough mixing, the content was kept in boiling water bath for 10 min and cooled, and the absorbance was recorded at 630 nm. Total sugar content was calculated as the equivalents of glucose.

Total Polyphenolic Content

Total polyphenolic content (TPC) was measured according to Singleton et al. (1999). For this, 100 mg of tissue sample was macerated in 2 ml of chilled 80% methanol. The debris was removed by centrifugation at 14,000 rpm for 15 min, and 100 μl supernatant was mixed with Folin Ciocalteu reagent (1 N). The reaction mixture was kept in boiling water bath for 1 min and cooled. The absorbance was noted at 650 nm by taking catechol as the standard.

Estimation of Antioxidant Enzymes

Enzyme extract of shoot and root was prepared by crushing 1 g of tissue in ice-cold phosphate buffer [4.0 ml of 100 mM, 0.5 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5]. Tissue debris was removed by centrifugation at 14,000 rpm, and the supernatant was stored at −20°C.

Catalase activity was determined with the reaction mixture (3 ml) containing potassium phosphate buffer (50 mM, pH 7.0), hydrogen peroxide (12.5 mM), and the enzyme extract (50 μl) (Luck, 1974). The enzyme reaction was monitored in terms of decreasing absorbance at 240 nm for 1 min.

Superoxide dismutase activity was determined in 3-ml reaction mixture containing methionine (13.33 mM), EDTA (0.1 mM), phosphate buffer (50 mM), sodium carbonate (50 mM), nitro blue tetrazolium chloride (75 μM), 100 μl of enzyme, and riboflavin (2.0 μM) (Dhindsa et al., 1981). The mixture was illuminated for 15 min under white fluorescent light, followed by dark exposure for 15 min. The absorbance was recorded at 560 nm. Illuminated and non-illuminated reaction mixtures without enzyme served as control. Fifty percent reduction of absorbance compared to control was considered as one unit of the enzyme.

Peroxidase activity was determined in 3 ml of the reaction mixture containing potassium phosphate buffer (50 mM, pH 6.1), guaiacol (16 mM), hydrogen peroxide (2.0 mM), and enzyme extract (100 μl) (Reddy et al., 1995). Progress of the reaction was monitored in terms of the formation of guaiacol tetramers at 470 nm.

Ascorbate peroxidase activity was estimated as described by Nakano and Asada (1981). To the 3.0 ml of reaction mixture having potassium phosphate buffer (50 mM, pH 7.0), ascorbic acid (0.5 mM), EDTA (0.1 mM), and hydrogen peroxide (0.1 mM), the enzyme extract (100 μl) was added for determining ascorbate peroxidase (APX). The change in absorbance of the reaction mixture for 30 s was recorded at 290 nm.

Quantitative Real-Time PCR

Fresh leaf tissues were frozen in liquid nitrogen and subjected to RNA extraction using RNeasy Plant Mini Kit (Qiagen, Leusden, Netherlands) as per the manufacturer's instructions. Purified RNA was used for the synthesis of cDNA using Verso cDNA Synthesis Kit (Thermo Scientific, United States). Amplification and quantitation of reference and target genes were done in a 96-well (cfx96) real-time PCR system (Bio-Rad, United States) using DyNAmo ColorFlash SYBR Green qPCR master mix (Thermo Scientific, United States). The reaction program consisted of initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing/extension at 60°C for 60 s with the lid temperature 105°C, and fluorescence recording at each cycle. The primers used for the quantification of gene transcripts related to the antioxidant enzymes were catalase (CAT; forward 5′-CCATGAGATCAAGGCCATCT-3′, reverse 5′-ATCTTACATGCTCGGCTTGG-3′), manganese superoxide dismutase (MnSOD; forward 5′-CAGAG GGTGCTGCTTTACAA-3′, reverse 5′-GGTCACAAGAGGG TCCTGAT-3′), APX (forward 5′-GCAGCTGCTGAAGGA GAAGT-3′, reverse 5′-CACTGGGGCCACTCACTAAT-3′) (Baek and Skinner, 2003), and peroxidase (POD; 5′-CAG CGACCTGCCAGGCTTTA-3′, reverse 5′-GTTGGCCC GGAGAGATGTGG-3′) (Jiang et al., 2012). The Ct value of a constitutive reference transcript of wheat actin (5′-CGAAACCTTCAGTTGCCCAGCAAT-3′, reverse 5′-ACCATCACCAGAGTCGAGCACAAT-3′) (Dong et al., 2013) was used to normalize gene expression.

Statistical Analysis

Unless described separately, all the experiments were conducted in triplicate. The numerical data were statistically analyzed using two-way analysis of variance with *post hoc* Duncan's multiple-range test using SPSS 16.0 (Windows 8.0). A clustered heat map was plotted to establish the relationship among the measured physicochemical attributes of the wheat seedling. Pearson's correlation analysis of the treatments was also performed using SPSS 16.0 and Past3. Difference at 95% confidence level was considered as significant.

RESULTS

Identification and Functional Characterization of the Bacterium

The bacterium isolated from the leaf surface of the soybean crop was identified as *Nocardioides* sp. NIMMe6 (accession: LC140963) on the basis of 16S rRNA gene sequence similarity (**Figure 1A** and **Table 1**). BIOLOG Gen III assay revealed the metabolic flexibility of *Nocardioides* sp. NIMMe6. The bacterium utilized amino acid and hexose sugar as carbon source with high affinity. It also metabolized carboxylic acid and derivatives, carboxylic esters, and fatty acids (**Figure 1B** and **Supplementary Figure S3**). The identified bacterium was able to grow on Ashby's N-free mannitol agar media, thereby qualitatively showing its ability to manage nitrogen from the atmosphere and sustain under N-limited conditions. It has failed to solubilize phosphate but has shown an orange halo zone in the CAS medium, indicating siderophore production ability (**Table 1**). The bacterium was tolerant to 10% NaCl concentration in the medium, with optimal growth at 4%, and was negative

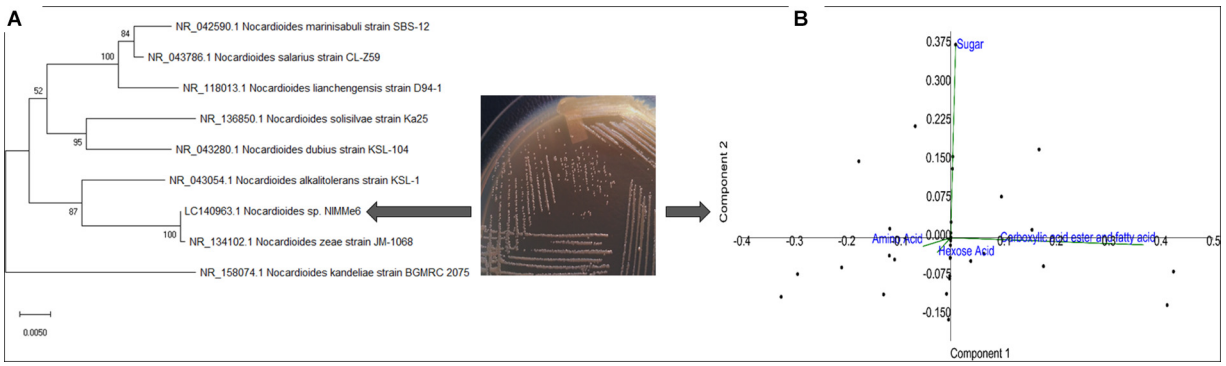


FIGURE 1 | Characterization of the methylotrophic bacterium from soybean leaf surface. Phylogenic positioning of the strain using MEGA 7 (neighbor-joining, bootstrap: 1,000 replicates) revealed its identity as *Nocardioides* sp. **(A)**. Biolog GEN III profiles of the strain indicated high metabolic plasticity **(B)**.

TABLE 1 | Identification of the bacterial strain and its functional attributes relating to plant growth promotion.

Strain	Identity	GenBank accession	N_2 fixation	PO_4 solubilization	Indole-3-acetic acid (μg/ml)	EPS	Siderophore	NaCl tolerance
M6	*Nocardioides* sp. *NIMMe6*	LC140963	++	–	45.6	–	+	0–10%

to epoxypolysaccharide production (**Table 1**). The growth of the bacterium in the salt (NaCl) concentration ranging from 1 to 10% exhibited a characteristic trend, with reduced growth and an extended lag-time at no salt as well as at high salt (8–10%). However, the bacterium showed an increasing trend of growth from 2% salt and continued until 4% of salt, where peak growth was noted, after which the growth started decreasing and continued until 10% of NaCl (**Supplementary Figure S2**).

Quantification of IAA and SA in BCFE

Bacterial culture filtrate extract was obtained in the solution of ethyl acetate and methanol (1:1 v/v), which was passed through the BCFE-trapped XAD 16 resin. XAD resin has an excellent capacity to enrich different biological molecules including phenolic compounds, flavonoids, peptides, antimicrobial, pharmaceuticals, etc., and has been extensively used for the selective extraction of biomolecules from complex microbial culture media (Liu et al., 2016; Salazar et al., 2017; González-Menéndez et al., 2019) and other biological mixtures, such as adlay bran (Yang et al., 2016), apple skin Wang et al. (2020), *Glycyrrhiza* leaves (Dong et al., 2015), and wheat gluten hydrolysates (Yang et al., 2018), as well as extraction of pharmaceuticals in wastewater (Yu and Wu, 2011). Thus, to achieve maximal entrapment of microbial secretions and minimize the interference of residual medium impurities in the crude biomolecule mixture, we specifically used XAD-16 resin to trap the biomolecules secreted by *Nocardioides* sp. in LB medium. Due to the presence of tryptone and yeast extract, the LB medium allowed a luxurious production of biomolecules by providing multiple kinds of substrates, particularly in the form of amino acid precursors. The recovery of the dried BCFE was 16.8 mg l^{-1}. *Nocardioides* sp. NIMMe6 produced and secreted into the medium a high amount of IAA (45.6 μg ml^{-1} of culture filtrate) (**Supplementary Figures S1A,B**). The secretion of IAA into the culture medium by *Nocardioides* sp. NIMMe6 was authenticated by HPLC (Rt 4.978 min) and MS spectra (*m/z* 129.1) (**Supplementary Figure S1A**). The bacterium also secreted salicylic acid (9.37 μg ml^{-1}) into the culture medium, which was further verified by HPLC (Rt 6.177 min) and MS analysis (*m/z* 138.1) (**Supplementary Figure S1B**). Both of these prominent secondary metabolites, of which IAA is a phytohormone and salicylic acid is a signaling molecule, have been considered as elicitors in plants against abiotic stresses (Sorty et al., 2016; Ding and Ding, 2020; **Figure 2**).

Impact of Bacterium Inoculation and BCFE Priming on Wheat

Inoculation with *Nocardioides* sp. NIMMe6 and seed priming with the BCFE resulted in a substantial impact on wheat grown under salinity stress condition (**Table 2**). BCFE priming enhanced seed germination (94.0% at 5 dS m^{-1}) equivalent to that of non-saline control. High salinity condition of 10 dS m^{-1} lowered germination to 92% instead in BCFE-primed seeds. The bacteria-inoculated seeds also showed a germination percentage in the range of 91–92%, with no prominent diminishing effect of saline conditions. However, non-inoculated and non-primed seeds showed a significant reduction in germination at 10 dS m^{-1} compared to non-saline condition (**Table 2**). Vigor index (VI), which reflects the ability of seeds to produce normal seedlings, was improved due to seed priming with BCFE than in inoculated and non-inoculated conditions. Compared to the primed seeds (VI 1,820.9 at 5 dS m^{-1}), the bacteria-inoculated seeds showed a VI of 1,514.5, while non-inoculated seeds resulted in a VI of 1,241.7. It indicated a better performance of BCFE seed priming than microbial inoculation (**Table 2**). A similar trend was also evident in the case of shoot and root length, shoot–root ratio, and biomass accumulation. On these parameters,

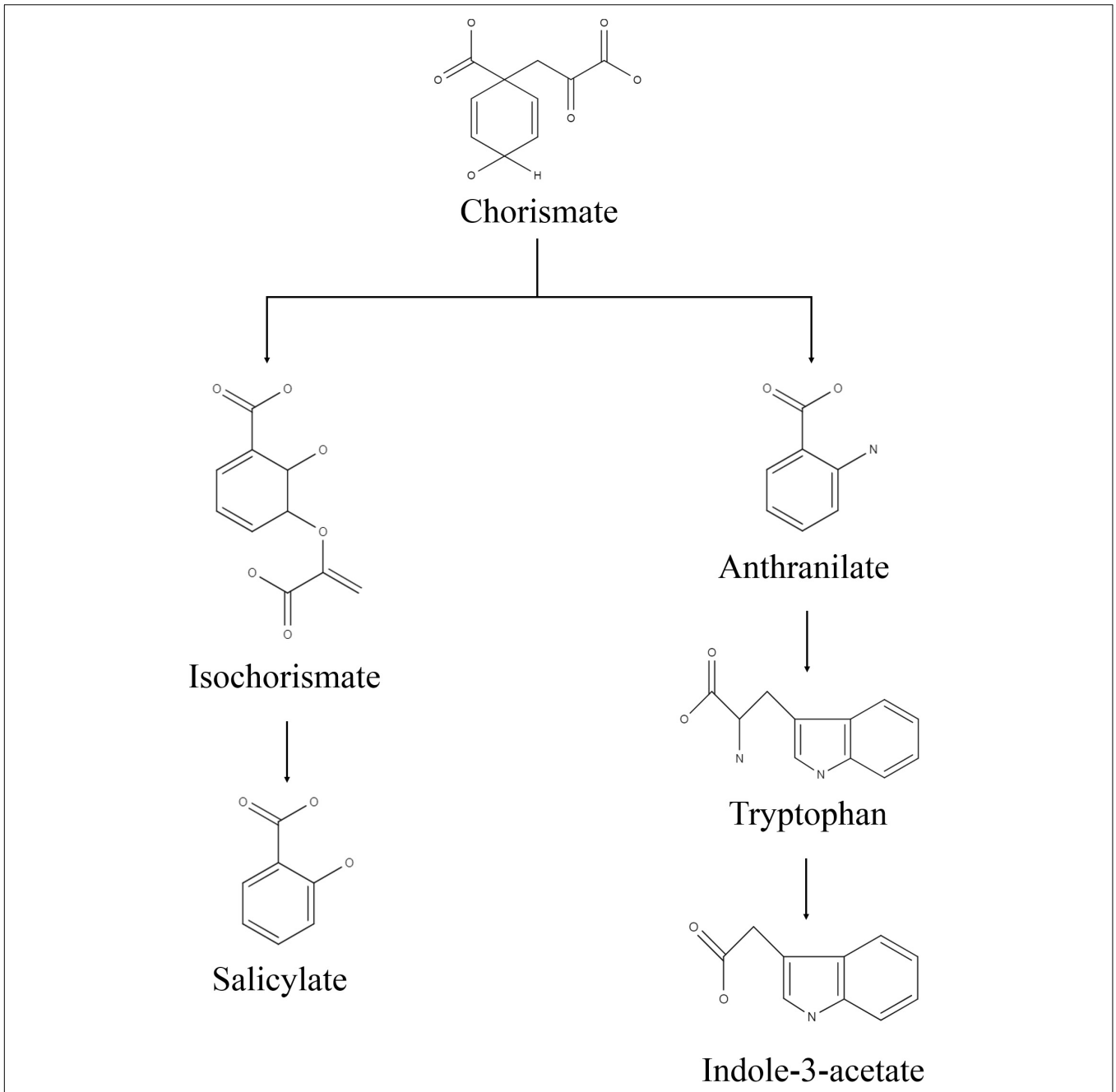


FIGURE 2 | Predicted biosynthesis pathway implemented by the isolate for production of indole-3-acetic acid and salicylic acid.

the performance of BCFE seed priming under saline and non-saline condition was high compared to bacterial inoculation and non-inoculation (**Table 2**).

Biochemical Analysis of Wheat Seedlings

Priming of seeds with BCFE not only enhanced seed quality and plant growth parameters but also increased the content of protein, sugars, and total phenolics in shoot and root as compared to bacteria-inoculated and non-inoculated plants under salinity stress (**Table 3**). It was apparent that plants grown with bacterial inoculation and BCFE priming under saline conditions (5 and 10 dS m^{-1}) accumulated a high content of protein, sugar, and total polyphenolics in comparison to non-saline, non-inoculated, and non-primed plants.

Total Protein Content

Under salinity stress of 5 and 10 dS m^{-1}, BCFE priming enhanced protein content up to 42 mg g^{-1} in fresh shoot and 28 mg g^{-1} in fresh root tissue, respectively. Bacterial inoculation resulted in 31.77 mg protein g^{-1} at 5 dS m^{-1} and 36.72 mg

TABLE 2 | Growth attributes of salinity-stressed wheat seedlings under the influence of the treatments with *Nocardioides* sp. and bacterial culture filtrate extract (BCFE).

EC (dS m^{-1})	Treatment	Germinationa[a],%	Vigor index[b]	Shoot length[c]	Root length[d]	Biomass[d]	Shoot/root[e]
0	*Nocardioides* sp.	92.50 ± 1.73ab	1, 700 ± 127.40ab	10.03 ± 0.39ab	8.34 ± 0.98b	0.014 ± 0.003a−c	1.211 ± 0.102ab
5		92.00 ± 3.27ab	1, 514.5 ± 169.29bc	8.24 ± 1.45c	8.20 ± 0.16b	0.015 ± 0.004ab	1.006 ± 0.176c
10		91.75 ± 2.50ab	1, 018.4 ± 195.60e	5.84 ± 1.36d	5.25 ± 0.71d	0.012 ± 0.001bc	1.104 ± 0.114bc
0	BCFE	94.00 ± 5.16a	1, 875 ± 138.08a	10.37 ± 0.52a	9.58 ± 0.66a	0.017 ± 0.002a	1.084 ± 0.041bc
5		94.00 ± 6.93a	1, 820.9 ± 167.89a	9.93 ± 0.47ab	9.43 ± 0.32a	0.017 ± 0.004a	1.052 ± 0.029bc
10		92.00 ± 7.30ab	1, 558 ± 156.94bc	8.98 ± 1.11bc	7.96 ± 0.28b	0.014 ± 0.001a−c	1.132 ± 0.165bc
0	Control	91.00 ± 3.83ab	1, 373 ± 109.50cd	8.56 ± 0.41c	6.51 ± 0.62c	0.011 ± 0.001bc	1.325 ± 0.150a
5		85.50 ± 7.55bc	1, 241.7 ± 179.55d	6.58 ± 0.52d	7.90 ± 0.53b	0.012 ± 0.001c	0.832 ± 0.040d
10		81.25 ± 1.89c	764.5 ± 53.35f	3.27 ± 0.31e	6.14 ± 0.37c	0.011 ± 0.002c	0.534 ± 0.058d
	Mean square error	24.796	22457.008	0.706	0.322	5.04E-006	0.012
	R^2	0.465	0.879	0.902	0.888	0.610	0.835

Values in the same data series represented by different letters are significantly different (p = 0.05) in Duncan's multiple-range test. [a]Percent of seeds successfully germinated. [b]Seedling length × germination percentage. [c]Length in millimeters. [d]Weight in milligrams (dry). [e]Ratio obtained by dividing the shoot length with the root length.

TABLE 3 | Biochemical characteristics of wheat seedlings treated with *Nocardioides* sp. and its bacterial culture filtrate extract (BCFE) under various salinity stress conditions.

		Protein[a]		Phenolic compounds[a]		Sugar[a]	
EC (dS m^{-1})	Treatment	Shoot	Root	Shoot	Root	Shoot	Root
0	*Nocardioides* sp.	34.640 ± 1.110c	23.095 ± 1.541b−d	1.195 ± 0.121b−d	1.068 ± 0.109ab	52.40 ± 1.19d	67.96 ± 4.11c
5		31.770 ± 1.200d	25.215 ± 1.849a−c	1.245 ± 0.052a−c	1.045 ± 0.097bc	52.92 ± 0.98d	59.69 ± 2.40d
10		36.723 ± 0.746bc	25.728 ± 2.576ab	1.300 ± 0.086ab	1.073 ± 0.121ab	64.49 ± 2.39b	60.56 ± 2.71d
0	BCFE	38.163 ± 0.896b	26.115 ± 2.357ab	1.318 ± 0.087ab	1.203 ± 0.068ab	56.06 ± 2.97c	71.91 ± 2.23b
5		42.003 ± 0.978a	28.953 ± 3.570a	1.348 ± 0.057a	1.218 ± 0.093a	58.75 ± 2.98c	65.56 ± 0.79c
10		42.013 ± 0.834a	28.758 ± 2.511a	1.315 ± 0.097ab	1.208 ± 0.059ab	73.58 ± 0.80a	77.17 ± 1.01a
0	Control	26.380 ± 1.586e	21.308 ± 2.497de	1.025 ± 0.111e	0.908 ± 0.162c	45.00 ± 2.21e	50.59 ± 2.72f
5		24.630 ± 2.994ef	18.370 ± 1.823e	1.108 ± 0.079de	0.890 ± 0.109c	51.87 ± 2.73d	55.88 ± 2.63e
10		23.123 ± 2.486f	21.823 ± 1.805c−e	1.135 ± 0.037c−e	0.893 ± 0.078c	51.93 ± 1.70d	57.86 ± 1.93de
	Mean square error	2.593	5.539	0.007	0.011	4.644	6.093
	R^2	0.960	0.0727	0.673	0.670	0.947	0.932

Values in the same data series represented by different letters are significantly different (p = 0.05) in Duncan's multiple-range test. [a]Concentration in milligram per gram of fresh tissue.

g^{-1} at 10 dS m^{-1} fresh shoot tissue. Saline condition alone significantly lowered the shoot protein content up to 24.63 and 23.12 mg g^{-1} of fresh tissue at 5 and 10 dS m^{-1}, respectively, compared to non-saline condition (26.38 mg protein g^{-1} fresh tissue) (**Table 3**). Protein content from root also varied, and seed priming with BCFE performed better than the control. Both microbial inoculation and BCFE priming improved protein content in the shoot and root tissues when the seedlings were under saline condition.

Accumulation of Soluble Sugars

The increase in sugar content in plant parts at high salinity stress indicated altered osmotic changes as a mechanism toward stress adaptation. Maximum sugar content was found in BCFE-primed seedlings (73.58 and 77.17 mg g^{-1} fresh tissue, respectively) at 10 dS m^{-1}. Compared to BCFE-primed seedlings, the bacteria-inoculated seedlings showed less sugar content, which was much lower in non-inoculated and non-primed seedlings (control) (**Table 3**).

Total Phenolic Content

In BCFE-primed seedlings, shoot and root accumulated a high content of total phenolics (1.348 and 1.218 mg g^{-1} fresh tissue, respectively) (**Table 3**). The bacteria-inoculated seedlings showed TPC of 1.245 and 1.3 mg g^{-1} of fresh shoot tissue and 1.045 and 1.073 mg g^{-1} of fresh root tissue, respectively, at 5 and 10 dS m^{-1}. It remained minimum (1.108 and 1.135 mg g^{-1}) in fresh shoot tissue and (0.890 and 0.893 mg g^{-1}) in fresh root tissue, respectively, at 5 and 10 dS m^{-1} in non-inoculated and non-primed seedlings.

Antioxidant Enzyme Activity in Wheat Seedlings

Bacterial inoculation and BCFE priming actively enhanced the level of antioxidative plant enzymes, namely, CAT, SOD, POD, and APX (**Table 4**). Salinity stress (5 and 10 dS m^{-1}) maximally enhanced the CAT activity in shoot tissues of the plants primed with BCFE (0.039 μM of H_2O_2 reduced mg^{-1} protein min^{-1}). Bacterial inoculation, however, showed a low CAT activity in the shoot. However, in the root, a reverse trend of CAT activity was

TABLE 4 | Antioxidant enzyme activity in wheat seedlings treated with *Nocardioides* sp. and bacterial culture filtrate extract (BCFE) under varying salinity stress conditions.

		Catalase[a]		Superoxide dismutase[b]		Peroxidase[c]		Ascorbate peroxidase[d]	
EC (dS m^{-1})	Treatment	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	*Nocardioides* sp.	0.022 ± 0.001e	0.020 ± 0.001b	15.80 ± 1.16e	5.51 ± 0.63e	0.038 ± 0.001bc	0.056 ± 0.002b	0.122 ± 0.007bc	0.126 ± 0.006cd
5		0.030 ± 0.001c	0.013 ± 0.001e	20.74 ± 0.98c	8.56 ± 0.88cd	0.035 ± 0.001cd	0.052 ± 0.002c	0.127 ± 0.005b	0.139 ± 0.011c
10		0.024 ± 0.001d	0.017 ± 0.001c	18.89 ± 0.94d	13.62 ± 0.49b	0.039 ± 0.001bc	0.047 ± 0.002*d*	0.125 ± 0.008bc	0.115 ± 0.010d
0	BCFE	0.027 ± 0.001b	0.032 ± 0.001a	22.64 ± 1.35b	9.06 ± 1.48cd	0.041 ± 0.005ab	0.071 ± 0.002a	0.134 ± 0.016*b*	0.180 ± 0.012a
5		0.039 ± 0.001a	0.018 ± 0.001c	23.46 ± 0.60b	9.78 ± 1.16c	0.044 ± 0.004a	0.072 ± 0.002a	0.187 ± 0.008a	0.135 ± 0.007c
10		0.039 ± 0.001a	0.015 ± 0.001d	25.48 ± 0.63a	17.09 ± 0.85a	0.043 ± 0.002a	0.054 ± 0.002bc	0.136 ± 0.007b	0.159 ± 0.008*b*
0	Control	0.019 ± 0.001f	0.013 ± 0.001de	11.57 ± 0.91f	3.47 ± 0.74f	0.032 ± 0.003d	0.038 ± 0.002e	0.101 ± 0.006d	0.100 ± 0.007e
5		0.020 ± 0.002f	0.012 ± 0.001e	14.95 ± 1.67e	7.47 ± 2.04d	0.032 ± 0.003d	0.044 ± 0.005d	0.112 ± 0.011cd	0.077 ± 0.005f
10		0.020 ± 0.001ef	0.013 ± 0.001e	15.46 ± 2.25e	8.47 ± 1.01cd	0.032 ± 0.003d	0.016 ± 0.002f	0.122 ± 0.006bc	0.093 ± 0.007e
Mean square error		1.30E-006	1.14E-006	1.610	1.277	7.23E-006	4.09E-00	7.97E-005	7.25E-005
R^2		0.983	0.976	0.941	0.939	0.794	0.998	0.895	0.946

Values in the same data series represented by different letters are significantly different (p = 0.05) in Duncan's multiple-range test. [a]Millimolar of H_2O_2 reduced per milligram of protein per minute. [b]Units of enzyme per milligram of protein. [c]Specific enzyme activity per milligram of protein. [d]Millimolar of ascorbate reduced per milligram of protein per minute.

observed. Both the microbe-inoculated and the BCFE-primed seedlings showed a high activity than the non-inoculated control plants. SOD was also enhanced due to bacterial inoculation and BCFE priming under saline condition (**Table 4**). In the shoot and the root tissues of BCFE-primed plants, maximum SOD activity was observed (25.48 and 17.09 U mg^{-1} protein at 10 dS m^{-1}).

Moderate saline condition (5 dS m^{-1}) increased the level of POD in shoot and root tissues primed with BCFE (0.044 and 0.072 specific enzyme activity mg^{-1} protein, respectively). Bacterial inoculation also enhanced the POD level in plants grown under salinity condition. Priming enhanced the APX activity in shoot at 5 dS m^{-1} (**Table 4**). Under salinity condition, the activity was low in the root tissues of the primed seedlings. We observed that priming significantly enhanced the activity of CAT, SOD, POD, and APX enzymes, which was low in bacteria-inoculated plants and the control.

Gene Expression

The antioxidant genes were upregulated in the seedlings having salinity stress (**Figures 3A–D**). Although bacterial inoculation significantly enhanced gene expression over the control, the extent of upregulation was quite less than that induced by BCFE priming. The expression of *CAT* gene increased with the increasing stress condition in both the bacterial inoculation and BCFE priming at all levels of stress. However, a plateau was observed at 5 dS m^{-1}, indicating no further upregulation of gene expression (**Figure 3A**). The expression of *MnSOD* and *POD* genes exhibited a marked upregulation at 5 dS m^{-1} that continued at 10 dS m^{-1} (**Figures 3A,B**). *APX* exhibited a characteristic trend in the control, and in the treatments the gene was intensively upregulated at 5 dS m^{-1} (**Figure 3D**).

Correlation Analysis

The cluster heat map prominently reflected physiological and biochemical responses of wheat seedlings inoculated with *Nocardioides* sp. NIMMe6, primed with BCFE and grown under saline condition (**Figure 4A**). Under non-saline condition (control), the performance of BCFE priming was better than the bacterial inoculation. Low scores (tending toward red) for several studied parameters of non-inoculated and non-primed plants indicated a higher performance of BCFE priming and bacterial inoculation on wheat. Pearson's correlation analysis revealed a complex intertwined physicochemical trait (**Figures 4B–D** and **Supplementary Tables S1A–C**). Phenotypic traits showed a positive correlation of root length with vigor index (r = 0.974 and 0.921, respectively) due to the influence of bacterial inoculation, which further enhanced the correlation between biomass and VI (r = 0.916). A positive correlation between sugar content in root with germination and POD activity in root with germination, vigor, and shoot and root length was seen due to the bacterial inoculation (**Figure 4B** and **Supplementary Table S1A**). Total phenolic content in shoot exhibited a strong negative correlation with shoot length (r = −0.998; **Figure 4B**). CAT activity in root, POD in shoot and root, and APX in shoot showed a significant ($p \leq 0.05$) positive correlation with shoot–root ratio, total phenolic content in root, shoot length, and SOD activity in shoot, respectively. Such positive correlations reflected a synergistic role of antioxidant enzymes and total phenolics in imparting important plant functions to alleviate abiotic stress. In BCFE-primed plants, biomass and germination (%), protein content in root and shoot, and CAT activity were positively correlated. SOD activity in root was positively linked with sugar content in shoot, while that of POD in root had a positive correlation with germination and biomass, respectively (**Figure 4C** and **Supplementary Table S1B**). Such correlations reflected an interlinked associative role of antioxidant and physicochemical status in the shoot and root development in bacteria-inoculated and/or BCFE-primed plants grown under saline conditions.

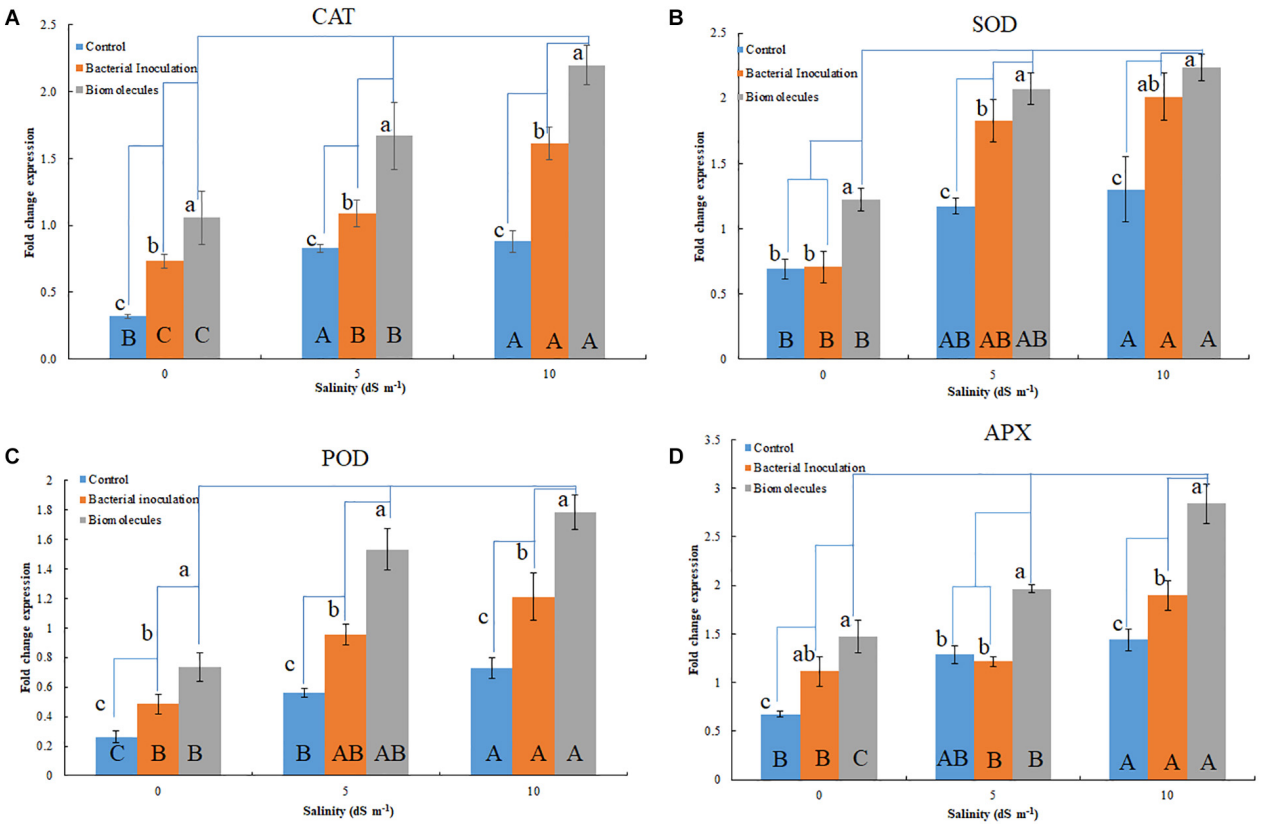


FIGURE 3 | Quantitative gene expression of antioxidant enzymes; *CAT* **(A)**, *MnSOD* **(B)**, *POD* **(C)**, and *APX* **(D)** in plants inoculated with *Nocardioides* strain and bacterial culture filtrate extract. Lowercase indicate comparison within a group (salinity level); while, the uppercase indicates comparison between groups (salinity levels).

DISCUSSION

Methylotrophic bacteria are important members of the phyllosphere community (Iguchi et al., 2015). They particularly inhabit plant leaf surface that facilitates the availability of methanol by virtue of stomatal leakage (Fall and Benson, 1996; Abanda-Nkpwatt et al., 2006). Previously, we have reported pink-pigmented methylotrophic bacteria from the phyllosphere of different crop plants such as sugarcane, pigeon pea, mustard, potato, and radish (Meena et al., 2012). In the present work, we isolated and characterized methylotrophic bacteria from the leaf surface of soybean, a crop of high commercial importance in India. The work was in continuation of our earlier efforts to obtain beneficial methylotrophic plant-growth-promoting rhizobacterial (PGPR) organisms from the phyllosphere of crop plants. The common association of colonizing bacterial species on the plant phyllosphere (Sy et al., 2005) and the potential role of methylotrophic species as PGPRs and mitigators of abiotic stresses in crop plants have been described (Kumar et al., 2019).

The PGP capabilities of methylotrophic bacteria are mainly attributed to their unique ability to synthesize a diverse array of biomolecules (Delmotte et al., 2009). These organisms secrete signaling molecules, phytohormones, vitamin B12, polysaccharides, and osmoprotectants into their habitats (Omer et al., 2004; Bustillos-Cristales et al., 2017). Plants in association with these microorganisms can get strength to sustain under biotic and abiotic stress conditions due to the impact of the phytohormones (Ojuederie et al., 2019). However, investigations that provide direct evidence on the beneficial impact of biomolecule-rich BCFE from methylotrophic bacteria on growth promotion and stress mitigation in crop plants are lacking.

We identified ***Nocardioides*** sp. NIMMe6 on the basis of 16S rRNA sequence similarity. The multiple carbon source utilization profile of this bacterium indicated its metabolic plasticity for better survival under nutritionally diverse habitats (Bochner, 2009). The capability of the isolate to actively utilize hexose sugars, amino acids, carboxylic acid ester, and fatty acids indicated that the bacterium can successfully inhabit chemically diverse habitats and thus can flourish in the dynamic environment of plant rhizosphere where a rich pool of metabolites exists (Berendsen et al., 2012). The actinobacterium ***Nocardioides*** sp. NIMMe6 showed specific PGP traits like siderophore and phytohormone production and survival under N-deficient condition which encouraged its impact assessment on wheat plants. A probable mechanism of SA and IAA production is depicted in **Figure 2**. The role of IAA in seed germination and development under salinity stress is known (Sorty et al., 2016; Korver et al., 2018). SA is a phenolic acid widely produced in both

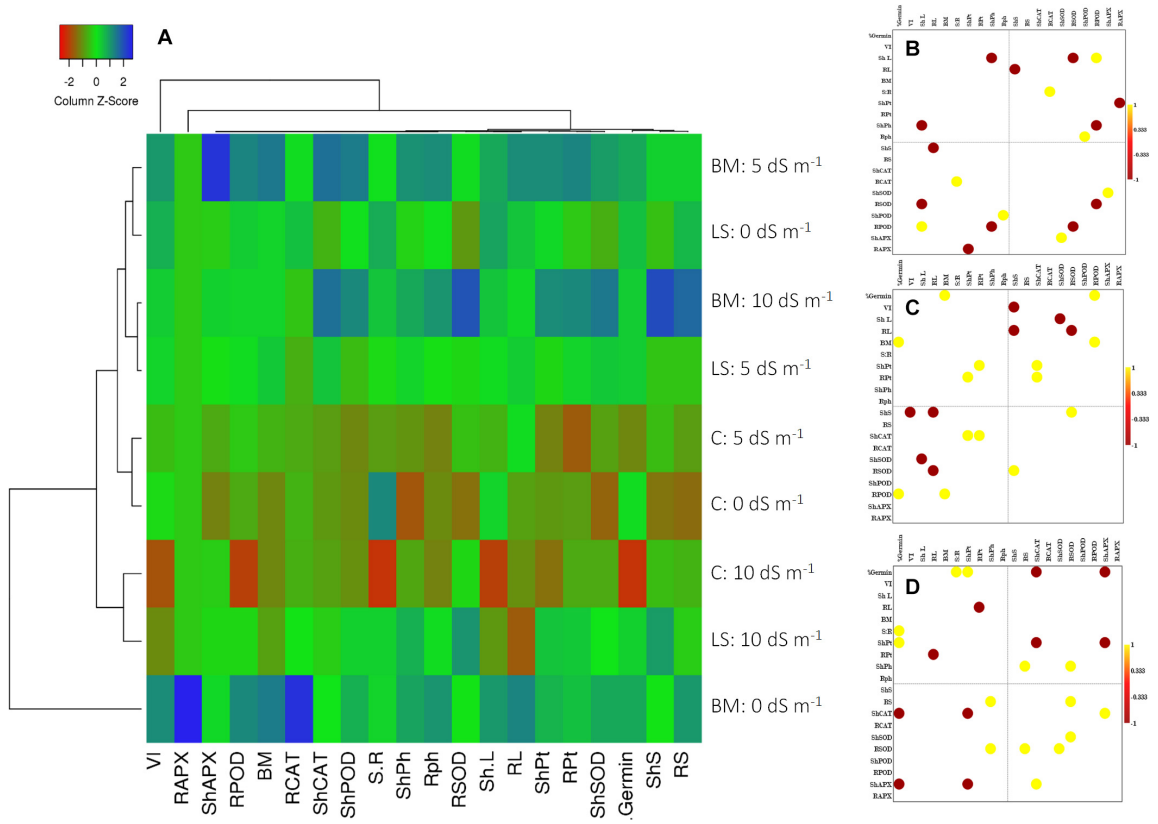


FIGURE 4 | Clustered heat map **(A)** and Pearson's correlation plots of the measured physicochemical traits of wheat seedlings under the influence of treatments with live strain **(B)** and bacterial culture filtrate extract **(C)** and control **(D)**. Values with $p \leq 0.05$ are included in the correlation plots.

prokaryotes and plants. SA activity in plants is mainly linked as a messenger for triggering local, and systemic acquired resistance under stress conditions (Conrath, 2006; An and Mou, 2011). We identified both IAA and SA in the culture filtrate of ***Nocardioides*** sp. using HPLC and MS (**Supplementary Figure S1**). This unique metabolic feature of the bacterium further endorsed its selection as a candidate strain for BCFE production in this study. The salt tolerance ability of the bacterium was the additional beneficial trait for applying it on wheat, a crop reported to suffer from saline conditions and whose early developmental stages, including the germination stage, are more vulnerable (Oyiga et al., 2016). Thus, there were reasonable reasons to assess the impact of inoculation of the bacterium and its culture filtrate extract on the wheat plants grown under saline conditions. Several workers have reported the impact of microbial inoculants on crop plants grown in soils of various EC levels. Agronomically, a soil having an EC value exceeding 4 dS m^{-1} of its saturated extract at room temperature and having exchangeable sodium of 15% is supposed to be saline (Yamaguchi and Blumwald, 2005). At this EC value of the soil, most crops show negative growth performance, although even lower ECs also exhibit yield reduction in crops (Manchanda and Garg, 2008). The positive impact of the inoculation of ***Glomus intraradices*** on bean (***Phaseolus vulgaris***) grown in soil of EC value 3.1 dS m^{-1}, ***Bacillus megaterium*** on maize grown in soil of EC 2.59 dS m^{-1}, and arbuscular mycorrhiza on ***Jatropha curcas*** grown in soils ranging from 1.7 to 8.5 dS m^{-1}. EC has been described (Dodd and Pérez-Alfocea, 2012). The comparative growth and development profile of wheat genotypes at different EC levels ranging from 0 to 12 dS m^{-1} was also evaluated by Kumar et al. (2017). Loss in germination percentage to the tune of 35–44% was recorded at 9 and 12 dS m^{-1} EC, respectively, in wheat varieties. As per Food and Agriculture Organization reports, the tolerance level of wheat is reported to be[2] 6 dS m^{-1}. Therefore, the assessment of the impact of microbial inoculation and BCFE in wheat at moderate, 5 dS m^{-1}, to relatively high EC level of 10 dS m^{-1} seems agronomically rightful and appropriate.

Inoculation of bacterium and priming of BCFE improved all plant parameters studied for growth and development of seedlings. Seedlings grown under saline conditions responded against stress in terms of low germination, vigor index, shoot and root length, and biomass accumulation. In general, a decrease in phenotypic characters like seedling biomass, moisture content, seed germination, and vigor index following salinity stress has been reported as a natural phenomenon (Hasanuzzaman et al., 2013). The impact of bacterial inoculation and BCFE priming significantly reversed the declining growth parameters among seedlings grown under stress conditions (5 and 10 dS m^{-1}). With increasing salinity condition, the BCFE-primed wheat seedlings

[2]http://www.fao.org/3/y4263e/y4263e0e.htm; visited on 20.4.2020

exhibited better physiological performance (germination, vigor index, shoot and root length, and biomass), indicating a significant role of BCFE priming in strengthening the seedlings against salinity stress. The impact is supposed to be due to the direct implications of the metabolites reported in the BCFE.

Cell-free inoculants have been considered as upcoming tools to mitigate abiotic stress(es) under a changing climate scenario (Bashan et al., 2016; Vassilev et al., 2017). Due to their characteristic ability to produce a variety of biomolecules, including plant growth hormones, siderophores, vitamins, phenolic compounds, volatiles, a variety of enzymes, and biocontrol agents, PGP microbes can be potential candidates toward designing cell-free inoculae (Sorty et al., 2016; Sreevidya et al., 2016; Mendes et al., 2017). The bacterium *Nocardioides* sp. NIMMe6 secreted into the culture filtrate IAA and SA. Both of these metabolites have a significant role in plant growth promotion (Egamberdieva et al., 2017) and induction of plant responses against stresses (Ku et al., 2018).

Our results witnessed both evidences. The BCFE which was shown to contain IAA and SA, when used as a priming agent on seeds, resulted in healthy seedlings under saline conditions, and plant growth was even better than bacterial inoculation itself. At the same time, quantitative changes at the level of antioxidant enzyme activity and regulation of antioxidant genes were also observed in BCFE-primed seedlings. It was therefore assumed that BCFE priming not only supported seedling growth but also helped in inducing stress-mitigating mechanisms in plant seedlings. The higher impact of BCFE on root and shoot length, germination, and vigor index in wheat can be attributed to the availability of IAA and SA compounds in higher quantity that favor plant growth. SA is structural component of siderophores (Djavaheri et al., 2012) and helps plants to mount vital responses against abiotic stress conditions (Rivas-San Vicente and Plasencia, 2011). IAA is a plant growth promoter under normal or abiotic stress and helps to maintain hormonal balance for adequate homeostasis (Fu and Harberd, 2003). Inoculation with live bacterial cells showed improved impact as compared to non-inoculated and non-primed plants. The performance of BCFE priming on seeds was comparatively more impactful. This effect may be attributed to the fact that the growth of bacterial inoculant cells under salinity-stressed habitat becomes challenging and the performance of the live inoculant is hampered due to non-favoring cellular development (O'Callaghan, 2016).

Reactive oxygen species that generate oxidative stress in plants (Foyer and Noctor, 2005) critically damage vital organelles and induce cellular apoptosis (Kumar et al., 2015). The overall oxidative challenge is maintained in plants through a delicate balance between ROS formation and concurrent scavenging by enzymatic and non-enzymatic antioxidants (Keunen et al., 2013). The production and the accumulation of phenolic compounds and antioxidant enzymes and the activation of the glutathione system that actively scavenge ROS to prevent consequent damage to cellular components are some of the known mechanisms to overcome stresses (Blokhina et al., 2003). Although plants respond to abiotic stresses by inducing various intrinsic mechanisms within their cells, microbial inoculation leads to enhanced mechanisms that significantly overcome ROS accumulation (Meena et al., 2017). The antioxidant enzymes in plants challenged with abiotic stress reflect cumulative enzymatic response in terms of cellular oxidative stress management. To measure superoxide radical management in tissues, we monitored the activity of SOD enzyme. Similarly, the activity of ascorbate peroxidase, peroxidase, and catalase reflected the efficiency of peroxide (H_2O_2) management in wheat seedlings under saline conditions. The improved activity of SOD, CAT, POD, and APX in both root and shoot tissues reflected activated enzymatic mechanisms that help plants to reduce the severity of salinity stress. The increased level of SOD indicated an antioxidant enzyme-based management of superoxide radicals. Similarly, treatment-dependent alterations in the expression of CAT in plants grown under stress were observed (Scandalios, 2005). Our report on the enhanced level of antioxidant enzyme activity under salinity condition aligns with the previous observations of Yang et al. (2008). BCFE priming significantly induced CAT in shoot at a higher salinity level. The enhanced activity of APX and POD also helped in antioxidant enzyme-based management of oxidative damage in wheat.

The management of a plant's stress level is also achieved by the regulation of various genes that code for the antioxidant enzymes CAT, SOD, APX, and POD to detoxify oxidative radicals (Xie et al., 2019). The upregulation of gene transcripts related to the antioxidant enzymes in inoculated and primed wheat was observed. The result of the upregulation of the four genes certifies the beneficial role of *Nocardioides* sp. NIMMe6 inoculation and BCFE priming in wheat plants grown under saline conditions. Induction of plant abiotic stress tolerance due to the overexpression of genes linked with the antioxidant enzyme by microbial inoculation has been reported (Gururani et al., 2013). We have shown that, along with bacterial inoculation on seeds as inoculants, the BCFE from the bacterium can also be used for stress alleviation in wheat seedlings due to its impact on the modulated biochemical and molecular mechanisms in plants. The BCFE showed a relatively better performance over bacterial live cell inoculation under salinity stress.

Potential benefits of microbial inoculation to plants under abiotic stress conditions have been reported (Singh et al., 2011; Nadeem et al., 2014). The limitations of the live cells of microbial inoculants applied under stress conditions that lower down the inoculant performance have also been discussed (Naylor and Coleman-Derr, 2018). The application of BCFE from the potential microbial species, therefore, could be a viable option. From the potential species having the capacity of becoming a microbial inoculant, specific functional secondary metabolite could be produced through induction of metabolic flux under modified culture conditions (Baral et al., 2018). This approach may offer a more practical, efficient, and sustainable alternative to maintain a plant's performance under unfavorable conditions. However, a critical evaluation under field conditions is necessary to warrant more applicability for recommendation at a large scale.

CONCLUSION

We have explored an actinobacterium, *Nocardioides* sp. (LC140963), for *in vitro* production and secretion into the medium of bioactive compounds like salicylic acid and indole acetic acid. These biomolecules were extracted from bacterial culture filtrate and utilized in the form of BCFE for salinity stress mitigation in wheat through seed priming. The findings suggested that the incorporation of BCFE is efficient over microbial inoculation for mitigation of salinity stress in wheat seedlings. Seed priming with BCFE actively enhanced both the physicochemical status and the oxidative enzymes and helped in the modulation of gene in wheat seedlings. Therefore, the results strongly endorse the strategic utilization of BCFE rich in biomolecules secreted by plant-growth-promoting microbial strain(s) for the strategic management of salinity stress tolerance in crop plants. More specific work will further warrant large-scale applicability under field conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the DNA data Bank of Japan; LC140963; https://www.ncbi.nlm.nih.gov/nuccore/LC140963.

AUTHOR CONTRIBUTIONS

KM conceptualized the work. KM, UB, and AS designed and conducted the experiments. KM, UB, AS, DS, SK, and VG gene expression studies, and biochemical experiments. KM, AS, UB, and GW prepared the draft of the manuscript. All authors edited and improved the manuscript document.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.02091/full#supplementary-material

FIGURE S1 | Indole-3-acetic acid and salicylic acid were identified from the pool of bacterial culture filtrate extract (BCFE) secreted by the strain using high-performance liquid chromatography and liquid chromatography–mass spectrometry approaches **(A,B)**. The photographs present the morphological appearance of the strain and BCFE diluted in absolute methanol.

FIGURE S2 | Growth characteristics of *Nocardioides* sp. under increasing salt stress conditions from 0 to 10% of NaCl.

FIGURE S3 | Metabolic behavior of *Nocardioides* sp. in the presence of a variety of carbon sources and inhibitory environments as evident from the Biolog Gen III assay.

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Nodule and Root Zone Microbiota of Salt-Tolerant Wild Soybean in Coastal Sand and Saline-Alkali Soil

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Soil salinization limits crop growth and yield in agro-ecosystems worldwide by reducing soil health and altering the structure of microbial communities. Salt-tolerant plant growth-promoting rhizobacteria (PGPR) alleviate plant salinity stress. Wild soybean (*Glycine soja* Sieb. and Zucc.) is unique in agricultural ecosystems owing to its ability to grow in saline-alkali soils and fix atmospheric nitrogen *via* symbiotic interactions with diverse soil microbes. However, this rhizosphere microbiome and the nodule endosymbionts have not been investigated to identify PGPR. In this study, we investigated the structural and functional rhizosphere microbial communities in saline-alkali soil from the Yellow River Delta and coastal soil in China, as well as wild soybean root nodule endosymbionts. To reveal the composition of the microbial ecosystem, we performed 16S rRNA and *nifH* gene amplicon sequencing on root nodules and root zones under different environmental conditions. In addition, we used culture-independent methods to examine the root bacterial microbiome of wild soybean. For functional characterization of individual members of the microbiome and their impact on plant growth, we inoculated isolates from the root microbiome with wild soybean and observed nodulation. *Sinorhizobium/Ensifer* accounted for 97% of the root nodule microbiome, with other enriched members belonging to the phyla Actinobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, and Gemmatimonadetes; the genera *Sphingomonas*, *Microbacterium*, *Arthrobacter*, *Nocardioides*, *Streptomyces*, *Flavobacterium*, *Flavisolibacter*, and *Pseudomonas*; and the family Enterobacteriaceae. Compared to saline-alkali soil from the Yellow River Delta, coastal soil was highly enriched for soybean nodules and displayed significant differences in the abundance and diversity of β-proteobacteria, δ-proteobacteria, Actinobacteria, and Bacteroidetes. Overall, the wild soybean root nodule microbiome was dominated by nutrient-providing *Sinorhizobium/Ensifer* and was enriched for bacterial genera that may provide salt resistance. Thus, this reductionist experimental approach provides an avenue for future systematic and functional studies of the plant root microbiome.

Keywords: wild soybean, salinity, root nodule, microbiome, illumina sequencing, 16S rRNA, *nifH*

INTRODUCTION

Soil salinization is a common abiotic stress that restricts crop growth and yield in agro-ecosystems worldwide. The Yellow River Delta is one of the three largest river deltas in China and is becoming a major region for agricultural development (Jing et al., 2019); however, crop production is limited by high soil salinity, which reduces the water-extraction capacity of roots and has a devastating effect on plant metabolism. In addition, high soil salinity disrupts cellular homeostasis and results in the uncoupling of major physiological and biochemical processes; thus, the reclamation of stressed soils is critical for meeting the food demands of the ever-increasing population and improving soil quality (Li et al., 2016b; Etesami and Maheshwari, 2018).

The microbiome that symbiotically inhabits the interior of plant roots and saprophytically interacts with soil particles in the rhizosphere is vital for promoting plant growth, fixing nitrogen *via* nodulation, and protecting plants from stress. Salt-tolerant plant growth-promoting rhizobacteria (PGPR) have displayed great potential for alleviating plant salinity stress (Akram et al., 2016; Backer et al., 2018; Chu et al., 2019; Kearl et al., 2019; Rodriguez et al., 2019). These beneficial soil microbes reside in the rhizosphere and, together with root exudates, can provide plants with nutrients, growth hormones, antioxidants, and systemic resistance, even under high salt concentrations (Ahmad et al., 2013; Li et al., 2014; Kong et al., 2015; Agler et al., 2016). Indeed, salt-tolerant PGPR and their metabolites isolated from halophyte species in saline soils can play key roles in mitigating salinity stress and enhancing crop yield (Bouhmouch et al., 2005; Albdaiwi et al., 2019; Chauhan et al., 2019; Chen et al., 2019; Saghafi et al., 2019). Different microbes have been associated with various plants and growth environments, suggesting the existence of specific microbe-host interactions (Perez-Jaramillo et al., 2019). In addition, some endophytes, including *Sphingomonas*, *Bacillus*, *Enterobacter*, and *Pantoea* species, can stimulate plant growth under saline conditions (Li et al., 2016a). Moreover, diazotrophs have been isolated from the nodule and root surface of legumes or the root surface of other plants (Fan et al., 2018). The same plant is generally associated with more than one diazotroph, which display different patterns of regional distribution and frequency. The most common diazotrophs identified so far are *Azospirillum*, *Herbaspirillum*, *Enterobacter*, *Klebsiella*, *Azotobacter*, *Beijerinckia*, *Bacillus*, and *Pseudomonas*, while other diazotrophs belong to the *Lactobacillus* and *Halobacillus* (Dos Santos et al., 2012).

Wild soybean (*Glycine soja* Sieb. and Zucc.) is widely distributed throughout China, northeast Russia, Korea, and Japan (Ge et al., 2010; Jing et al., 2018; Xie et al., 2019) and is characterized by greater cold hardiness, salt tolerance, and disease resistance than cultivated soybean *Glycine max* Merr (Jing et al., 2018; Xie et al., 2019). Consequently, wild soybean is of high economic value, particularly for the cultivation of advanced soybean varieties (Jing et al., 2018; Ohigashi et al., 2019); however, its root zone microbiome and nodule endosymbionts have not yet been investigated to identify PGPR by next generation sequencing.

Nitrogenases are widespread in bacteria and archaea, providing them with a competitive advantage in environments depleted of bio-available nitrogen, which affects PGPR function (Dos Santos et al., 2012; Fan et al., 2019). The ability to fix nitrogen is widely, but sporadically, distributed among archaea and bacteria, including the families Proteobacteria, Firmicutes, Cyanobacteria, Actinobacteria, and Chlorobi (Dos Santos et al., 2012). *NifH* is used as a marker gene to detect nitrogen-fixing microorganisms in the environment (Igai et al., 2016; Fan et al., 2019), and nitrogen-fixing Rhizobiales have been identified in the special root nodules of crop legumes, such as alfalfa, beans, peas, and soy, which provide 20% of food protein worldwide (Mnasri et al., 2012; Egamberdieva et al., 2013; de Almeida Lopes et al., 2016; Sharma et al., 2016; Singh et al., 2019).

In this study, we combined metagenomic approaches and *NifH* Illumina sequencing to characterize wild soybean rhizosphere and nodule microbiomes at a deep taxonomic resolution. In addition, we investigated the structure and function of the microbiota at the wild soybean root-soil interface and in the nodules of coastal sand and saline-alkali soil using comparative computational approaches. Metagenomic results were correlated and confirmed by the culture and isolation of microbial communities, while their effect on nodulation was verified using root infection assays. Together, this reductionist experimental approach provides an avenue for future systematic and functional studies of the plant root microbiome.

MATERIALS AND METHODS

Sampling Sites

The samples used in this study were obtained from the Yellow River Delta (37°39′43.58″N, 118°40′48.19″E, north of the Shangdong Peninsula, China) and coastal sand from Qingdao near the Huanghai sea coastline (36°7′58″N; 120°26′42″E, south of the Shangdong Peninsula, China). Soil salinity, electrical conductivity (EC), and pH were measured using a 1:2.5 soil:water solution. Briefly, 10 g of air-dried soil was dissolved in 25 ml of ddH_2O, mixed completely for 30 min, and filtered using filter paper. The filtrate was measured using pH and conductivity meters before being evaporated to obtain salt. According to the Food and Agriculture Organization (FAO of the United Nations) World Reference Base for Soil Resources, the soil from the Yellow River Delta was calcaric fluvisol and had a pH of 7.94, soil salinity of 1.96, soil organic matter of 1.01%, and total nitrogen of 0.11/100 g (Jing et al., 2019), with an EC of 565 ± 33 μS/cm. The coastal sand had a pH of 6.5, soil salinity of 0.56%, soil organic matter of 0.41%, total nitrogen of 0.60 mg/100 g, and EC of 155 ± 64 μS/cm. Although these soils were not highly salinized, cultivated soybean was not able to grow normally, yet wild soybean was able to grow normally and produce yield. Since rhizospheric soil was not readily obtained from the sand samples, we used the root zone for further experiments. Briefly, surface soil (2 cm deep) was removed and soil within 5 cm of the plant stem was moved using a shovel. The root was then lifted out carefully,

with surrounding soil designated as the root zone. Root-attached nodules were washed thoroughly with sterilized distilled water and 50 nodules were sent for sequencing.

Library Preparation, Sequencing, and Bioinformatic Analysis of High-Throughput Data for the Rhizospheric Soil and Nodules

Total metagenomic DNA was extracted from rhizospheric soil and nodule samples using a FastDNA spin kit for soil (MP Biomedicals, LLC, Santa Ana, CA, USA) according to the manufacturer's protocols and verified using 0.8% agarose gel electrophoresis. Extracted DNA was amplified using a 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGT CATCCCCACCTTCC-3') universal primer set targeting the V5–V7 region of the bacterial 16S ribosomal RNA (rRNA) genes as well as a *nifH*1 460–476 (5'-ADNGCCATCATYT CNCC-3') and *nifH*2 115–131 (5'-TGYGAYCCNAARGCNGA-3') universal primer set targeting the *nifH* genes (Zehr and McReynolds, 1989). An AxyPrepDNA gel extraction kit was used to purify the PCR products and remove salts and proteins to construct a MiSeq library. The PCR products were also checked by 2% agarose gel electrophoresis before and after gel extraction. DNA sequence degeneracy was described according to the International Union of Pure and Applied Chemistry Conventions: Y = C/T; S = G/C; R = A/G; B = C/G/T; D = G/A/T; H = T/C/A; N = A/G/C/T; W = A/T; and I = inosine. The most degenerated *nifH* primers pairs according to the conserved amino acid sequence were used and the PCR products were examined using 1.5% agarose gel electrophoresis, purified using a Qiagen gel extraction kit (Qiagen, Hilden, Germany), and sequenced on an Illumina (San Diego, CA, USA) MiSeq PE300 platform by Allwegene Genomics (Beijing, China). Raw data were deposited in BioProject under accession numbers PRJNA597572 and PRJNA597574 for 16S rDNA and *nifH*, respectively.

After low quality, ambiguous reads had been filtered and chimeric sequences had been removed using UCHIME[1] (Edgar et al., 2011), high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity and bacterial taxonomy was assigned phylogenetically using the Ribosomal Database Project (RDP) classifier (Cole et al., 2014). The raw data analysis of the *nifH* gene fragments was carried out in a similar manner to the 16S rRNA high-throughput sequencing and submitted to the Non-Redundant Protein Sequence Database and Nucleotide Sequence Database from National Center for Biotechnology Information (NCBI nr/nt database). Next, α-diversity indices used to estimate bacterial diversity (Shannon and Simpson) and richness (Chao1 and ACE) were calculated based on OTUs using Mothur[2] (Schloss et al., 2009). Venn diagrams were constructed using Venny[3] and community sequencing data were subjected to taxonomic diversity analysis using QIIME (Caporaso et al., 2010). Principal co-ordinates analysis (PCoA) was conducted on Bray-Curtis dissimilarity matrices of OTUs at 97% cut-off in R Studio to reveal community-level differences between treatments (McMurdie and Holmes, 2013). Sequencing and data analysis were carried out by Allwegene Genomics (Beijing, China).

[1]http://drive5.com/uchime
[2]https://www.mothur.org/
[3]http://bioinfogp.cnb.csic.es/tools/venny/index.html

Isolation and Identification of Nodule Bacteria

To isolate nodules, plants were gently uprooted and taken to the laboratory, where root samples were washed thoroughly under running tap water, surface-sterilized in a 3% sodium hypochlorite solution with 0.02% Tween 20 for 3 min, rinsed three times in sterilized distilled water, and dry-blotted onto sterilized filter paper (Lopez-Gomez et al., 2017). The nodules were then ground in a sterilized mortar, streaked onto a Luria-Bertani (LB) agar plate and incubated at 30°C for 2 days or onto a yeast extract mannitol agar (YMA) plate and incubated at 30°C for 5 days. Purified colonies were preserved in 20% (v/v) glycerol at −80°C for long-term storage and in slants for regular use.

Isolated strains were identified according to Singh et al. (2019). Briefly, genomic DNA was isolated using a Wizard® Genomic DNA Purification Kit (Promega) and 16S rRNA was amplified by PCR using 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-GGTT-ACCTTGTACGACTT-3') universal primers with an Applied Biosystems PCR system. Amplified PCR products were purified by 1.2% agarose gel electrophoresis (Bio-Rad, Shanghai, China) and sequenced from both directions at Qingke Biotech (Qingdao, China). Sequence annotations were compared to the EzBioCloud database (Yoon et al., 2017).

Construction of Artificial Culture System for Isolate Nodulation

Two-hundred wild soybean seeds collected from the Yellow River Delta were scarified by immersion in concentrated H_2SO_4 (98.3% vol/vol) for 5 min to make the seed coat thinner and more conducive to germination. Then, the seeds were immediately washed with cool running sterile water for 5–10 min and germinated on 1.0% water-agar plates at 25°C in the dark (Lopez-Gomez et al., 2017). Once rooting and germination had occurred after approximately 2 days, the seedlings were transferred to 100 ml of sterile nitrogen-free liquid medium [3 g $Ca(NO_3)_2$•$4H_2O$, 0.46 g $CaSO_4$, 0.075 g KCl, 0.06 g $MgSO_4$•$7H_2O$, 0.136 g K_2HPO_4•$2H_2O$, 0.075 g iron citrate, and 1 ml of trace element solution (2.86 g H_3BO_3, 1.81 g $MnSO_4$, 0.22 g $ZnSO_4$, 0.80 g $CuSO_4$•$5H_2O$, and 0.02 g H_2MoO_4 in 1000 ml ddH_2O), and 1,000 ml of ddH_2O] according to Chen and Wang (2011) in 21 grass test tubes (200 × 40 mm) scaffolded with filter paper. Two bacterial isolates were from the Yellow River Delta and one was from the coastal region. For each bacterial isolate, five tubes were used as the experimental group and two as the control group, with three seedlings per tube. After 2 days, the seedlings were inoculated into 1 ml of nitrogen-free liquid medium containing *Sinorhizobium* sp., (c. 10^9 cell/ml) grown in tryptone-yeast extract (TY) medium (5 g tryptone, 3 g yeast extract, 0.7 g $CaCl_2$•$2H_2O$,

1,000 ml ddH_2O, and pH 6.8–7.0) and washed twice in logarithmic phase by suspension and centrifugation in nitrogen-free liquid medium to remove the TY medium nutrients. Plants were grown in a controlled environmental chamber for 2 weeks with a 16/8 h light-dark cycle, 23/18°C day-night temperature, and 55/65% relative humidity. Control plants were cultivated in the same nutrient solution without *Sinorhizobium* sp., inoculation.

Statistical Analysis

Results are presented as the mean ± standard deviation of three independent experiments. Statistical differences were determined by one-way analysis of variance followed by Tukey's test in SPSS version 17.0 (IBM, Armonk, NY, USA), with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

Bacterial Community Composition in the Root Zone of Saline-Alkali Soils

To investigate the effect of saline-alkali soils on the root and nodule microbiomes of wild soybean, we analyzed the root zone microbiome of wild soybean grown in two saline-alkali soil types from the Yellow River Delta near to Dongying city (SDY) and coastal soil (Ss). A total of 1,528 ± 39 and 1,797 ± 246 OTUs were obtained from the clean sequences from the SDY and Ss root zones, respectively (**Supplementary Table S1**), whereas only 358 ± 209 and 164 ± 10 OTUs were obtained from the nodules of wild soybean grown in these regions, respectively. We found that the barren sand root zone carried more OTUs than the fertile soil; however, our previous study identified 191 ± 13 OTUs in nodules using PacBio's circular consensus sequencing for full-length bacterial 16S rRNA (Zheng et al., 2020), similar to the 164 ± 10 OTUs obtained here. The dataset was rarefied to an even sequencing depth of 20,000 sequences and 2,423 bacterial OTUs were identified. The Shannon and Simpson diversity indices and Chao1 richness estimator for the 16S V5-V7 replicon sequencing of these samples are shown in **Figure 1** and **Supplementary Table S2**, while the Venn diagram is shown in **Supplementary Figure S1**.

Although we found no significant difference in the number of clean sequences between the root zones and nodules (**Supplementary Table S3**), the number of OTUs did differ significantly, with the SDY nodules having around one-tenth the number of OTUs of the SDY root zone. In addition, there were approximately twice as many OTUs in the nodules of the Ss samples than in the SDY samples.

Overall, 99.67 ± 0.34% of the sequences obtained from the root zone of wild soybean from Ss samples were assigned to a bacterial taxonomy, compared to 95.89 ± 1.63% of those in the SDY samples. The nine most abundant phyla (>1% sequences) comprised 95.42 ± 0.33 and 96.75 ± 0.24% of the root zone community in Ss and SDY samples, respectively (**Supplementary Table S3**), with 39.67 ± 2.87% and 39.67 ± 3.09% of the sequences belonging to Proteobacteria (**Supplementary Table S4**; **Figure 2A**). The second most abundant phylum was Actinobacteria (26.00 ± 2.94 and 24.00 ± 3.74% in Ss and SDY root zones, respectively), while other phyla identified in the root zones included Bacteroidetes (10.67 ± 1.70 and 9.00 ± 0.82%), Chloroflexi (6.00 ± 0.05 and 6.67 ± 0.47%), Acidobacteria (3.67 ± 0.90 and 6.00 ± 0.02%), Gemmatimonadetes (2.67 ± 0.47 and 4.67 ± 0.47%), Planctomycetes (3.00 ± 0.83 and 3.67 ± 0.47%), and Firmicutes (1.30 ± 0.50 and 1.00 ± 0.01%). The Ss root zone samples also included Saccharibacteria (1.33 ± 0.47%), which only accounted for significantly less than 0.20 ± 0.00% of the root zone abundance in SDY samples. Another significant difference in relative abundance was observed for Verrucomicrobia (0.90 ± 0.14 and 2.00 ± 0.00% in Ss and SDY root zones, respectively) as well as Acidobacteria, which could be due to differences in pH between the root zones of wild soybean grown in coastal sand (pH 6.5) and saline-alkali soil from the Yellow River Delta (pH 7.6). At the genus level, *Sinorhizobium/Ensifer*, which is the dominant genus in nodules, accounted for just 1.59 ± 0.40 and 1.87 ± 0.20% of the species in Ss and SDY root zones, respectively (**Supplementary Table S5**; **Figure 2B**). The relative microbial abundance in six root zone samples and six nodule samples at the order and family levels, as determined by 16S Illumina sequencing, is shown in **Supplementary Figure S2**.

Identification of Nodule Bacterial Community Composition

The 16S rRNA sequences amplified from the uncultured root nodule samples were assigned to 14 phyla and an overview of the dominant phyla and genera is provided in **Supplementary Tables S4** and **S5**. Based on relative abundance, the most common phyla (>0.1%) in the coastal nodule samples (nS) and Yellow River Delta nodule samples (nD), respectively, were Proteobacteria (98.458 ± 0.045 and 99.703 ± 0.002%), Actinobacteria (0.533 ± 0.340 and 0.167 ± 0.047%), Bacteroidetes (0.177 ± 0.158 and 0.037 ± 0.017%), Chloroflexi (0.123 ± 0.122 and 0.047 ± 0.009%), Acidobacteria (0.113 ± 0.092 and 0.043 ± 0.012%), and Gemmatimonadetes (0.117 ± 0.095 and 0.043 ± 0.009%), indicating that nS samples displayed a higher diversity and proportion than nD samples. As predicted, the most prominent Proteobacteria were *Sinorhizobium/Ensifer* (97.330 ± 1.701 and 98.661 ± 0.471%); however, the proportion differed (85.67 ± 6.29%) from PacBio's circular consensus sequencing for full-length bacterial 16S rRNA gene in nD samples (Zheng et al., 2020).

To investigate the diversity of bacteria related to saline tolerance and plant growth promotion, we focused on the minor dominant genera beyond *Sinorhizobium/Ensifer*. Besides Proteobacteria, Actinobacteria was the most abundant phylum in the two types of nodule samples. *Microbacterium*, *Arthrobacter*, *Nocardioides*, and *Streptomyces* were the most predominant Actinobacteria in nS samples, with relative abundances of over 0.03%, while *Blastococcus* and *Patulibacter* had relative abundances of over 0.005%. In the α-proteobacteria, *Sphingomonas* (60.0, 68.3% in Sphingomonadaceae) accounted for 0.086 ± 0.009 and 0.016 ± 0.004% of all bacteria in the nS and nD samples, respectively, while *Variibacter* (77.0, 67.1% in Xanthobacteraceae) accounted for 0.015 ± 0.006 and 0.013 ± 0.004%. Other identified

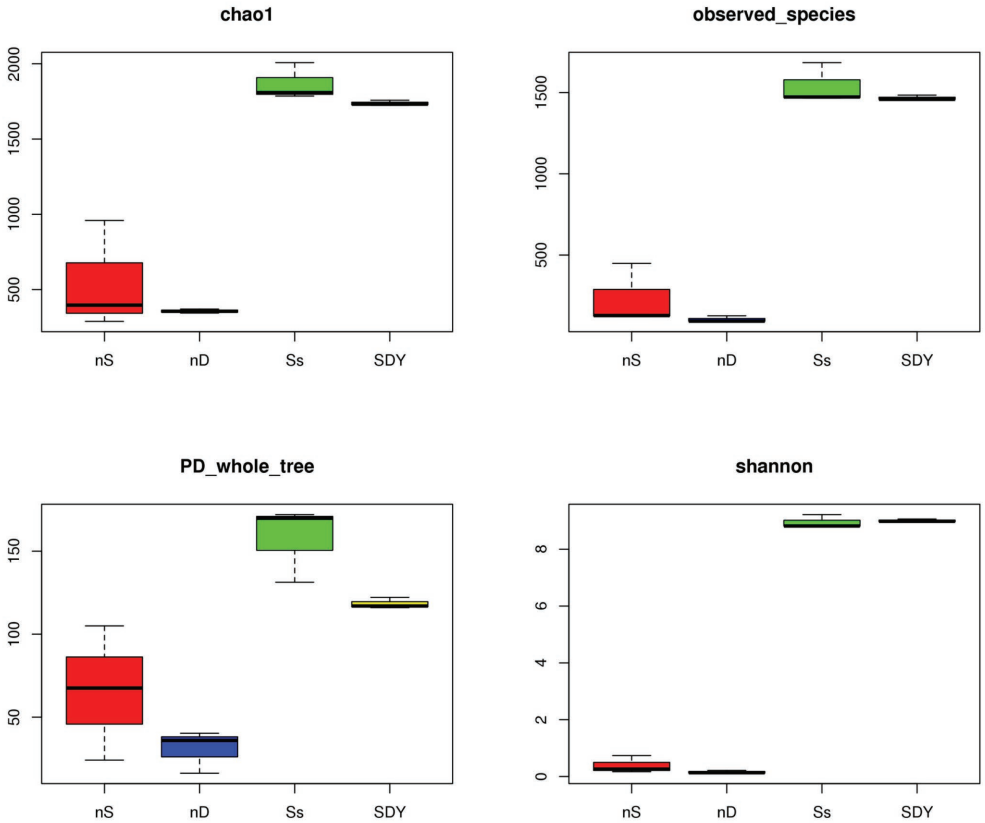

FIGURE 1 | Alpha diversity indices of 16S rRNA sequences in the two nodule samples and two root zone samples from wild soybean.

α-proteobacteria genera included *Devosia*, *Pedomicrobium*, and *Microvirga* (**Supplementary Table S5**). In the γ-proteobacteria, some OTUs were only identified as belonging to the Enterobacteriaceae family (38.3 ± 14.5 and 31.7 ± 21.3% in the nS and nD samples, respectively), which have been reported to be PGPR (Agler et al., 2016). *Pseudomonas* was the predominant genus identified, accounting for 0.032 ± 0.008 and 0.027 ± 0.007% in Ss and SDY root zones of all bacteria, while *Acinetobacter* and *Pseudoxanthomonas* had relative abundances of >0.01%. *Flavobacterium* (24.0 ± 10.7%) was the predominant Bacteroidetes genus in nS samples, accounting for 0.042 ± 0.010% of all bacteria, but only making up 2.0 ± 1.5% of *Bacteroidetes* in nD samples. *Flavisolibacter* was another dominant Bacteroidetes genus (**Supplementary Table S5**). *Bacillus* (59.5%), *Paenibacillus* (18.6%), *Halobacillus* (21.4%), and *Lysinibacillus* (10.4%) were the most predominant Firmicutes genera in nS samples, constituting 0.022 ± 0.005, 0.008 ± 0.003, 0.004 ± 0.002, and 0.004 ± 0.001% of all bacteria, respectively (**Supplementary Table S5**).

Comparison of Bacterial Community Composition in Different Soil Samples

Although Ss and SDY samples displayed similar proportions of Actinobacteria (26:24%), we found that levels were more than three times higher in nodule samples, suggesting that Actinobacteria may be involved in saline tolerance in the rhizosphere of wild soybean grown in coastal regions (Bhatti et al., 2017). In α-proteobacteria, nS samples had more than five times as many *Sphingomonas* species than nD samples; however, it has previously been reported that these root zones display similar proportions of *Sphingomonas* species (Menon et al., 2019). Similarly, nS samples also harbored a higher proportion of the γ-proteobacteria Enterobacteriaceae and *Pseudomonas* families, which have also been reported as PGPR (Agler et al., 2016; Chu et al., 2019). Despite thorough cleaning before environmental DNA extraction, the surface of the nodules contained some rhizosphere bacteria from the root zone. Although the salinity of the coastal sand was lower than that of fertile soil from the Yellow River Delta, its sandy characteristics result in a lower water holding capacity, meaning that the relative salinity of the sand was actually higher than that of the soil.

The nodules had similar proportions of γ-proteobacteria and thus could be used as a control to compare changes in the ratio of different bacteria. We found that the pH 7.9 alkali soil had a higher relative abundance of Acidobacteria than the pH 6.5 coastal sand, indicating that Acidobacteria may play an important role in the degradation of plant residues due to a high organic matter content (Kielak et al., 2016). Moreover, the relative abundance of Acidobacteria in nS samples was nearly three times that in nD samples, suggesting that

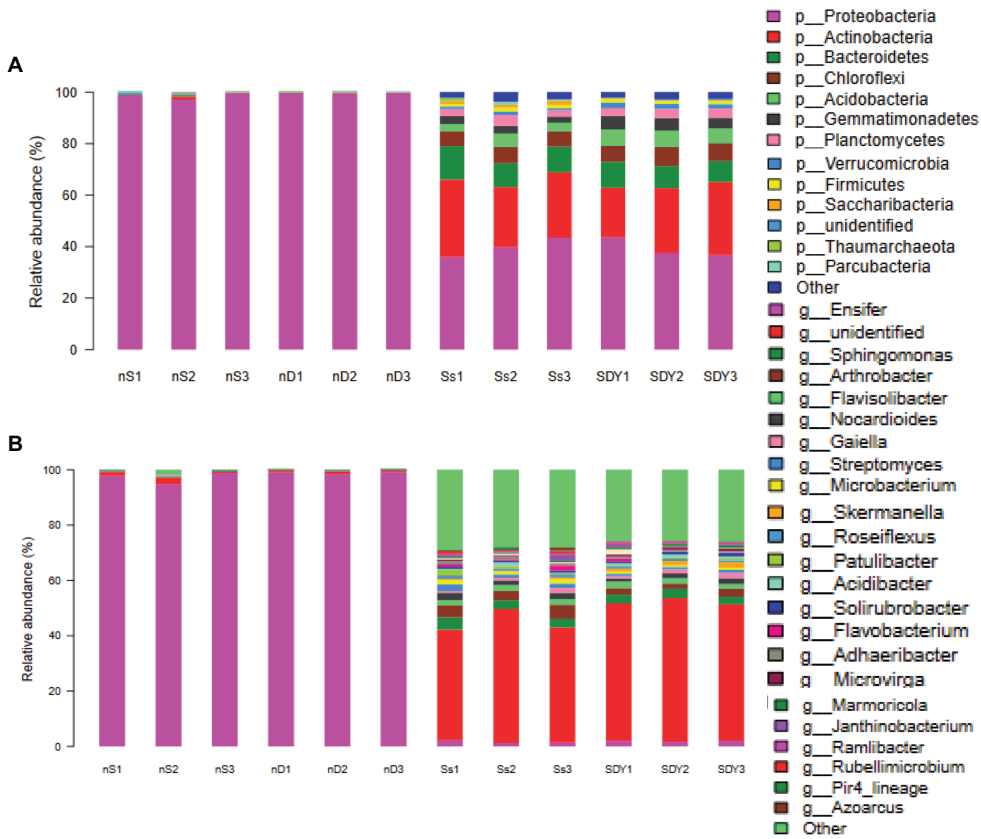

FIGURE 2 | Bacterial community composition related to the root of wild soybean. Relative abundance at the **(A)** phylum and **(B)** genus levels.

root exudates provide an ecological niche for the enrichment of Acidobacteria as symbionts. To our knowledge, this is the first study to investigate the relationship between the nodule and root zone microbiomes of wild soybean to understand PGPR in saline-alkali soils.

Beta Diversity Analysis of 16S rRNA Sequences in Root Zone and Nodule Samples

Analysis of bacterial community composition revealed that the microbiomes of the coastal sand root zone or nodule samples were richer and phylogenetically more diverse than those of the saline-alkali soil in the Yellow River Delta. Therefore, we quantified the major components driving differences between samples (β-diversity) using unconstrained principal coordinates analysis (PCoA) on weighted UniFrac distances. We found a clear separation along axis 1 (explaining 95.67% of the overall variation) and confirmed the general pattern that root zones and nodules harbor distinct microbiomes (**Figure 3A**). Axis 2 explained 1.81% of the overall variation and mainly separated the root zone samples, with no obvious clustering observed between wild soybean grown in the two soil types, suggesting that growth conditions have negligible effects on β-diversity. Hierarchical clustering was performed using the unweighted pair group method with arithmetic mean to understand the relationship between these samples, indicating that the two root zones had similar microbiomes but formed two branches, as shown in **Figure 3B**.

To further understand the relationship between these samples, we performed principal component analysis (PCA) based on OTU relative abundance (**Supplementary Figure S3**). We found that each sample type formed a cluster, indicating reliable biological replication and that the bacterial composition statistic correctly reflected the microbiomes of the nodules or root zone of wild soybean grown in coastal sand or saline-alkali soil from the Yellow River Delta. Moreover, this analysis revealed that soil type predominantly shaped the assembly of the rhizosphere microbiome (Liu et al., 2019), demonstrating that rhizobial evolution in different geographic locations is related to soil type, altitude, and spatial effects (Zhao et al., 2014).

Composition Analysis of *nifH* Genes in Root Zones and Nodules

The most degenerated *nifH* primer pairs were used to amplify the environmental DNA extracted from the nodules and root zone, following which the Illumina sequencing data were

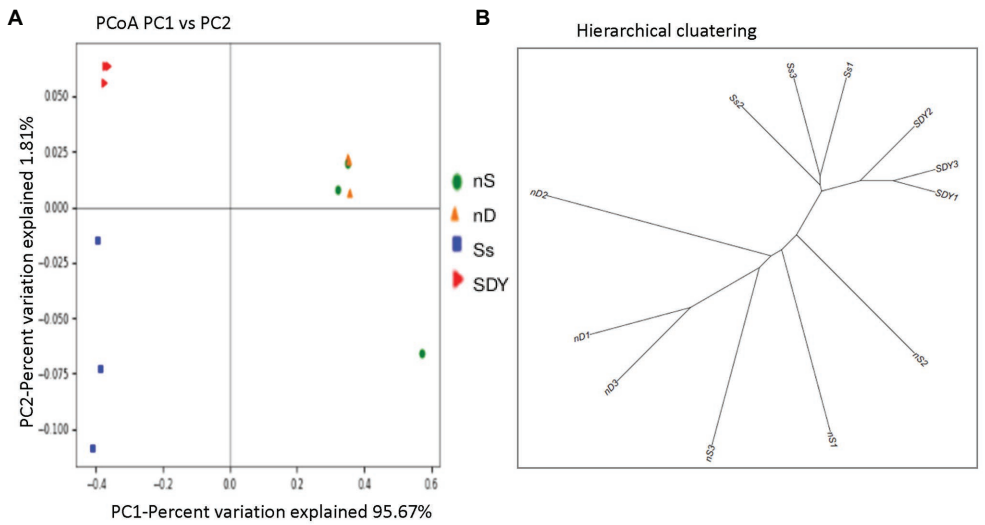

FIGURE 3 | Unconstrained principal coordinate analysis (PCoA) of weighted *Unifrac* distances for root zone and nodule samples **(A)**. Hierarchical clustering analysis **(B)**.

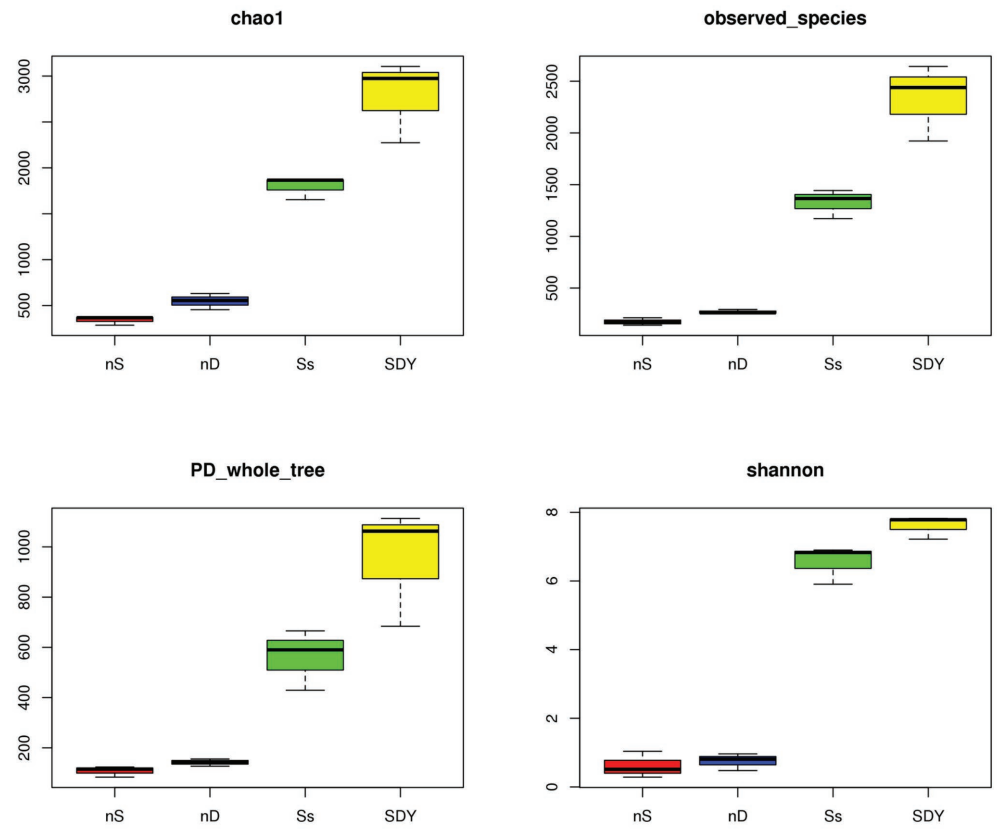

FIGURE 4 | Alpha diversity indices of *nifH* sequences in the two nodule samples and two root zone samples from wild soybean.

summarized based on the PCR products (**Supplementary Table S6**). The α-diversity indices for *nifH* replicon sequencing of two root zone and nodule samples are listed in **Figure 4** and **Supplementary Table S6**. A total of 67,017 ± 4,505 and 68,701 ± 6,364 clean sequences were obtained from nS and nD nodule samples, whereas around twice as many

(131,744 ± 27,556 and 148,306 ± 22,497) were obtained from the Ss and SDY root zone samples (**Supplementary Table S7**). OTU identification revealed that there were approximately eight times as many OTUs in the root zones as in the nodules, indicating that root zone diversity was much richer (**Supplementary Table S7**). The length of the *nifH* PCR products varied from 240 to 480 bp, indicating that the degenerated *nifH* primer pairs were able to amplify as many different *nifH* genes as possible (**Supplementary Figure S4**).

The Venn diagram of *nifH* OTUs identified 165 shared OTUs in the four different soybean root-related microbiomes (**Supplementary Figure S5**), while the associated α-diversity indices for *nifH* replicon sequencing are shown in **Supplementary Table S7**. In the nodules, 96.657 ± 0.943 and 93.342 ± 2.645% of sequences were identified as *Sinorhizobium*/*Ensifer* (**Supplementary Table S8**), but may not all have been from the same single species. For instance, a previous study isolated and identified *Ensifer fredii*, *Ensifer morelense*, *Rhizobium radiobacter*, and a putative novel *Rhizobium* species from the root nodule of wild soybean in northwest China, all of which formed a single lineage related to *E. fredii* in *nodA* and *nifH* gene phylogenies, suggesting that symbiotic genes are laterally transferred between species (Zhao et al., 2014). In the root zones, the relative abundance (>1%) of *nifH* genes in Ss and SDY samples was as follows: Cyanobacteria (49.000 ± 20.607 and 37.667 ± 8.055%), Proteobacteria (32.323 ± 9.843 and 31.333 ± 3.091%), unidentified (5.672 ± 0.473 and 10.333 ± 2.357%), delta/epsilon (10.000 ± 7.204 and 3.667 ± 0.471%), Firmicutes (0.668 ± 0.231 and 4.104 ± 0.393%), Actinobacteria (0.238 ± 0.047 and 9.000 ± 2.160%), and Euryarchaeota (0.100 ± 0.007 and 1.733 ± 1.159%; **Supplementary Table S9**; **Figure 5**), respectively. In Cyanobacteria, *Nostoc* and *Cylindrospermum* were the predominant genera in Ss or SDY samples (**Supplementary Table S10**), respectively, while *Bradyrhizobium* (32.7 ± 4.7%) and *Methylobacter* (17.7 ± 6.2%) were the predominant Proteobacteria and *Frankia* was the predominant Actinobacteria in both root zones. In Firmicutes, *Cellulosilyticum*, *Paenibacillus*, *Clostridium*, and *Bacillus* were identified to contain *nifH* genes, with almost half of all *Paenibacillus* isolates having been reported to fix nitrogen in soils (Xie et al., 2014). The relative abundance of *nifH* gene fragments from Illumina sequencing at the order, family, and species level are explored in detail in **Supplementary Figure S6**.

PGPR can fix nitrogen *via* non-symbiotic bacteria, thus enabling a biologically inactive form of nitrogen to be readily used by organisms (Singh et al., 2020; Soares et al., 2020). Since diverse microbial communities use this process to fulfill their nitrogen demands (Liu et al., 2012), the phylogenetic diversity of *nifH* genes, a molecular marker of

FIGURE 5 | *nifH* composition related to the root of wild soybean. Relative abundance at the **(A)** phylum and **(B)** genus levels.

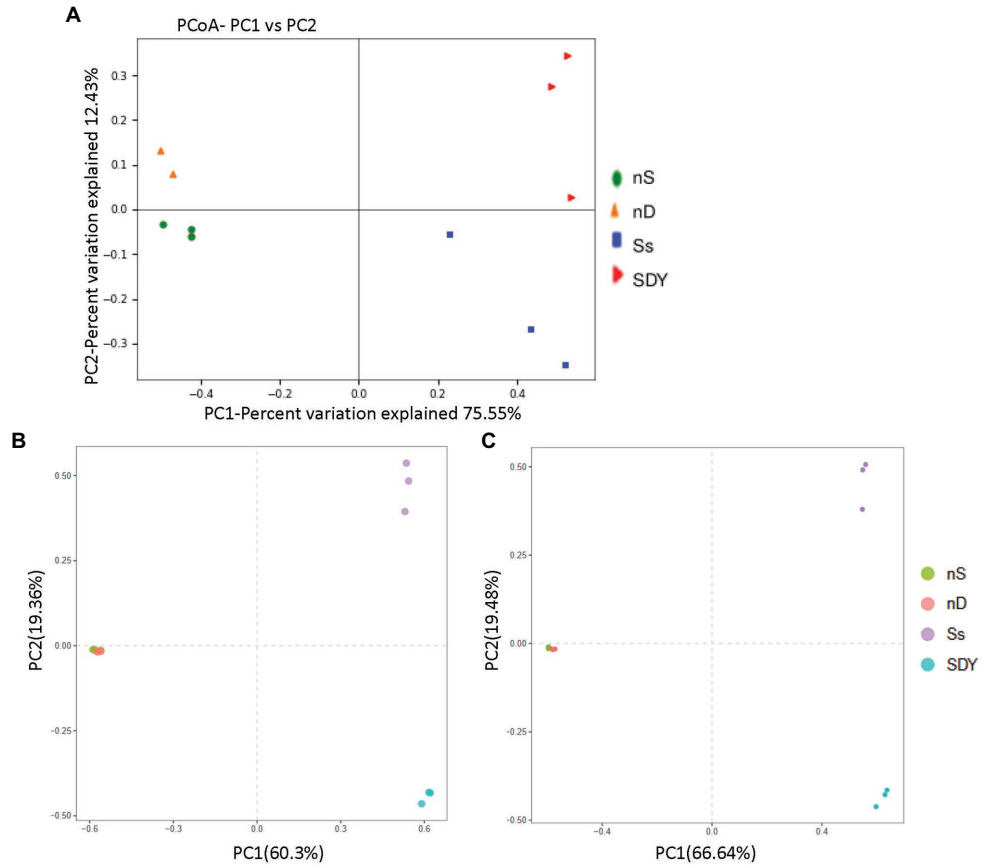

FIGURE 6 | Principal coordinate analysis (PCoA) of weighted *Unifrac* distances based on *nifH* operational taxonomic units (OTUs) **(A)** and principal component analysis (PCA) based on OTU relative abundance **(B)** and diff-OTUs relative abundance **(C)** in root zone and nodule samples.

nitrogen fixation (Gaby et al., 2018), has been examined extensively in the microbiota of various plants and invertebrates, including humans (Yamada et al., 2007; Xiao et al., 2010; Liu et al., 2012; Igai et al., 2016). At the phylum level, we found that Cyanobacteria accounted for 49.0% of the sequences identified in coastal sand, but only 37.7% of those in the Yellow River Delta samples. Although unexpected, this result was consistent with a previous study which found that Geobacter and Cyanobacteria are important functional components of the nitrogen-fixing community in agricultural soil based on *nifH*-RNA transcriptomic sequencing analysis (Calderoli et al., 2017). We found no difference in the relative abundance of *nifH* sequences belonging to proteobacteria in the two root zones (approximately 32 and 31% in Ss and SDY root zones, respectively); however, the ratio of *nifH* belonging to α-, β-, γ-, and δ-proteobacteria did differ. Previously, the γ-proteobacteria *Pseudomonas* were isolated as the main nitrogen-fixing bacterial isolates from three rhizosphere soil samples taken from mangrove plants in the Dongzhaigang National Mangrove Nature Reserve of China (Liu et al., 2012). Using a soil DNA extraction and PCR-cloning-sequencing approach the study analyzed 135 clones and identified 27 unique *nifH* sequence phylotypes, most of which were closely related to sequences from uncultured bacteria; however, the other seven were identified as nitrogen-fixing α-proteobacteria (*Bradyrhizobium*, *Rhodospirillum*, and *Rhodobacter*) or *Archaea* (Liu et al., 2012). In this study, significant differences in *nifH* relative abundance were observed between Firmicutes (0.67 and 4.10%), Actinobacteria (0.24 and 9.00%), and one Archaea phylum Euryarchaeota (0.10 and 1.73%) in Ss and SDY samples, respectively. Conversely, no significant difference was observed in the nodule samples due to the overwhelming relative *nifH* abundance, which may be the result of host selection in different environments.

Beta Diversity Analysis of *nifH* Sequences in Root Zone and Nodule Microbiomes

nifH composition analysis revealed that *nifH* sequence diversity appeared to be richer in nD and SDY samples (**Figure 5**). To understand this similarity, we quantified the major components driving β-diversity using unconstrained PCoA on weighted UniFrac distances. We found a clear separation along axis 1 that explained 75.55% of all variation and confirmed a general pattern with root zones and nodules harboring distinct *nifH* genes (**Figure 6A**). Conversely, axis 2 explained 12.4% of the

overall variation and mainly separated the root zone samples. To further understand the relationship between these samples, we performed PCA on OTU relative abundance (**Figures 6B,C**). Each sample type formed a cluster, indicating that biological replication was reliable and that the bacterial composition statistic correctly reflected the *nifH* gene set in the nodules and root zones of wild soybean grown in coastal sand or saline-alkali soil in the Yellow River Delta.

Isolated Members of Wild Soybean Nodule Microbiota

To identify potential PGPR from the rhizosphere and endophytes of wild soybean roots grown in saline-alkali soil, we isolated bacteria from wild soybean nodules. A total of 277 cultured bacteria were characterized, including 180 from nS samples and 97 from nD samples (**Figure 7**). The α-proteobacteria genus *Sinorhizobium/Ensifer* dominated the cultured bacteria and accounted for 81 nS isolates and 38 nD isolates (43.0% of all isolates), the majority of which were able to grow in 2% NaCl + LB medium. A previous study investigated the diversity and biogeography of *G. soja*-nodulating rhizobia by characterizing 155 nodule isolates from seven sites in northwest China by 16S rRNA PCR-RFLP and the sequence analysis of multiple core genes (16S rRNA, *recA*, *atpD*, and *glnII*). Among the isolates, 80 were *Ensifer fredii*, 19 were *Ensifer morelense*, 49 were *Rhizobium radiobacter*, and seven were putative novel *Rhizobium* species (Zhao et al., 2014). In this study, we identified all isolates as *Ensifer americanum* based on 16S rRNA, while the γ-proteobacteria *Enterobacter* (nine isolates) and *Pantoea* (six isolates) were isolated from nS and nD samples, respectively.

Of the Firmicutes, *Bacillus* was the second most predominant bacterial genus, accounting for 40 nS isolates and 41 nD isolates, representing 29.2% of all bacteria and 71.1% all Firmicutes (81/114).

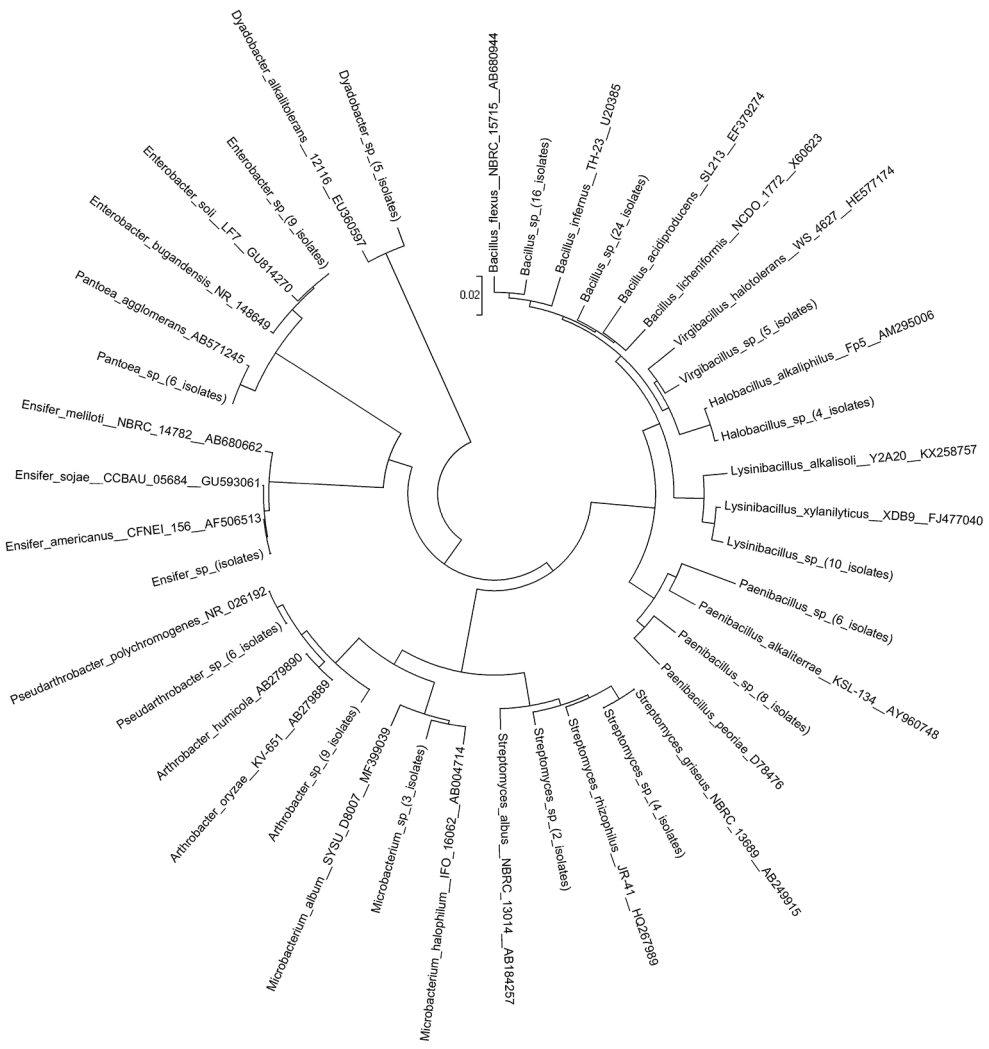

FIGURE 7 | Phylogenetic tree of 16S rRNA gene sequences showing the relationship between the representative and reference isolates. The neighbor-joining (NJ) tree was derived from a 16S rRNA gene sequence distance matrix (Kimura two parameter).

FIGURE 8 | Wild soybean nodule formation **(A)** when inoculated with *Sinorhizobium/Ensifer* and **(B)** without inoculation with *Sinorhizobium/Ensifer*. **(C)** Root nodule formation when inoculated. **(D)** Nodule size and shape.

Bacillus sp., has been reported to possess versatile traits that protect plant against diverse abiotic stresses, including heat, cold, and freezing (Tiwari et al., 2017). Indeed, *Paenibacillus* (14 isolates) have been reported to provide their host with multiple benefits, including nitrogen fixation, phosphate solubilization, and biocontrol (Grady et al., 2016). *Lysinibacillus* (10 isolates), *Virgibacillus* (five isolates), and *Halobacillus* (four isolates) were also isolated and identified from the nS and nD samples.

Actinobacteria and Bacteroidetes accounted for 8.7 and 1.8% of all isolates, respectively. We identified four Actinobacteria genera (24 isolates) with more than one representative isolate, including *Arthrobacter* (nine isolates), *Streptomyces* (six isolates), *Pseudarthrobacter* (six isolates), and *Microbacterium* (three isolates), which were mainly from nS samples, with the exception of *Arthrobacter*. Conversely, only one Bacteroidetes genus *Dyadobacter* (five isolates) was isolated from nS samples. Our previous study at the species level identified four genera with high relative abundance: *Enterobacter*, *Chryseobacterium*, *Stenotrophomonas*, and *Flavobacterium* (Zheng et al., 2020); however, only *Enterobacter* spp. were isolated in this study, which have been reported to be microsymbionts of *G. soja* (Zhao et al., 2014).

Functional Study of Wild Soybean Root Microbiota

To investigate the effect of the microbiota isolates on wild soybean growth and nodule formation, we developed a sterile hydroponics cultivation method using nitrogen-free medium and evaluated its potential to examine plant-microbiota interactions. When inoculated with *Sinorhizobium/Ensifer* isolates, nodule formation was observed in the wild soybean seedlings with less than 2 weeks of cultivation and plant biomass was slightly higher than in seedlings without inoculation, with approximately half as many nodules as formed in the wild (**Figure 8**; **Supplementary Figure S7**).

Our experimental hydroponics cultivation system enabled us to test the effects of microbiota members isolated from root nodules on plant growth and thus represents a possible approach to advance our functional understanding of the root microbiome. Consequently, we believe that our study is a pioneering example of novel laboratory research and future studies with different isolation media and growth conditions will allow us to broaden the reference stock to investigate PGPR in saline-alkali soil.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the NCBI BioProject under accession numbers PRJNA597572 and PRJNA597574.

AUTHOR CONTRIBUTIONS

YY, RS, and CZ generated ideas, directed the work, conducted experiments, and wrote and edited the manuscript. YY and CJ isolated and characterized the initial bacterial isolates, including sequence analysis. CM and SM prepared some figures. CM, CJ, YL, and CZ obtained funding. All coauthors reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.523142/full#supplementary-material

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Growth-Stimulatory Effect of Quorum Sensing Signal Molecule *N*-Acyl-Homoserine Lactone-Producing Multi-Trait *Aeromonas* spp. on Wheat Genotypes Under Salt Stress

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Salinity is one of the major threats to agricultural productivity worldwide. Soil and plant management practices, along with inoculation with plant-beneficial bacteria, play a key role in the plant's tolerance toward salinity stress. The present study demonstrates the potential of acyl homoserine lactone (AHL)-producing plant growth promoting rhizobacteria (PGPR) strains of *Aeromonas* sp., namely, SAL-17 (accession no. HG763857) and SAL-21 (accession no. HG763858), for growth promotion of two wheat genotypes inherently different for salt tolerance potential. AHLs are the bacterial signal molecules that regulate the expression of various genes in bacteria and plants. Both *Aeromonas* spp., along with innate plant-growth-promoting (PGP) and salt tolerance traits, showed AHL production which was identified on tandem mass spectrometry as C6-HSL, 3-OH-C5-HSL, 3-OH-C6-HSL, 3-oxo-C7-HSL C10-HSL, 3-oxo-C10-HSL, 3-OH-C10-HSL, 3-oxo-C12-HSL and C6-HSL, and 3-oxo-C10-HSL. The exogenous application of purified AHLs (mix) significantly improved various root parameters at 200 mM NaCl in both salt-sensitive (SSG) and salt-tolerant (STG) genotypes, where the highest increase (≈80%) was observed where a mixture of both strains of AHLs was used. Confocal microscopic observations and root overlay assay revealed a strong root colonization potential of the two strains under salt stress. The inoculation response of both STG and SSG genotypes was evaluated with two AHL-producing strains (SAL-17 and SAL-21) and compared to non-AHL-producing *Aeromonas* sp. SAL-12 (accession no. HG763856) in saline (EC = 7.63 ms/cm^2) and non-saline soil. The data reveal that plants inoculated with the bacterial consortium (SAL-21 + SAL-17) showed a maximum increase in leaf proline content, nitrate reductase activity, chlorophyll a/b, stomatal conductance, transpiration rate, root length, shoot length, and grain weight over non-inoculated plants grown in saline soil. Both STG and SSG showed relative effectiveness toward inoculation (percent increase for STG: 165–16%; SSG: 283–14%) and showed a

positive correlation of grain yield with proline and nitrate reductase activity. Furthermore, principal component analysis (PCA) and categorical PCA analysis clearly showed an inoculation response in both genotypes, revealing the effectiveness of AHL-producing *Aeromonas* spp. than the non-AHL-producing strain. The present study documents that the consortium of salt-tolerant AHL-producing *Aeromonas* spp. is equally effective for sustaining the growth of STG as well as SSG wheat genotypes in saline soil, but biosafety should be fully ensured before field release.

Keywords: AHLs, wheat, *Aeromonas*, PGPR – plant growth-promoting rhizobacteria, halophilic

INTRODUCTION

Salinity is edaphic stress that has affected 45 million hectares out of 230 million hectares of irrigated land, causing annual losses of about US$ 12 billion worldwide (FAO, 2020), and is a major threat to global agricultural productivity. There are two types of salinity: primary salinity which occurs in arid and semi-arid regions due to low average rainfall, excessive weathering of rocks, and improper drainage in soils containing high salt contents (Bui, 2013) and secondary salinity which is mainly caused by human activities such as land clearing, inappropriate irrigation practices, and excessive use of chemical fertilizers (de Wit et al., 2011). Salinity decreases the agricultural production of all major crops and deteriorates the structure and the ecological functioning of the soil. It imposes ion toxicity, osmotic and oxidative stresses, limits water uptake from soil, consequently causing nutrient deficiency, especially phosphorous (P) because P ions precipitate with Ca ions (Bano and Fatima, 2009). Salinity also affects photosynthetic efficiency, leaf area, stomatal conductance, and chlorophyll contents.

Plants have also co-evolved the adaptation mechanisms (Gupta and Huang, 2014) against salinity. The first phase of plant response to salinity is characterized by the release of phytohormones, mainly abscisic acid (Ismail et al., 2014), the expression of reactive oxygen species (ROS)-scavenging enzymes (Bharti et al., 2013), and the accumulation of osmoprotectants such as proline (Sneha et al., 2013; Iqbal et al., 2014). The exogenous application of nitric oxide (NO) and nitrate reductase-mediated NO production are also reported as abiotic stress coping strategy in plants, such that they are involved in the homeostasis of ROS in plants (Sun et al., 2015; Pan Q.-N. et al., 2019). The second phase of plant response is characterized by Na^+ exclusion from xylem parenchyma cells *via* plasma membrane porter HKT1 (Lv et al., 2012; Munns et al., 2012), SOS 1 Na^+/H^+ antiporter (Ariga et al., 2013), Na^+ storage into vacuoles *via* vacuolar Na^+/H^+ antiporter (Kronzucker and Britto, 2011), or Na^+ compartmentalization (Garcia de la Garma et al., 2015).

Many strategies to induce salinity tolerance in plants have been discussed, including genetic engineering of regulatory elements, manipulation of ion transport and transporters, membrane transports, RNAi technology, QTLomics, alternative splicing, and exploring the halobiomes as a gene pool for conferring salt tolerance (Wani et al., 2020). Halobiome is referred to as a group of halophilic and/or halotolerant bacteria, algae, fungi, and plants that can withstand a high-saline environment. It is now generally accepted that plant performance and activities can only be characterized and understood completely if the plant, plus the intimately associated microbiota, is considered. The role of microorganisms in plant growth promotion, nutrient management, and disease control is well established (Naqqash et al., 2020). These microorganisms colonize the rhizosphere/endorhizosphere of plants and promote the growth of plants through various direct and indirect mechanisms (Lugtenberg and Kamilova, 2009; Yang et al., 2009; Upadhyay et al., 2012). The term induced systemic tolerance has been proposed for plant growth promoting rhizobacteria (PGPR)-induced physical and chemical changes that result in enhanced tolerance to abiotic stress. Hence, PGPR acts as an effective strategy to mitigate the detrimental effects of stress along with improved plant growth. PGPR inoculation improves nutrient uptake under stress (Dodd and Pérez-Alfocea, 2012; Han et al., 2014), e.g., *Pseudomonas* sp. inoculation enhances chlorophyll content in maize under salinity while *Klebsiella oxytoca* inoculation improves nutrient uptake in cotton (Nadeem et al., 2007; Wu et al., 2014). However, the inoculation efficiency is higher under normal conditions compared to the stressed condition because stress not only affects the growth and the physiology of plants but also rhizosphere functioning (Hashem et al., 2015). The rhizosphere is a hotspot for microbial diversity and activity and is affected by various abiotic and biotic factors, including nutrients, pH, moisture, and pathogens (Egamberdieva et al., 2010). Microbes native to saline and hypersaline habitats have well-developed physiological pathways and survival mechanisms to cope with the harsh conditions (Torres et al., 2019) and have shown positive effects on plants under salt stress (Ahmad et al., 2015; Singh and Jha, 2016a; Rajput et al., 2018). Under stress conditions, the plant hormone ethylene endogenously regulates plant homeostasis, which results in reduced root and shoot growth. In the presence of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase-producing bacteria, plant ACC is sequestered and degraded by bacterial cells to supply nitrogen and energy. Furthermore, by removing ACC, the bacteria reduce the deleterious effect of ethylene, ameliorating stress and promoting plant growth.

Acyl homoserine lactones (AHLs) are quorum sensing (QS) molecules produced by root-associated bacteria and represent novel elicitors or inducers of biotic and abiotic stress tolerance in plants. They induce rapid changes in the morphology, physiology, and gene expression of roots and shoots (Schikora

et al., 2016), trigger a collective response to change cell density (Papenfort and Bassler, 2016), produce antifungal/antimicrobial molecules and antibiotics (Chapalain et al., 2013), influence colonization and association with the host, and induce host defense mechanism against pathogens (González and Marketon, 2003). QS-related studies from saline and hypersaline habitats have been mainly reported from family *Halomonadaceae* (Llamas et al., 2005; Tahrioui et al., 2013) and recently *Desulfovibrio vulgaris* and *Desulfobacterium corrodens* (Sivakumar et al., 2019). None of the studies reported on AHL production from PGPR strains isolated from saline and hypersaline rhizosphere.

Aeromonas spp. have been reported as PGPR and/or biocontrol agent from the rhizosphere of different crop plants, including rice (Mehnaz et al., 2001; Sánchez-Matamoros et al., 2018), soybean (Safni and Antastia, 2018), bean and cotton (Inbar and Chet, 1991), chickpea and mustard (Kundu et al., 2009), and wheat (Chibani et al., 2016; Rajput et al., 2018), but AHL-producing PGPR *Aeromonas* sp. have not been studied until now. AHL-producing *Aeromonas hydrophila* KOR1 was isolated from mangrove rhizosphere (Yin et al., 2015), *Aeromonas caviae* strain YL12 was from plant-based compost material (Lim et al., 2014), and *Aeromonas sobria* was from the spoilage of *Scophthalmus maximus* L. (Li et al., 2016), but these strains were not characterized as PGPR.

This study was based upon the hypothesis that AHL-producing plant-beneficial bacteria may serve as inducers of salt tolerance in plants with concomitant plant growth promotion. The present study has demonstrated the production of different AHLs, varying in acyl chain length (C5–C12), from halo-tolerant, plant-beneficial *Aeromonas* spp. strains isolated from wheat rhizosphere and their subsequent growth-promoting effect on two wheat genotypes (salt tolerant and salt sensitive) under salt stress. Plant inoculation further showed their root colonization potential in saline and non-saline soil. Our results provide evidence that AHLs modulate root architecture, and the inoculation of both AHL-producing *Aeromonas* spp. shows an elevated effect than that of non-AHL strain for plant growth and yield under salt stress. Therefore, the utilization of these bacteria as biofertilizer offers a sustainable solution for crop (wheat) cultivation in saline lands.

MATERIALS AND METHODS

Bacterial Strains and Wheat Genotypes Used

Three test strains *Aeromonas* spp., [SAL-12 (accession no. HG763856), SAL-17 (accession no. HG763857), SAL-21 (accession no. HG763858)], biosensor strain *Chromobacterium violaceum* CV026, reference strain *Rhizobium leguminosarum* strain 8401, and *R. leguminosarum* A34 which is a derivative of strain 8401 containing symbiotic plasmid pRL1J1, along-with the wheat genotypes NW-10-1111-7 (salt-tolerant genotype; STG), and NW-5-1212-I (salt-sensitive genotype; SSG) are mentioned in **Table 1** with a short description and growth conditions. The *16S rRNA* gene sequences of *Aeromonas* spp. strains SAL-17, SAL-21, and SAL-12 were aligned to highly similar sequences using multiple sequence alignment, and phylogeny was determined by maximum likelihood method (Jukes and Cantor, 1969) using a MEGA6 software package (Kumar et al., 2016).

Biochemical and Physiological Characterization

The *Aeromonas* sp. strains used were already reported as PGPR (Rajput et al., 2018). For further characterization, they were tested for tolerance range for NaCl (0.5–10%), pH (6–8), and temperature (4–42°C). Biochemical tests were carried out as described previously (Miñana-Galbis et al., 2002): Gram staining, motility, glucose oxidation–fermentation, oxidase and catalase activity, production of a brown diffusible pigment, hydrogen sulfide production from cysteine and thiosulfate, acid production from carbohydrates, hydrolysis of urea, and utilization of substrates as sole carbon and energy sources. Arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activity (Moeller's method) were determined as described by Smibert and Krieg (1994). The hemolytic activity of strains was tested by spot inoculating the cells onto nutrient agar plates containing 5% sheep blood. The plates were incubated at 28 ± 2°C and observed for hemolysis.

Identification and Analysis for AHLs

Detection of AHLs

Initial screening of AHL production was done by the overlay assay (McClean et al., 1997). Briefly, 100 μl of an overnight-grown culture of test strain was spot-inoculated onto a Luria broth (LB) agar plate. The indicator strain *C. violaceum* CV026 (mini Tn5 negative mutant for violacein production) was grown individually in TY medium, mixed with semi-solid LB agar (0.7%) cells, and spread onto the test strain, and the plates were streaked with the reference strains. The bacterial strain SAL-12 was used as AHL negative control. The plates were incubated at 28 ± 2°C for 24 h and observed for the development of purple color. The AHLs produced by test strains/reference strains diffused through the agar and stimulated violacein synthesis (blue/purple pigmentation) in *C. violaceum* CV026 which cannot synthesize its own AHLs.

For the confirmation of the AHL system in *Aeromonas*, a primer pair was designed to amplify a ≈750-bp sequence from the regulatory gene of the LuxR-type transcriptional regulator in *Aeromonas* spp. from the sequences available in the database. Lux gene was amplified using the primer pair P1 = 5′-ATGAAACAAGACCAACTGCT-3′/P4 = 5′-AAGCTTAATGCCACTGCTCACC-3′ using the following conditions: initial 5 min denaturation at 95°C, followed by 30 cycles at 95°C for 60 s, 57°C for 30 s, 72°C for 45 s, and a final extension step of 72°C for 10 min.

TLC and ESI-MS/MS Analysis of AHLs

Bacterial strains were individually grown at 28 ± 2°C for 3 days in LB broth with constant shaking at 200 rpm. AHLs were extracted twice from spent supernatant using an equal volume of acidified ethyl acetate (0.1% glacial acetic acid v/v) and confirmed by overlay assay as described earlier (Hanif et al., 2020). Extraction

TABLE 1 | Bacterial strains, growth conditions, and wheat genotypes used in this study.

Strain	Description	Growth conditions	Purpose	Source/References
Chromobacterium violaceum CV026	mini-Tn5 mutant of ATCC 31532; violacein negative	LB + kanamycin (25 μg/ml), 28 ± 2°C	Biosensor/indicator strain for AHLs detection; detect and respond to AHLs (C_4–C_8 in length) by producing purple pigment violacein	McClean et al., 1997
Rhizobium leguminosarum A34	Derivative of strain 8401; carries a symbiotic plasmid pRL1J1	YEM/TY, 28 ±2°C	Reference strain for AHLs production; produce C_4–C_8HSLs	Downie et al., 1983
Aeromonas sp. strain SAL-17	Wheat rhizosphere isolates from Biosaline Research Station-II (BSRS-II) Pakka Anna (31°24/N and 73°05/E)	LB, 28 ± 2°C	Test strains	Rajput et al., 2018
Aeromonas sp. strain SAL-21	Wheat rhizosphere isolates from Biosaline Research Station-II (BSRS-II) Pakka Anna (31°24/N and 73°05/E)	LB, 28 ± 2°C	Test strains	Rajput et al., 2018
Aeromonas sp. strain SAL-12	Wheat rhizosphere isolates from Biosaline Research Station-II (BSRS-II) Pakka Anna (31°24/N and 73°05/E)	LB, 28 ± 2°C	Negative strain for AHLs	Rajput et al., 2018

Parentage of wheat genotypes used in this study

Wheat genotype	Genotype description	Parentage/ pedigree	Origin	References
NW-10-1111-7	Salt tolerant	NARC-241/Bhittai-1111-7	Pakistan	Saleem et al., 2015
NW-5-1212-I	Salt sensitive	NARC 41/Bhittai-18 Pakistan		Saleem et al., 2015

and subsequent reverse phase-thin layer chromatography (RP-TLC) of AHLs were performed as described (Imran et al., 2014; Ali et al., 2016) on glass-backed C18 reverse phase plates (Merck) developed with an overlay of the exponentially grown culture of CV026.

For electrospray ionization (ESI) analysis, the AHL extracts were purified by solid phase extraction (SPE) (Li et al., 2006), and ESI–mass spectra were obtained by infusion with 5% formic acid on a mass spectrometer (LTQ XL Linear Ion Trap Mass Spectrometer from Thermo Scientific, United States) equipped with and ESI probe. All conditions were set as described previously (Ali et al., 2016), and data were acquired in positive and negative total ion full-scan mode (mass scan range: m/z 50–500). Various AHL peaks produced during full scan were subjected to tandem mass spectrometry (MS/MS) to confirm their chemical structures based on the fingerprints of their daughter ion peaks produced during fragmentation. The structures of AHLs and the fragmentation schemes were generated using Chem Bio Draw Ultra 12.0. The functions of AHLs already reported in the literature were assigned to those detected in the present study.

Plant Inoculation Assays

Formulation of Halo-Tolerant PGPR Inoculum

Due to the difference in the AHL production ability and different PGPR activities of both strains (Rajput et al., 2018), the bacterial strains were inoculated individually; a consortium containing bacterial strains SAL-21 and SAL-17 was formulated as well. Before this, both strains were tested for compatibility by a standard well-cut method (Rajendran et al., 2011). After confirmation of compatibility, the bacteria were grown separately in LB medium overnight up to an optical density (OD) of 0.45; the cells were harvested by centrifugation and mixed (1:1 ratio) in 0.85% saline to get a consortium of halo-tolerant bacteria (PGPR-consortium) for plant inoculation. A non-AHL-producing *Aeromonas* sp. strain SAL-12 was used as negative control in pot experiment.

Root Colonization and QS Detection Under Induced Salinity Under Monoxenic Condition

Seeds of salt-tolerant wheat genotype (NW-10-1111-7) were surface sterilized with 2% sodium hypochlorite for 5 min, washed thrice with sterile distilled water, and germinated in the dark in sterile plastic plates containing 1% water agar supplemented with 200 mM NaCl at 25 ± 2°C. After germination, 3-day-old seedlings were inoculated with bacterial strains (SAL-17 and SAL-21) and grown for 10 days at day/night temperature of 25/20°C and light/dark periods of 16/8 h. The roots were transferred to new LB agar plates overlaid with biosensor strain CV026 and incubated at 30 ± 2°C. Another experiment was set up with three replicates for root colonization analysis under a confocal laser scanning microscope (CLSM) using the same conditions. The roots were detached from the seedlings after 10 days aseptically and stained for 4–5 min in 20–30 μl methyl acridine orange dye. The roots were washed with sterile water and observed under a CLSM (Fluo view, FV 1000, Olympus) attached with a digital monitoring system for capturing the fluorescence image. The samples were excited using the argon–ion laser line

at 502–525 nm (for acridine orange), and fluorescence of the samples was detected. The fluorescent images were captured using FluoView software (Olympus).

Effect of AHL Treatment on Wheat Roots Under Axenic Condition

Seeds of both wheat genotypes were surface sterilized with 2% sodium hypochlorite for 5 min and washed thrice with sterile distilled water. Purified AHL mixes (200 μl) from both strains were mixed individually in 15 ml of 0.8% water agar medium and poured as a thin layer onto the water agar plate. For the mix-AHLs treatment, AHL extracts from both strains were mixed in a 1:1 ratio and mixed in water agar before pouring into the plates. Sterilized seeds were placed on the plate and germinated in the dark. The experiment was conducted in a completely randomized design with four replicates each. At 7 days after germination, the seedlings were removed from agar, and the roots were washed with distilled water and scanned using Rhizoscanner (EPSON Perfection V700Photo, Epson America, Inc. United States), equipped with WinRHIZO software (Regent Instruments Co. Canada). The roots were also observed under a light microscope (Leica DMLS) for the development of root hairs, and photographic images were recorded using digital camera.

Pot Experiment: Effect of AHL-Producing *Aeromonas* spp. on Wheat Growth in Saline Soil

A pot experiment was carried out in sterilized saline soil (BSRS-II) in the wheat growing season. The seeds of wheat genotypes were inoculated separately with AHL-producing *Aeromonas* spp. SAL-17 and SAL-21, a mix of both SAL-17 + SAL-21 (consortium), and non-AHL-producing *Aeromonas* sp. SAL-12. Non-inoculated seeds in saline soil and non-saline soil were set as controls. The experiment was set up in a completely randomized design with five replicates of each treatment, and the plants were grown in natural wheat growing season. The plants were evaluated for different stress-related and agronomic parameters at 45–50 days after germination, while yield data were recorded at maturity.

Total Proline Contents

Free proline contents from wheat leaves were measured according to the method of Bates et al. (1973). Fresh leaves (0.5 g) were extracted in 10 ml of 3% sulfosalicylic acid. Then, 2.0 ml of the filtrate was mixed with 2.0 ml of acid ninhydrin, followed by 2.0 ml of glacial acetic acid. The samples were incubated at 100°C for 60 min and cooled in an ice bath, and 4.0 ml of toluene was added to the solution and mixed vigorously. The chromophore-containing toluene was aspirated, and the absorbance read as 520 nm on a spectrophotometer (IRMECO U2020). Proline concentration in the samples was determined from a standard curve and calculated on a fresh weight basis.

Nitrate Reductase Activity

Nitrate reductase activity from wheat leaves was measured by homogenizing leaves in a chilled mortar and pestle with 100 mM potassium phosphate buffer (pH 7.4), containing 7.5 mM cysteine, 1 mM ethylenediamine tetraacetic acid (EDTA), and 1.5% (w/v) casein. The homogenate was centrifuged at 10,000 × *g* for 15 min at 4°C. Nitrate reductase activity was determined as described (Robin, 1979). The extract was incubated in a reaction mixture containing 100 mM potassium phosphate buffer (pH 7.4), 10 mM EDTA, 0.15 mM NADH, and 0.1 M KNO_3 at 30°C for 30 min. The reaction was stopped by 100 mL of 1.0 M zinc acetate. The absorbance of the supernatant was determined at 540 nm after diazotation of nitrite ions with 5.8 mM sulfanilamide and 0.8 mM *N*-(1-naphthyl)-ethylenediamine-dihydrochloride.

Chlorophyll Contents and Gas Exchange Parameters

Chlorophyll a and b were determined using 500 mg fresh leaf extracted overnight with 80% acetone and centrifuged at 10,000 × *g* for 5 min. The absorbance of the supernatant was estimated using a spectrophotometer at 480-, 645-, and 663-nm wavelength against the solvent, and chlorophyll contents were calculated according to Arnon (1949).

Measurements of transpiration rate (E) and stomatal conductance (gs) were made on the third leaf from the top of each plant using an infrared gas analyzer (Analytical Development Company, Hoddeson, United Kingdom) on a sunny day from 10 to 11 a.m.

Morphological and Field Data

The parameters studied for morphological data at 25 dpi were plant fresh weight and shoot and root length and at 75 dpi were shoot and root (length, fresh weight, and dry weight) and plant biomass along with the weight of 1,000 grains. Five plants from each replicate and 15 plants per treatment were uprooted at maturity, and the mean was calculated for each treatment.

Statistical Analysis

Data were analyzed statistically by analysis of variance technique, using the Statistix (version 8.1) software, and the least significant difference test (Fisher LSD) at 5% probability was used to compare the differences among treatment means. The data presented in this work are the average of at least 15 plants per treatment; means ± standard deviations are given in the figures. Graphs were constructed using Microsoft Excel (2016) and assembled using Corel Draw (R 12). Pearson/Spearman's correlations were calculated at 1,000 bootstrap analysis at 0.05 level (two-tailed). Categorical principal component analysis was performed using IBM SPSS software package version 20 (SPSS, Inc. Chicago, IL, United States).

RESULTS

Biochemical and Physiological Profiling of *Aeromonas* Species

Cells of *Aeromonas* spp. SAL-21, SAL-17, and SAL-12 are motile and Gram-negative. Growth occurs at 25–37°C, 0–10% NaCl (w/v), and pH 6.5–9.5. Optimum growth temperature is 28 ± 2°C. All three strains are positive for oxidase and catalase tests. The brown pigment is not produced by any species. SAL17

and SAL-12 are positive for alanopine dehydrogenase (ADH) and β-galactosidase tests but negative for lactate dehydrogenase (LDH) and octopine dehydrogenase (ODH). SAL-21 is negative for ADH, LDH, and ODH but positive for the β-galactosidase test. Only SAL-21 cannot hydrolyze urea. All strains produce H_2S and utilize sodium citrate and malonate except SAL-12. Acid is produced from arabinose, mannitol, sucrose, sorbitol, maltose, succinate, rhamanose, inositol, and melibiose from all *Aeromonas* spp. in this study. The β-hemolytic activity was not found in any strain. All the biochemical and the physiological test results of *Aeromonas* spp. strains have been summarized and compared with the already reported *Aeromonas* species in **Table 2** for the phenotypic and the biochemical differentiations. The plant-growth-promoting traits of these *Aeromonas* spp. strains are already published (Rajput et al., 2018), and their phylogenetic tree is shown in **Supplementary Figure S1**.

Analysis of AHLs

The strains SAL-17 and SAL-21 produced purple color on LB agar plates overlaid with biosensor strain *C. violaceum* CV026, indicating the production of AHLs compared with the positive control (**Figure 1A**). AHLs were extracted from the cell-free supernatant of strains SAL-17 and SAL-21 and confirmed by plate overlay assay (**Figure 1B**). The strain SAL-12 did not show any purple color around the colony with the biosensor strain CV026. RP-TLC was further carried out to separate the extracted AHLs (**Figure 1C**). The comparison was done with the strain *R. leguminosarum* 8401 and a derivative of this strain named A34 containing pRL1J1 as reference for AHLs. Four spots were observed in the lane of SAL-17 and SAL-21 compared to six spots for pRL1J1 (**Figure 1C**). Both strains (SAL-17 and SAL-21) gave amplification with the *Aeromonas* Lux gene-specific primers, confirming the presence of LuxR-type regulators in them. The AHLs-negative strain SAL-12 did not show any amplification with these primers, indicating the absence of the Lux regulator.

AHL extracts were purified through SPE and were subjected to ESI–mass spectrometry analysis for the profiling of AHLs. The structure of the selected AHLs and their corresponding peaks were confirmed by MS/MS analysis (**Tables 3**, **4**). When an extract of SAL-17 was analyzed, eight AHLs (C6-HSL, 3-OH-C5-HSL, 3-OH-C6-HSL, 3-oxo-C7-HSL, C10-HSL, 3-oxo-C10-HSL, 3-OH-C10-HSL, and 3-oxo-C12-HSL + H_2O) were observed, and their structures were confirmed by tandem mass spectrometry (**Figures 2A,B**). Only two AHLs were confirmed by MS/MS analysis, in the case of SAL-21 C6-HSL and 3oxo-C10-HSL + H_2O (**Figures 2A,B**); the other two spots detected in TLC could not be detected in MS/MS analysis. Functional annotation of the AHLs was done by equating them with the published literature, and their putative roles were assigned (**Table 4**).

Plant Inoculation Assays

Root Colonization Under Induced Salinity

The development of purple color on the roots shows the bacterial attachment/colonization as seen by the production of AHLs during early seedling growth and root colonization (**Figures 3A,B**). Root colonization analysis by CLSM, carried out both in the salinized as well as non-salinized medium, showed the colonization of inoculated bacteria on the root surface and root hairs and their presence in close vicinity of the root epidermal cells under salt stress (**Figure 3C**). A higher number of cells were found on the root surface in the case of wheat grown under salt stress as compared (**Figure 3B**) to wheat grown under normal conditions (**Figure 3D**), while non-inoculated control plants did not show the presence of any bacterial cell on root surfaces (**Figures 3E,F**).

Effect of Axenic Supplementation of AHL Extract on Seedling Growth and Root Morphologies

Wheat seeds grown on AHL-supplemented water agar showed early seedling growth with longer roots and greener shoots compared to the seedlings grown without AHL supplementation (**Figure 4A1**). Microscopic observations of the root showed the development of more root hairs in roots grown in the presence of AHLs than those grown without AHLs (**Figure 4A2**).

The rhizoscan data show that the addition of AHLs in agar medium under salt stress significantly improved the root growth and the morphologies in both wheat genotypes (**Figure 4B1**). Both SSG and STG of wheat, treated either with AHLs of SAL-21 and SAL-17 or a mixture of both, showed a significant increase in different root parameters compared to the non-AHL-treated seedlings. Among all the AHL treatments, seedlings grown on AHL mixture showed the highest percent increase in all root parameters, and AHL extracts of strain SAL-17 showed the lowest. Furthermore, the response of the salt-tolerant genotype was comparatively higher than the salt-tolerant genotype under stress. The AHL-treated salt-sensitive genotype showed an increase of 35–86% in root length (**Figure 4B1**), 32–58% in projected area (**Figure 4B2**), 4–61% in surface area (**Figure 4B3**), 10–16% in average diameter (**Figure 4B4**), 20–47% in root volume (**Figure 4B5**), 30–78% in root tips (**Figure 4B6**), 58–117% in forks (**Figure 4B7**), and 33–266% in root crossing (**Figure 4B8**) over non-AHL-treated plants under salt stress, whereas the AHL-treated salt-tolerant genotype showed an increase of 27–74% in root length (**Figure 4B1**), 30–55% in projected area (**Figure 4B2**), 5–67% in surface area (**Figure 4B3**), 12–15% in average diameter (**Figure 4B4**), 16–40% in root volume (**Figure 4B5**), 37–73% in root tips (**Figure 4B6**), 52–110% in forks (**Figure 4B7**), and 66–333% in root crossing (**Figure 4B8**) over non-AHLs-treated plants under salt stress.

Effect of *Aeromonas* spp. Strain Inoculation on Wheat Growth in Saline Soil

Proline Contents and Nitrate Reductase Activity

Proline accumulation and nitrate reductase activity were significantly higher in the leaves of inoculated plants compared to non-inoculated plants grown with and without salt stress in both genotypes. Overall, leaf proline content, nitrate reductase activity, and the photosynthetic performance of inoculated plants in STG were significantly higher than in SSG inoculated plants (**Figure 5**).

The analysis of treatment response shows that the increase in leaf proline content was maximum in both genotypes where a

TABLE 2 | Key biochemical and physiological tests for the phenotypic differentiation of *Aeromonas spp.* strains SAL-17 and SAL-21 from reported species of genus *Aeromonas*.

Characteristics		1. *Aeromonas* sp. SAL-17	2. *Aeromonas* sp. SAL-21	3. *Aeromonas* sp. SAL-12	4. *A. piscicola*	5. *A. salmonicida*	6. *A. bestiarum*	7. *A. mollusorum*	8. *A. sobria*	9. *A. bivalvium*	10. *A. veronii*	11. *A. jandaei*	12. *A. hydrophila*	13. *A. popoffii*	14. *A. encheleia*
Cell shape		Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Brown pigment		–	–	–	–	+	–	–	–	–	–	–	–	–	–
Gram's reaction		–	–	–	–	–	–	–	–	–	–	–	–	–	–
Catalase		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility		+	+	+	+	–	+	+	+	+	+	+	+	+	+
NaCl tolerance (%)		0.5–6.5	0.5–6.5	0.5–6.5	0–3	0–5	0–1	0-3	0–3	0.5–6	0–1	0–3	0–3	0–1	0–3
pH tolerance (%)		6.5–9.5	6.5–9.5	6.5–9.5	6.5–7.5	4–5	6.5–7.5	8.5–9.5	6.5–7.5	5–9	6.5–7.5	8.5–9.5	6.5–7.5	6.5–7.5	8–9
Temperature tolerance (°C)		25–37	25–37	25–37	4–37	4–37	20–37	4–37	30–37	4–37	22–37	4–42	28–37	4–37	4–37
H_2S Production		–	–	+	+	+	+	–	+	–	–	+	+	±	–
Urea hydrolysis		+	–	+	+	nd	nd	–	–	–	–	nd		–	–
Arginine dihydrolase		–	–	–	+	+	nd	+	–	–	–	+	nd	+	+
Production of acid from	Lactose	+	+	-	–	–	–	–	–	–	–	+	–	–	–
	Arabinose	+	+	+	–	+	+	+	–	+	–	–	+	+	–
	Mannitol	+	+	+	+	+	nd	+	+	+	+	+	+	+	+
	Sucrose	+	+	+	nd	+	+	+	+	+	+	–	+	–	–
	Sorbitol	+	+	+	+	+	–	–	–	–	–	–	–	–	–
	Maltose	+	+	+	+	+	nd	+	nd	+	+	nd	nd	+	+
	Succinate	+	+	+	nd	+	nd		nd	nd	nd	+	nd	+	nd
	Rhamnose	+	+	+	–	–	±	–	–	–	–	–	–	–	–
	Inositol	+	+	+	–	+	nd	–	nd	–	–	–	nd	–	–
	Adonitol	–	–	–	–	+	nd	–	nd	–	–	–	nd	–	nd
	Melibiose	+	+	+	–	+	nd	–	–	–	–	–	–	–	–
	Raffinose	–	–	+	–	+	nd	–	–	–	–	–	–	–	–
Decarboxylation of	Lysine	–	–	–	+	+	+	–	+	+	+	+	+	–	–
	Ornithine	–	–	–	–	–	nd	–	–	–	+	–	–	–	–
Utilization of	Sodium citrate	+	+	nd	nd	–	+	nd	±	+	+	nd	nd	+	nd
	Sodium malonate	+	+	–	nd	–	nd	nd	nd	nd	–	nd	nd	+	nd
Clinical significance		No	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No

Data were taken from 1, 2, and 3 (from this study), 4 (Beaz-Hidalgo et al., 2009), 5 (Austin et al., 1989), 6 (Martínez-Murcia et al., 2005), 7 (Miñana-Galbis et al., 2004), 8 and 12 (Popoff and VéEron, 1976), 9 (Miñana-Galbis et al., 2007), 10 (Hickman-Brenner et al., 1987), 11 (Esteve et al., 2003), 13 (Huys et al., 1997), and 14 (Esteve et al., 1995). +, 80–100% of strains positive; –, 80–100% strains negative; nd, no data available.

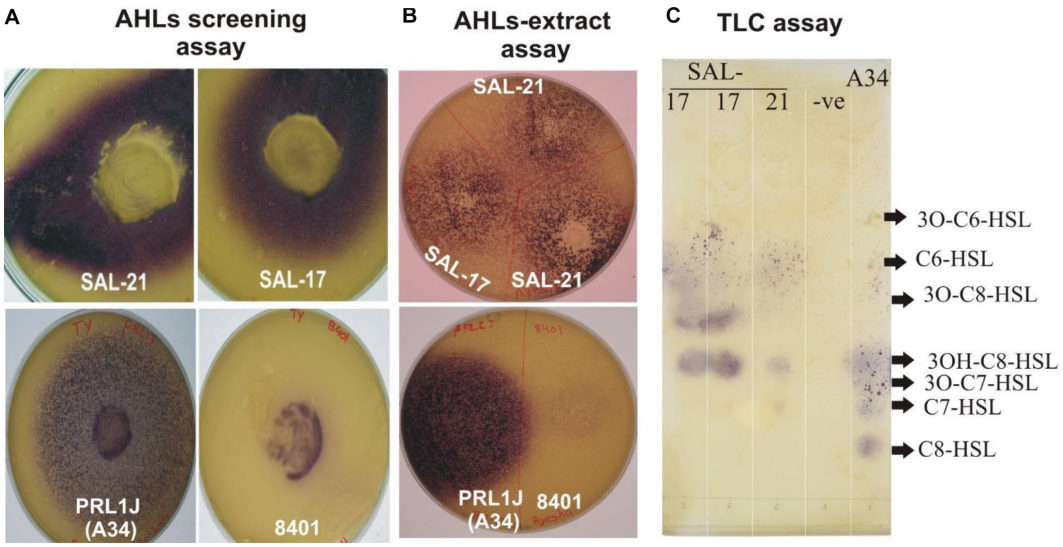

FIGURE 1 | (A) Acyl homoserine lactone (AHL) detection assay of *Aeromonas* spp. SAL-21 and SAL-17 with *Chromobacterium violaceum* CV026 showing purple coloration. *Rhizobium leguminosarum* A34 and 8401 refers to positive controls. **(B)** Plate assay of extracted AHLs. **(C)** Reverse phase-thin-layer chromatography of AHL extracts of SAL-17 and SAL-21 with the *C. violaceum* CV026 strain used as a biosensor and the *R. leguminosarum* strain A34 used as a positive control. From right, lane 1—positive control A34, lane 2—solvent control, lane 3—SAL-21, and lanes 4 and 5—SAL-17.

mixed inoculation of bacteria (consortium) was applied under saline soil, whereas the lowest was observed when both were grown without inoculation under normal soil (**Figure 5A**). Non-AHL-producing *Aeromonas* sp. SAL-12 also showed an increase in proline contents, but this increase was significantly lower than in the other bacterial inoculation treatments.

Nitrate reductase activity (NR) was significantly higher in STG than in SSG irrespective of bacterial treatments or the soils. The plants inoculated with the bacterial consortium and those inoculated with SAL-17 showed the maximum NR activity and the highest percent increase over the respective non-inoculated control. Similarly, SSG showed maximum NR activity in consortium-inoculated plants, although the activity was much lower than the corresponding treatment in STG (**Figure 5B**). The leaves inoculated with non-AHL-producing *Aeromonas* sp. SAL-12 showed a little increase in NR activities in both genotypes.

Stomatal Conductance, Transpiration Rate, and Chlorophyll Contents

Of the two genotypes, STG exhibited increased activities for all the gas exchange and photosynthetic parameters. The maximum response of inoculation was observed in the treatment where bacterial consortium was applied. In non-inoculated plants, stomatal conductance, transpiration rate, and chlorophyll contents were higher in normal soil compared to those in saline soil (**Figures 5C–F**).

Stomatal conductance was highest in STG, with an increase of 84% in the consortium, 57.6% in SAL-17, 24% in SAL-21, and 20% in SAL-12 inoculation, respectively, over the non-inoculated control. The salt-sensitive genotype showed an increase of 147.85% in the consortium, 120.36% in SAL-17, 103.57% in SAL-21, and 50.45% in SAL-12 inoculation, respectively (**Figure 5C**). This shows that, although stomatal conductance was higher in STG, the relative percent increase after inoculation was significantly higher in SSG.

Transpiration rate showed a similar trend and was highest in STG with an increase of 54.5% in the consortium, 27% in SAL-17, 24.5 in SAL-21%, and 18% in SAL-12 inoculation, respectively, over the non-inoculated control. The salt-sensitive genotype showed an increase of 75.05% in the consortium, 32.53% in SAL-17, 29.04% in SAL-21, and 21.26% in SAL-12 inoculation, respectively (**Figure 5D**).

Chlorophyll a band total chlorophyll contents were significantly high in STG plants after inoculation with the consortium. The improvement in chlorophyll contents was statistically less significant in SSG and other treatments (**Figures 5E,F**).

In general, the STG showed the maximum increase in all the stress parameters studied, but a comparative analysis of data revealed that the salt-sensitive genotype responded better to inoculation because the percentage increase was higher in SSG than in STG compared to the non-inoculated controls.

Growth and Yield

Analysis of growth and yield parameters of wheat showed a significant increase in inoculated wheat plants compared to non-inoculated controls (**Figure 6**) in both wheat genotypes. Overall, the salt-tolerant genotype inoculated with bacterial consortium showed the maximum growth and yield, whereas the non-inoculated salt-sensitive genotype in normal or saline soil showed the minimum.

The data regarding plant fresh weight (**Figure 6A**), root length (**Figure 6B**), and shoot length (**Figure 6C**) collected on the 25th day of inoculation showed a significant response of inoculation

TABLE 3 | Liquid chromatography–tandem mass spectrometry analysis of acyl homoserine lactones (AHLs) in spent culture supernatant of *Aeromona*s spp. SAL-17 and SAL-21.

Sr. #.	AHL type	m/z $(M + H)^+$	Relative abundance of isolated ions*	Daughter ions
1	3-OH-C5-HSL	202	+++	187, 185, 174, 159, 147, 144, 130, 123, 100, 85
2	C6-HSL	200	++	185, 182,172, 158, 156, 144, 130, 114, 102, 88
3	3-OH-C6-HSL	216	+	198, 173, 159, 146, 102, 84
4	3-oxo-C7-HSL	228	++	210, 199, 186, 172, 159, 145, 130, 120, 102, 84
5	C10-HSL	256	++++	238, 228, 214, 188, 186, 172, 159, 130, 102, 88
6	3-oxo-C10-HSL	270	+++	252, 242, 228, 214, 200, 185, 172, 159, 146, 120, 102, 88
7	3-OH-C10-HSL	272	++	254, 228, 214, 200, 186,172, 159, 146, 118, 102
8	3-oxo-C12-HSL	316	+	298, 272, 246, 222, 212, 184, 166, 152, 106, 102
9	C6-HSL	200	+	184, 182, 172, 139, 126, 102, 85
10	3-oxo-C10-HSL	288	++++	273, 270, 260, 244, 214, 188, 174, 160, 144, 140, 125, 106, 102, 88

in both genotypes, although the effect was significantly higher in STG than in SSG. A similar trend was observed for the data collected for growth parameters at 75 days after inoculation for shoot fresh weight (**Figure 6D**), root fresh weight (**Figure 6E**), shoot length (**Figure 6F**), and root length (**Figure 6G**).

A comparison of treatment means for the 1,000-grain weight (**Figure 6H**) showed that the response of the salt-tolerant genotype was maximum in all treatments compared with that of the salt-sensitive genotype. Furthermore, the STG plants inoculated with consortium showed the maximum grain weight than all other inoculation treatments.

The Relationship Among the Parameters

The whole data were subjected for correlation analysis using SPSS, and a direct positive relationship of root length was found with other morphological parameters of root, i.e., projected area ($r = 0.936^{**}$), surface area ($r = 0.876^{**}$), average diameter ($r = 0.896^{**}$), root volume ($r = 0.912^{**}$), tips ($r = 0.903^{**}$), forks ($r = 0.958^{**}$), and crossings ($r = 0.852^{**}$). The salt-tolerant genotype showed a specifically higher correlation coefficient ratio (r value). Plant fresh weight was found to be positively correlated with other plant morphological (dry weight and length), biochemical (nitrate reductase activity, proline contents), and physiological (chlorophyll contents) parameters ($r = 0.621^{**}$–0.958^{**}).

Linear regression effectively modeled the positive relationship of grain weight with the chlorophyll contents ($R^2 = 0.45$ for SSG; $R^2 = 0.533$ for STG), accounting for 70–82% of the total variance. Quadratic regression was observed for grain weight with nitrate reductase activity ($R^2 = 0.858$ for SSG; $R^2 = =0.880$ for STG) and proline contents ($R^2 = =0.917$ for SSG; $R^2 = =0.942$ for STG) (**Supplementary Figure S2**).

The CAT-PCA and the PCA captured more than 75–90% of the variance and demonstrated the key genotype difference in both soils and inoculation treatments. The CAT-PCA (**Figure 7A**) demonstrated that all the observed plant traits/parameters loaded onto the positive quadrant were strongly positively correlated to each other ($R^2 = 0.838$). The PCA showed that the inoculation response was similar in both genotypes, where the consortium-inoculated plants loaded positively while the non-inoculated plants loaded negatively on PCA (**Figure 7B**).

DISCUSSION

Various eco-physiological parameters of soil determine the microbial community and activity in the plant rhizosphere (Egamberdieva et al., 2010). Selection and subsequent plant inoculation of efficient PGPR strains compatible with local eco-physiological conditions can significantly improve a plant's nutritional status and their overall biotic and abiotic stress tolerance ability. Therefore, we selected wheat rhizosphere isolates from saline soil (BSRS-II) containing multiple plant-growth-promoting traits. They were previously identified as *Aeromonas* spp. with plant-growth-promoting traits (Rajput et al., 2018). The strains were clinically non-significant (negative for the beta-hemolytic reaction) and hence could be used for further studies. Phylogenetic analysis showed their relatedness, but biochemical comparison showed their key differences from other *Aeromonas* spp. strains (**Table 1**).

This study reports two AHL-producing *Aeromonas* spp. from saline-soil rhizosphere, their mass spectrometry analysis, and the subsequent effect on plant growth. AHLs identified in SAL-17 and SAL-21 strains include 3-OH-C5-HSL, C6-HSL, 3-OH-C6-HSL, 3-oxo-C7-HSL, C10-HSL, 3-oxo-C10-HSL, 3-oxo-C10-HSL + H_2O, 3-OH-C10-HSL, and 3-oxo-C12-HSL + H_2O. There are various AHLs reported from genus *Aeromonas* (**Supplementary Table S1**), but six AHLs (3-OH-C5-HSL, 3-OH-C6-HSL, 3-oxo-C7-HSL, 3-oxo-C10-HSL, 3-OH-C10-HSL, and 3-oxo-C12-HSL) identified in this study (**Table 3**) were not reported earlier, a feature that makes SAL-17 and SAL-21 different from other *Aeromonas* species. Being different in the genus *Aeromonas*, we deduced the function of these six AHLs from already published studies where they have been detected from other bacterial species. The functional annotation of the detected AHLs (**Table 4**) in the *Aeromonas* spp. SAL-17 and SAL-21 showed that these AHLs are mainly involved in the induction of systemic resistance against various pathogens, synthesis of phytohormones, and plant growth promotion. Exclusively C6-HSL plays its role in the induction of systemic resistance against biotic and abiotic stresses, root colonization, and biofilm formation by bacteria, root growth, and development. C6-HSL, 3-oxo-C-10-HSL, and 3-oxo-C12-HSL have a combined well-defined role against salt stress *via* the enhanced activity of superoxide dismutase (SOD),

TABLE 4 | Functional annotation of acyl homoserine lactones (AHLs) in spent culture supernatant of *Aeromonas* spp. SAL-17 and SAL-21 compared to others reported in literature.

AHL types detected in this study	Putative role/function	Detected previously in bacteria	Isolated from (host)or tested on plant	References
3-OH-C5-HSL	Putative role in symbiosis	*S. meliloti*	*Alysicarpus bupleurifolius* L. root nodules	Zarkani et al., 2013
C6-HSL	Production/regulation of phenazines, siderophore, chitinases, proteases and pyrrolnitrin, 2,4-DAPG, hydrogen cyanide, antifungal activity against pathogens, induced systemic resistance, systemic induction of ethylene– and salicylic acid-dependent defense-related genes, increased plant resistance to early infection, improved germination, growth, development, and productivity, root elongation, alteration of auxin to cytokinin ratio in roots and shoots, root colonization, synthesis of IAA, elevated defense response	*Pseudomonas* sp., *Burkholderia ambifaria*, *P. chlororaphis*, *Serratia liquefaciens*, *Serratia plymuthica*, *Pseudomonas fluorescens*, *Serratia plymuthica*	Wheat and maize roots, *Lycopersicon esculentum, Brassica napus* L. roots, transgenic *Nicotiana tabacum*, *Arabidopsis thaliana* L. roots, roots of *Brassica napus* subsp. *Napus* L., *Cucumis sativus* L., *Phaseolus vulgaris* L., *Lycopersicum esculentum* L., *Chlorella vulgaris* L. roots	Wood et al., 1997; Chin-A-Woeng et al., 2001; Zhou et al., 2003; Müller, 2006; Schuhegger et al., 2006; Wei and Zhang, 2006; von Rad et al., 2008; Müller et al., 2009; Pang et al., 2009; Bi et al., 2012; Cheng et al., 2018; Hu et al., 2018
	Low concentration protected against salt stress *via* enhanced activity of SOD, POD, CAT, and higher accumulation of MDA, stress-responsive, signal transduction and regulation and biosynthesis-related proteins	NA	*Arabidopsis thaliana* L. Col–0 roots	Ding et al., 2016
	Plant growth promotion including and development of lateral roots and NO accumulation in calyptra, enhanced K^+ uptake through membrane hyper-polarization	NA	*Hordeum vulgare* L. roots	Rankl et al., 2016
3-OH-C6-HSL, 3-oxo-C7-HSL	Antifungal activity, production of pyrrolnitrin, chitinase, protease siderophores and hydrogen cyanide, rhizosphere colonization, biocontrol activity	*Serratia* sp., *Ochrobactrum* sp.	*Triticum aestivum* L. stems, *Phaseolus vulgaris* L. roots	Liu et al., 2010; Imran et al., 2014

(Continued)

TABLE 4 | Continued

AHL types detected in this study	Putative role/function	Detected previously in bacteria	Isolated from (host)or tested on plant	References
3-oxo-C10-HSL, 3-oxo-C12-HSL	Biofilm formation, expression of defense-related, stress-responsive, flavonoid synthesis, phytohormonal and regulatory genes, salt stress protection mechanism, overall growth promotion	*Pseudomonas putida*, *Sinorhizobium meliloti*, *Pseudomonas aeruginosa*, *Burkholderia graminis*	*Lycopersicon esculentum* L. roots, *Medicago truncatula* L roots, transgenic *Lycopersicum esculentum* L (*Las*I)	Steidle et al., 2001; Steidle et al., 2002; Mathesius et al., 2003; Barriuso et al., 2008
C10-HSL	Post−embryonic root development including lateral and primary root growth and root hair development, adventitious roots formation through H_2O_2, NO and cGMP signaling, expression of IAA-responsive genes, induced systemic resistance and root development	NA	*Arabidopsis thaliana* L. roots, *Vigna radiata* L. roots, *Hordeum vulgare* L. roots	Ortíz-Castro et al., 2008; Bai et al., 2012; Hu et al., 2012; Sieper et al., 2014
	Calmodulin-regulated primary root growth	NA	*Arabidopsis thaliana* L. roots	Zhao et al., 2015
	Enhanced activity of critical photosynthetic enzymes including rubisco, maximal and actual photochemical efficiency was also enhanced	NA	*Chlorella vulgaris* roots	Dou et al., 2017
	Increased plant resistance against *B. cinerea via* jasmonic acid signaling under elevated CO_2	NA	*Lycopersicum esculentum* L. leaves	Hu et al., 2020
3-OH-C10-HSL	Root colonization in microcolonies, plant growth promotion, inhibition of plant defense responses	*Acidovorax radicis* N35	*Hordeum vulgare* L. roots	Han et al., 2016

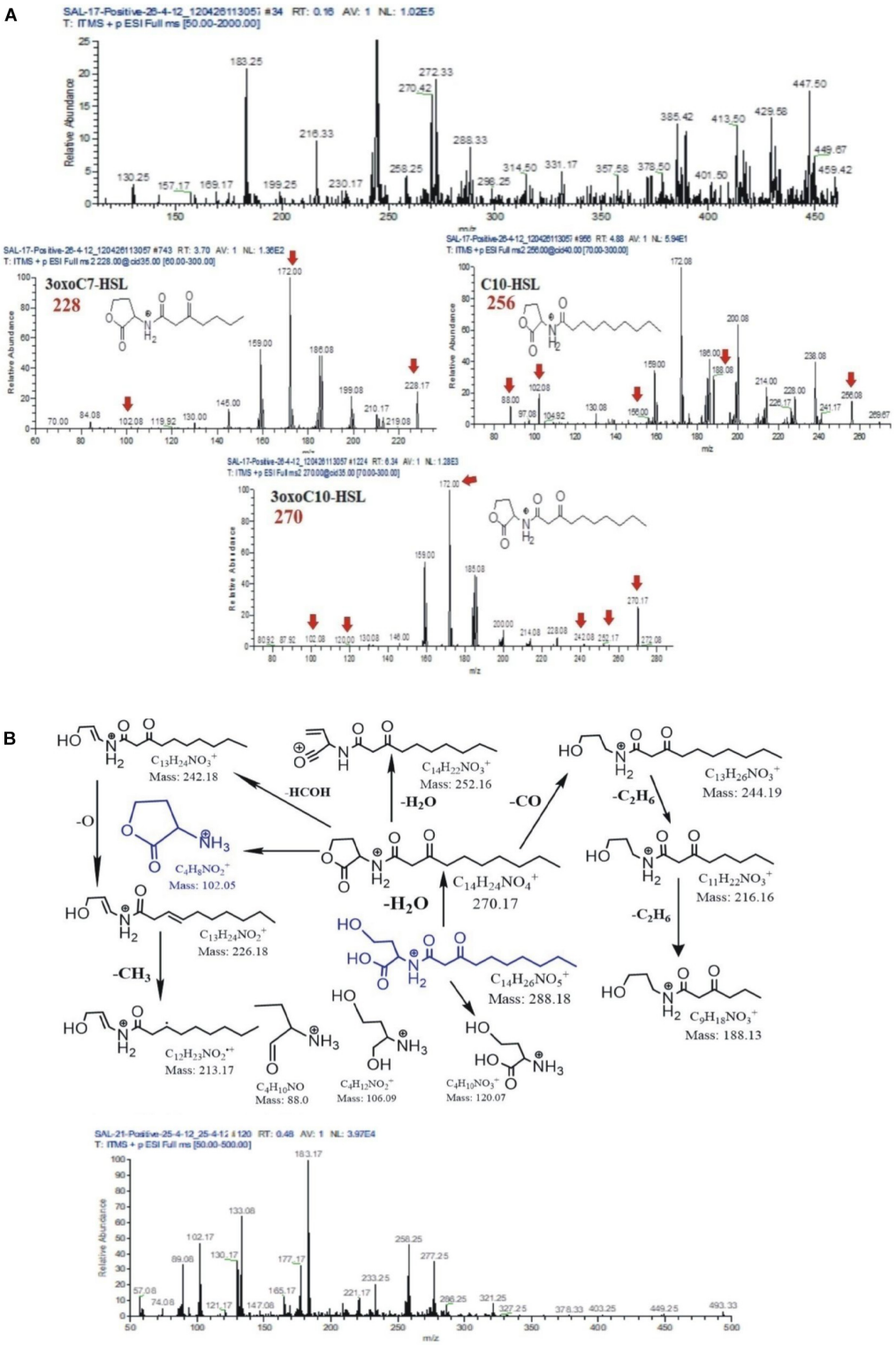

FIGURE 2 | (A) Mass spectrometry chromatogram (full MS) with peaks at $[M + H]^+$, $[M + H + H_2O]^+$, and $[M + Na]^+$ of acyl homoserine lactones (AHLs) extracted from strain SAL17. Protonated AHL $[M + H]^+$ peaks were at a very low intensity. The peaks were identified as $[M + H + H_2O]^+$ and $[M + Na]^+$ for the presence of AHLs by adding a water molecule or the formation of sodium adducts. **(B)** Fragmentation pattern of AHLs 3oxoC10-HSL m/z 288. All daughter ions generated from the fragmentation of m/z 288 are unambiguously assigned (above). Mass spectrometry chromatogram (full MS) with peaks at $[M + H]^+$, $[M + H + H_2O]$+, and $[M + Na]^+$ of AHLs extracted from strain SAL21 (below). Protonated AHLs $[M + H]^+$ peaks were at a very low intensity. The peaks were identified as $[M + H + H_2O]^+$ and $[M + Na]^+$ for the presence of AHLs by adding a water molecule or the formation of sodium adducts.

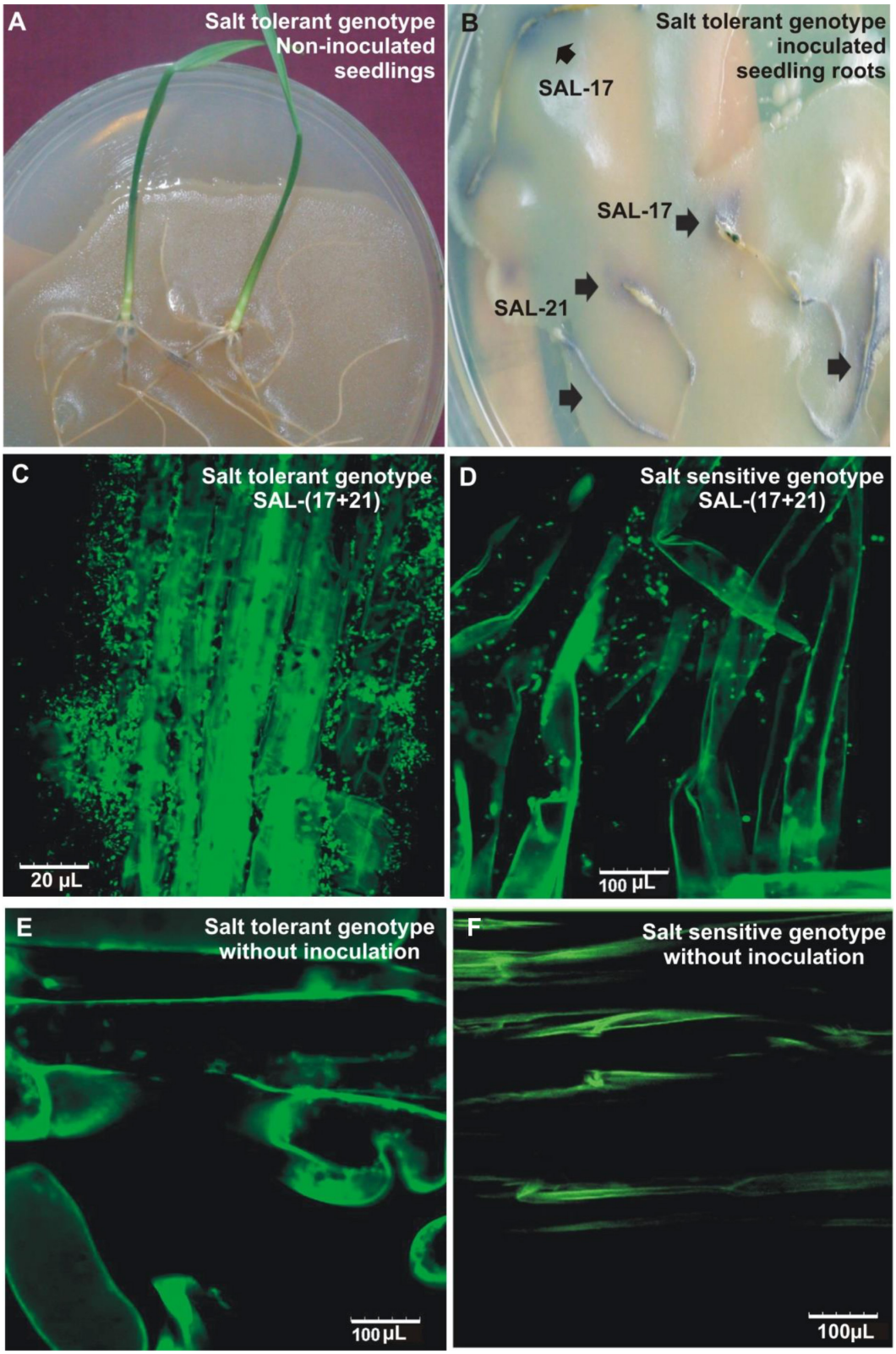

FIGURE 3 | Root colonization of salt-tolerant wheat genotype by bacterial inoculation and subsequent detection by plate overlay assay **(A,B)**. Confocal microscopic images of salt-tolerant **(C)** and salt-sensitive **(D)** wheat roots inoculated with *Aeromonas* spp. bacterial consortium under salt stress. Non-inoculated salt tolerant **(E)** and salt-sensitive **(F)** genotype grown in salt stress are shown as controls.

peroxidase (POD), and catalase (CAT) enzymes. 3-OH-C6-HSL is known to induce the production of antifungal metabolites by root-colonizing microbes, whereas 3-oxo-C7-HSL assists in the colonization process, and it may serve as biocontrol component in the rhizosphere. The signal molecules 3-OH-C6-HSL, 3-oxo-C7-HSL, 3-oxo-C-10-HSL, and 3-oxo-C12-HSL have been shown to protect against biotic and abiotic stresses. Furthermore, their role in biofilm formation, root colonization, and development of lateral and primary roots has also been described. The signal molecules C10-HSL and 3-OH-C1-HSL have been reported to control the primary and the secondary growth of root and colonization and the induction of plant defense response. Furthermore, they have a well-defined role in root growth and development, along with a significant impact on photosynthesis, induced stress resistance, and plant hormone signaling pathways. 3-Oxy-C10-HSL helps in biofilm formation and improves the growth of adventitious roots and the expression of indole-3-acetic acid (IAA)-responsive genes, while 3-OH-C10-HSL mediates plant root colonization, growth promotion, and induction of defense response. 3-Oxo-C12-HSL serves in biofilm formation and expression of stress-related, hormonal, and regulatory genes. The function of 3-OH-C5-HSL is not mentioned in the literature, but studies suggest that it might have some role in symbiosis as stated by Zarkani et al. (2013). The detection of this AHL type from *Aeromonas* spp. in the present study suggests that it might have some other functions in plants rather than just symbiosis. The same AHL from different bacteria exhibits fairly similar functions; detailed molecular studies are required for the validation of these functions.

The root colonization potential of a bacterium is necessary to develop a successful interaction with plant, and bacteria use AHL-mediated synchronized response to design and establish an efficient interaction between the host and its associated symbionts (Schenk and Schikora, 2015). Both strains (SAL-21 and SAL-17) possess IAA production ability (Rajput et al., 2018) and AHL production (this study) and exhibited their colonization ability on wheat roots in different experiments, i.e., confocal microscope analysis and plate assay. The confocal analysis showed that bacterial colonization is a little affected in the presence of salt. Furthermore, a modified plate overlay assay validated the root colonization and purple color, along with the growing seedling root, displaying that AHLs are being produced and might have a robust role in root development. AHL-mediated root colonizing

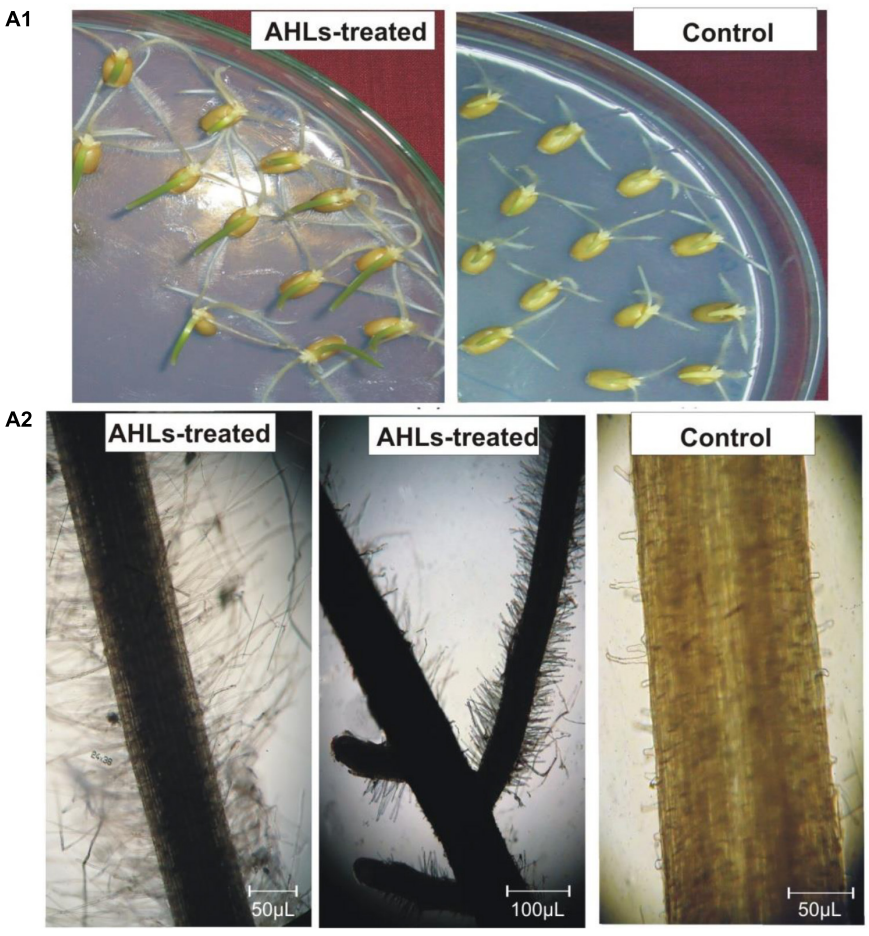

FIGURE 4 | Continued

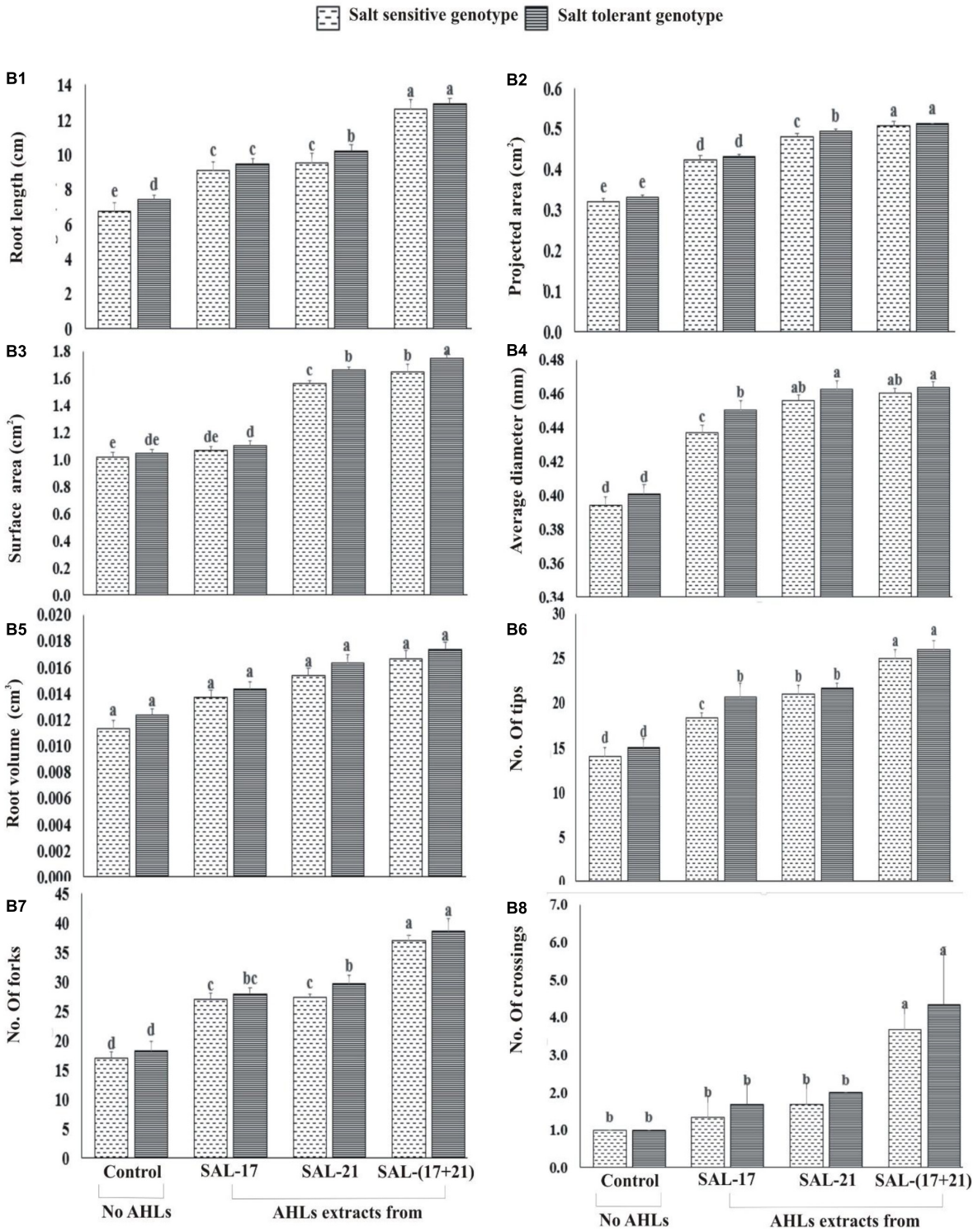

FIGURE 4 | (A1,A2) Effect of acyl homoserine lactone (AHL) supplementation on the seedling growth of wheat **(A1)** and AHL-mediated root hair development **(A2)** compared to control wheat seeds grown on water agar. The pictures were photographed at ×100 magnification and then cropped to remove the background. **(B1–B8)** Effect of AHL supplementation on 10-day-old wheat roots under induced salinity **(**200 mM NaCl**)** compared to non-treated control in terms of **(B1)** root length, **(B2)** projected area, **(B3)** surface area, **(B4)** average diameter, **(B5)** root volume, **(B6)** number of tips, **(B7)** number of forks, and **(B8)** crossings. Values are the mean of four replicates. Bars represent standard deviation. Values sharing the same letter do not differ significantly ($P \leq 0.01$) according to Fisher's least significant difference test.

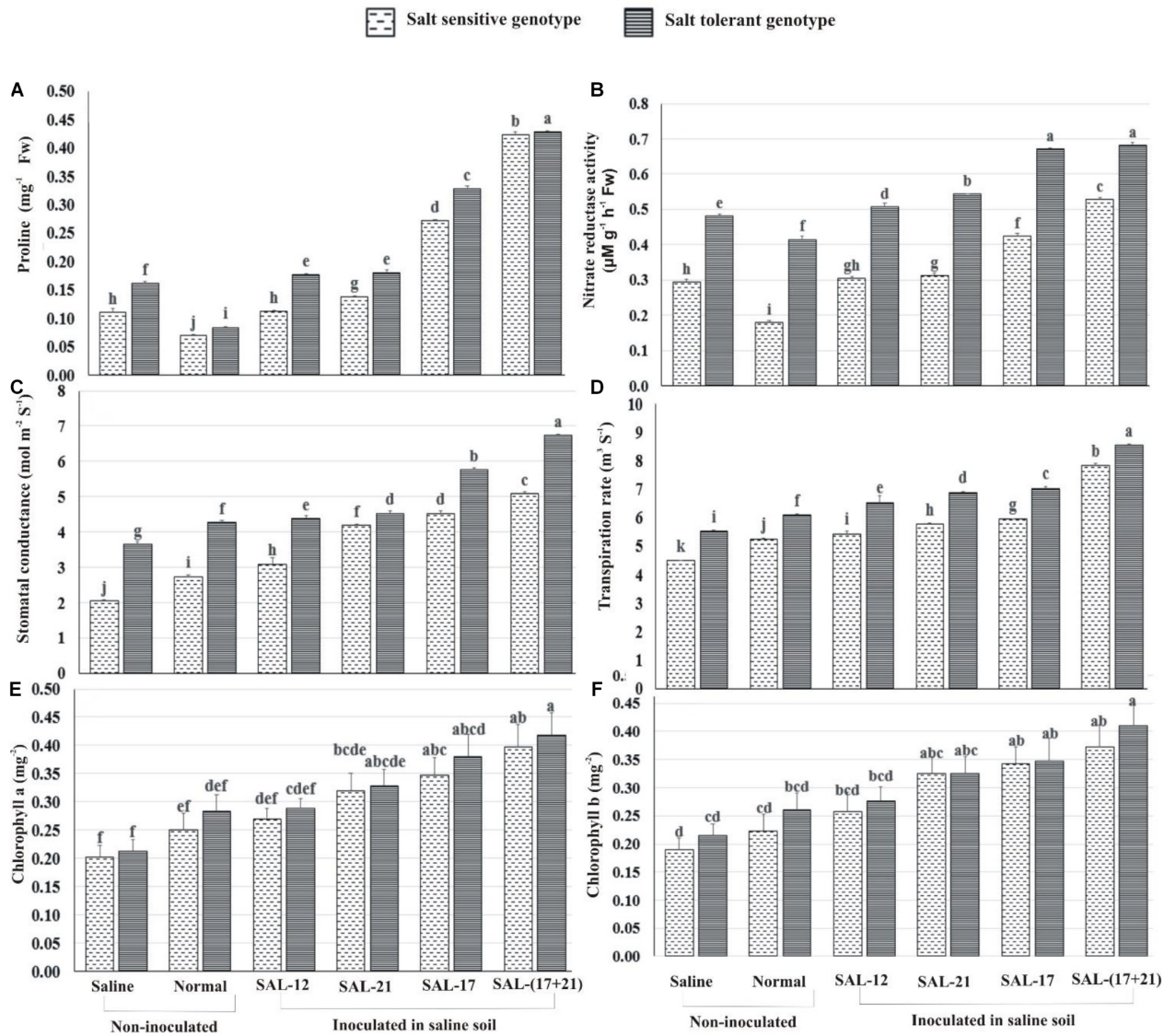

FIGURE 5 | Effect of acyl homoserine lactone-producing and non-producing *Aeromonas* spp. strain inoculation on the biochemical and the physiological parameters of wheat grown in saline soil compared to non-inoculated control: **(A)** proline contents at 45 dpi, **(B)** nitrate reductase activity at 45 dpi, **(C)** stomatal conductance at 45 dpi, **(D)** transpiration rate at 45 dpi, **(E)** chlorophyll a at 50 dpi, and **(F)** chlorophyll b at 50 dpi. Values are the mean of six replicates. Bars represent standard deviation. Values sharing the same letter do not differ significantly [$P \leq 0.05$ for **(A,B)** and $P \leq 0.01$ for **(C–E)**] according to Fisher's least significant difference test.

ability has been previously reported in rhizobia and genus *Pantoea* (Sutherland, 2001; Koutsoudis et al., 2006).

The root overlay assay revealed a likely role of AHLs in early root growth, which was further confirmed in a plate assay where purified AHLs were applied in growth medium and seeds were grown without bacterial inoculation. The data regarding root morphology establish the fact that the increase in root growth is the function of AHLs rather than of IAA. The plate overlay assay of extracted AHLs, RP-TLC analysis, and SPE steps ruled out the likelihood of the presence of IAA traces in the AHL extracts. The AHL extracts of these strains contain C6-HSL, which has a well-reported function in primary root elongation, auxin/cytokinin ratio alteration, transcriptional regulation, and biomass improvement (von Rad et al., 2008). The other AHL in the extract was C10-HSL, which enhances lateral root growth (Bai et al., 2012; Zhao et al., 2015) and shoot growth (Götz et al., 2007) in different plants. Purified AHL extracts from *Bradyrhizobium* sp. strain SR-6, which produces a wide variety of AHLs including C6-HSL, C10-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, *etc.*, significantly improved root hair development in wheat, along with increased nodulation in soybean (Ali et al., 2016).

Plant response was further evaluated by strain inoculation in saline soil. An IAA-positive but AHL-non-producing *Aeromonas* sp. strain SAL-12 indigenous to saline soil (Rajput et al., 2018) was used for comparison of inoculation response. SAL-12, along with both AHL-producing *Aeromonas* spp. SAL-17 and

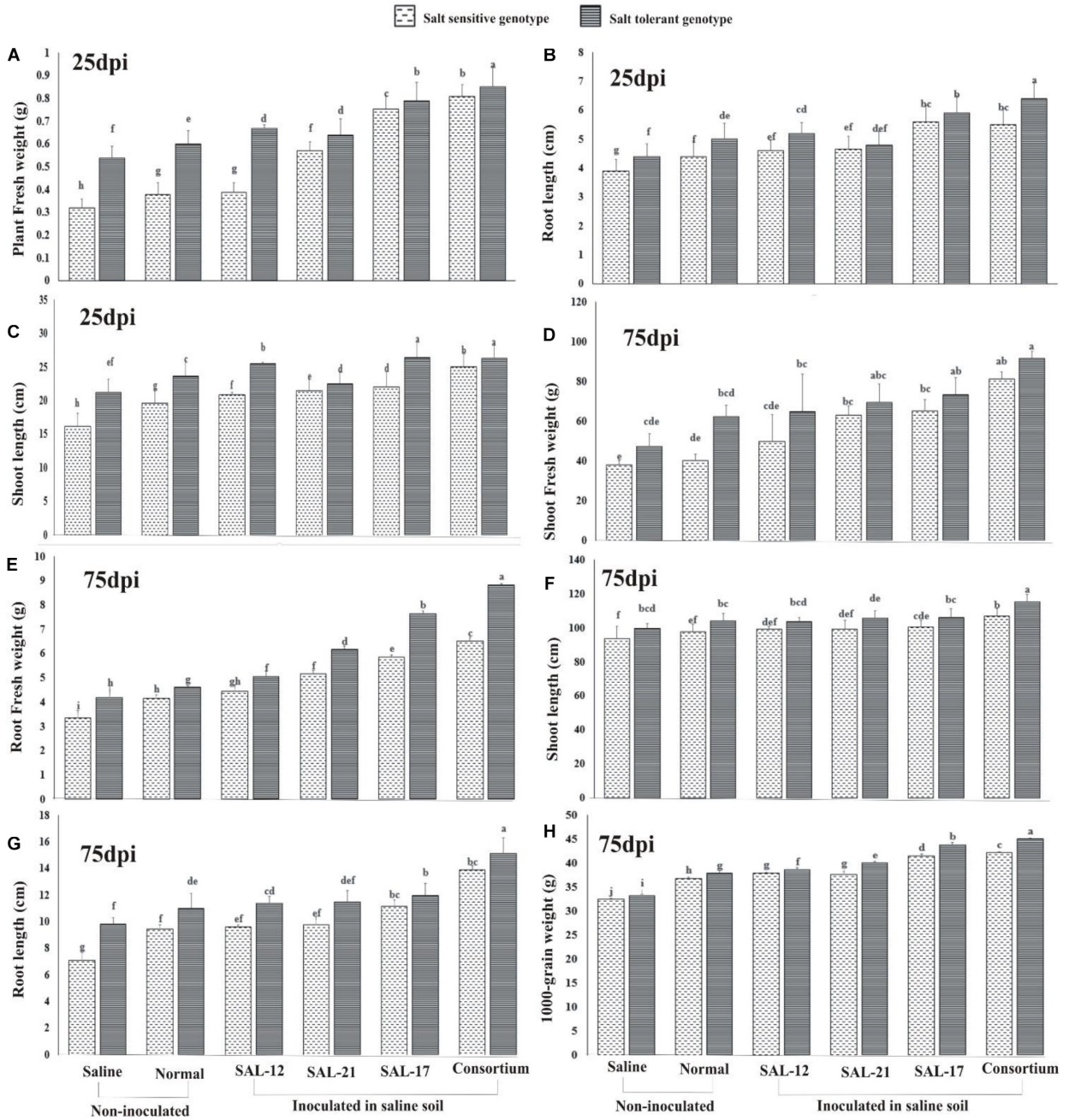

FIGURE 6 | Effect of acyl homoserine lactone-producing and non-producing *Aeromonas* spp. strain inoculation on the growth and the yield of wheat grown in saline soil compared to non-inoculated control: **(A)** plant fresh weight at 25 dpi, **(B)** shoot length at 25 dpi, **(C)** root length at 25 dpi, **(D)** shoot fresh weight at 75 dpi, **(E)** root fresh weight at 75 dpi, **(F)** root length at 75 dpi, **(G)** shoot length at 75 dpi, and **(H)** weight of 1,000 grains (after harvest). Values are the mean of six replicates. Bars represent standard deviation. Values sharing the same letter do not differ significantly ($P \leq 0.05$) according to Fisher's least significant difference test.

SAL-21, also exhibits ACC deaminase activity. IAA is a plant hormone that is involved in the stimulation of plant growth, and ACC deaminase has a well-known function in salt stress mitigation *via* the cutting synthesis of ethylene (Ahmad et al., 2011; Arshadullah et al., 2017; Sarkar et al., 2018; Afridi et al., 2019; Bharti and Barnawal, 2019; del Carmen Orozco-Mosqueda et al., 2020). Both these traits are a characteristic feature of any PGP candidate species because some studies have shown a synergistic effect of bacterial IAA and ACC deaminase. Both IAA and ACC deaminase have direct positive effects on root growth and root hair development, which help to enhance water and nutrient absorption from the soil (Príncipe et al., 2007). Our plant inoculation data demonstrated the significance of AHLs for plant growth under stress along with the role of

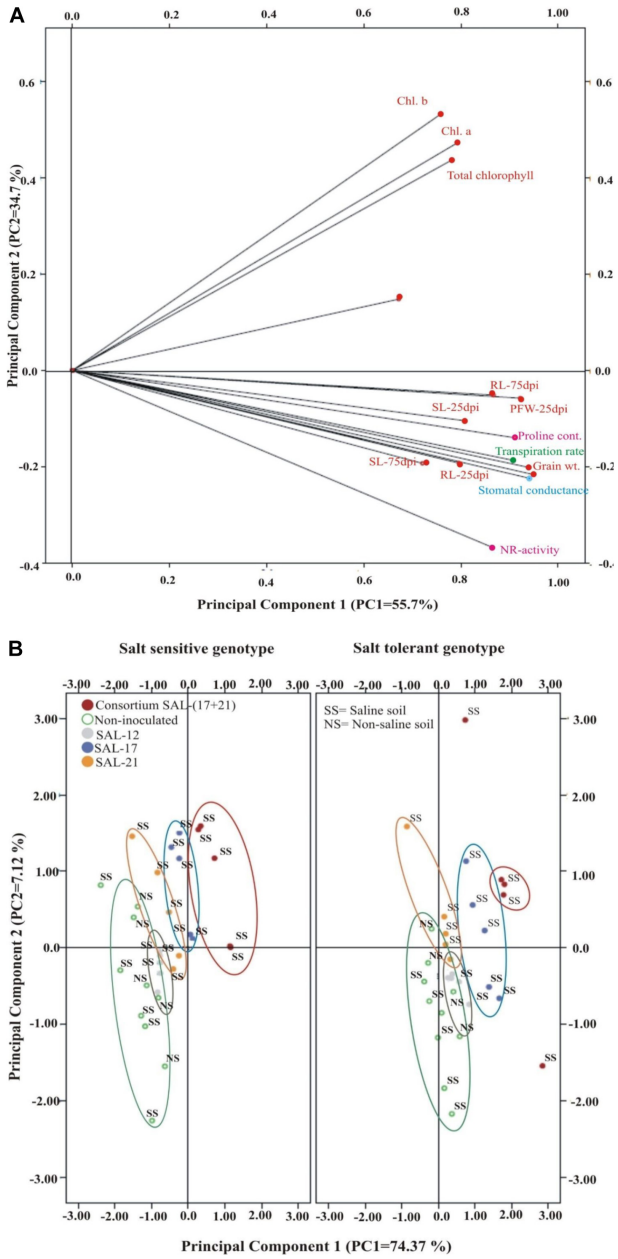

FIGURE 7 | (A,B) Categorical principal component analysis (CAT-PCA) of various plant traits measured across salt-tolerant and salt-sensitive genotypes grown in saline soil after different bacterial inoculation treatments (total variance explained: 90%; PC1 = 55.7%, PC2 = 34.7%). CAT-PCA is a non-linear PCA. Factor loadings in PC1 and PC2 are presented as vectors using external scale. PCA showing the response of salt-tolerant and salt-sensitive genotypes toward bacterial inoculation in saline soil compared to non-inoculated controls; loaded as genotypes (total variance explained : 81%; PC1 = 74%, PC2 = 7%).

IAA and ACC deaminase. For instance, if SAL-17 produces a wide variety of AHLs and higher IAA than SAL-21 and SAL-12, the response toward SAL-17 single inoculation and a mix inoculation (SAL-17 + SAL-21) was more pronounced on different biochemical, physiological, and growth parameters of wheat. AHLs directly or indirectly induce stress resistance in plants through QS-mediated production of metabolites (Ryu et al., 2013). It has been reported that inoculation of 3-oxo-C12-HSL-producing bacterial strains induce salt stress tolerance, metabolic regulation, and phytohormone response in tomato and *Medicago truncatula* (Mathesius et al., 2003; Barriuso et al., 2008). The data from this study have presented many folds increase in the NR activity. NR is the enzyme responsible for nitrate assimilation and the production of NO in plants (Chamizo-Ampudia et al., 2017). NR-mediated NO also has been reported as a key signaling molecule in leaf shape development (Pan Q.-N. et al., 2019), root geotropism (Vazquez et al., 2019), and various stress responses by plants. Although purified AHLs were not used in the experiment, still we speculate that AHLs have some role in the regulation of NR activity, which in turn induces salt stress tolerance in wheat because NR activity and proline contents directly correlate with AHL production. PGPR has a documented role to accumulate higher proline in plants under stress (Jha and Subramanian, 2014; Kumari et al., 2015; Singh and Jha, 2016b), and this study advocates this role along with some plausible role of AHLs, although it is still unclear how plants perceive these signals and how many are responsible to elicit these responses in plants. However, plants inoculated with the bacterial consortium (SAL-17 + SAL-21) showed a significantly ($P \leq 0.05$) higher response and a maximum percent increase for all parameters in both genotypes of wheat. Further experiments using purified and inclusive inoculum for each kind of AHL with different concentrations and gene knockout studies can elucidate the role of individual AHLs on plant growth.

The data regarding stomatal conductance, transpiration rate, and photosynthetic pigments show that these parameters were significantly increased ($P \leq 0.05$) in inoculated plants in both wheat genotypes under salt stress, wherein plants inoculated with AHL-producing *Aeromonas* sp. strains (SAL-17 and SAL-21) showed a better response in terms of percent increase than the plants inoculated with non-AHL-producing *Aeromonas* sp. strain (SAL-12). This may be attributed to the contribution of AHLs in the overall plant response. Salinity usually causes osmotic stress in plants (Upadhyaya et al., 2013), which leads to stomatal closure by altering the turgor potential of the guard cells. It is a feedback process to prevent water loss *via* transpiration (Buckley and Mott, 2013), but it also blocks the passage for CO_2, causing the photosynthetic activity to decrease. Not by themselves but the degraded products of AHLs have been reported to enhance stomatal conductance and transpiration rate in mung bean (Joseph and Phillips, 2003) because AHLs are not stable in the soil and readily degrade into their active constituents (Wang and Leadbetter, 2005). As acyl-HSLs in the rhizosphere are degraded, the bioavailability of nutrients to the roots and root-associated bacteria increases, which indirectly increases transpiration and growth (Joseph and Phillips, 2003). PGPR-mediated improvement in chlorophyll pigments and overall photosynthetic capacity is well established (Babaei et al., 2017; Afridi et al., 2019; Azarmi-Atajan and Sayyari-Zohan, 2020; Ji et al., 2020). A meta-analysis of 561 studies has suggested

the positive role of PGPR inoculation in K^+/Na^+ ratio ion homeostasis, Na^+ exclusion, and enhanced photosynthetic activity (Pan J. et al., 2019). Moreover, inoculated *Aeromonas* spp. strains also produce IAA.

Along with the biochemical and the physiological parameters, the growth and the yield parameters of plants were significantly ($P \leq 0.05$) improved in inoculated plants of both genotypes as compared to the non-inoculated control. It could be an accumulative effect of multiple PGP and stress tolerance traits and a wide range of AHLs. A significant contribution of AHLs becomes obvious when results for different growth parameters and yield from plants inoculated with AHL-producing strains SAL-21 and SAL-17 were compared with those of non-AHL-producing strain SAL-12. The role of PGPR in stress tolerance amelioration, plant growth, and yield improvement of several crops is well established (Van Loon, 2007). Plant-beneficial bacteria play a key role in the improvement of crop growth, nutrition, and yields and in sustaining soil productivity with low input of chemical fertilizers under stress (Islam et al., 2016; Yasmeen et al., 2019).

The results of the current study advocate that plant-associated beneficial *Aeromonas* spp. strains have a significant role in salt stress mitigation and overall plant growth improvement. Moreover, stress-resilient PGPR is the best choice to be used as inoculants under stressful conditions because they can sustain stress and maintain their PGP traits as well. This study indicates the contribution of AHLs in stress tolerance induction, but other plant-growth-promoting factors cannot be ruled out completely. Furthermore, plants can be engineered for AHL production to foster their interaction with beneficial microbes as previously reported for bioengineered plants (Scott et al., 2006). A study in which tomato plants were inoculated with AHL-producing strains and also bioengineered for production of short-chain and long-chain AHLs has concluded that AHLs promote plant growth and confer protection against salt stress (Barriuso et al., 2008). The current study opens future directions for the researchers to study the AHL regulation of microbial process and plant response modulation through induction of stress-responsive genes and signaling pathways.

CONCLUSION

This study has demonstrated a wide range of AHL production by the halotolerant plant-growth-promoting *Aeromonas* spp. strains SAL-17 and SAL-21, in which six are unique to the two strains being reported. Exogenous application of purified AHLs significantly increased the root morphology in wheat. Both strains showed the potential to colonize wheat roots and stimulate substantial growth under saline conditions in two different wheat genotypes. The inoculated plants showed higher proline contents, transpiration rate, stomatal conductance, chlorophyll contents, and nitrate reductase activity. The overall growth stimulation may be attributed to a synergistic response of the IAA, ACC-deaminase activity, and AHL production, of which the role of AHLs seems imperative. Future research involving AHL-deficient mutants, use of synthetic AHLs, and AHL-engineered plants will further validate the role of AHLs because, in this study, the comparison of inoculation results for the AHL-producing strains with a strain missing AHL production suggested their significant contribution toward salt stress mitigation and plant growth improvement. A comparison with phenotypically AHL-negative strain derivative using lactonase constructs would result in more direct evidence because the genetic background would be the same. This study concludes that multi-trait, non-pathogenic *Aeromonas* spp. strains are candidates of choice for the production of inoculum for saline soils. The strains should be used/released in the field only after implementation of biosafety parameters because some aeromonads have clinical significance. The study is of prime importance because 45 million hectares of salt-affected soil direly need an efficient solution for better cropping on a larger scale.

DATA AVAILABILITY STATEMENT

The data can be found at NCBI [SAL-17 (Accession No. HG763857) and SAL-21 (Accession No. HG763858)].

AUTHOR CONTRIBUTIONS

MN analyzed the data and wrote the manuscript. AA performed the AHL experiments as part of her MPhil research work. LR conducted the strain characterization and the pot experiments as part of her Ph.D. research. KF performed the confocal studies. SU helped in the AHL screening experiment. MA helped in the pot experiments. AI conceived and supervised the whole study and edited the manuscript. All the authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.553621/full#supplementary-material

Supplementary Figure 1 | Phylogenetic relationship of *Aeromonas* spp. Sal-12, Sal-17, and Sal-21 based on the sequences of 16S rRna along with closely related sequences obtained from GenBank. Boot strap value was 1,000, and

nucleotide sequence divergence is shown by a bar. The tree was generated in Mega6 using maximum likelihood method.

Supplementary Figure 2 | Grain yield response to proline contents and nitrate reductase activity as a function of bacterial inoculation in two different wheat genotypes grown in both saline and normal soil. Data from all treatments were jointly loaded on the graph to evaluate the overall response. The graph shows the quadratic relationship of grain yield to plant proline and nitrate reductase activity, with significantly high R^2 values.

Supplementary Table 1 | Occurrence and functional annotation of acyl homoserine lactones in spent culture supernatant of *Aeromonas* spp. reported in literature.

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Potential of Salt Tolerant PGPR in Growth and Yield Augmentation of Wheat (*Triticum aestivum* L.) Under Saline Conditions

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Soil salinity has emerged as a major obstacle to meet world food demands. Halo-tolerant plant growth promoting rhizobacteria (PGPR) are potential bioinoculants to enhance crop productivity in saline agriculture. Current work was aimed at studying individual or synergetic impact of salt tolerant PGPR on wheat growth and yield under saline conditions. A pot experiment was conducted on two wheat genotypes (Aas-11; salt tolerant and Galaxy-13; salt sensitive) inoculated with *Pseudomonas fluorescence, Bacillus pumilus,* and *Exiguobacterium aurantiacum* alone and in consortium. The salt tolerant variety (Aas-11) exhibited maximum root fresh (665.2%) and dry biomass (865%), free proline (138.12%) and total soluble proteins (155.9%) contents, CAT (41.7%) activity and shoot potassium uptake (81.08%) upon inoculation with *B. pumilus,* while improved shoot dry weight (70.39%), water (23.49%) and osmotic (29.65%) potential, POD (60.51%) activity, enhanced root potassium (286.36%) and shoot calcium (400%) were manifested by *E. aurantiacum.* Highest shoot length (14.38%), fresh weight (72.73%), potassium (29.7%) and calcium (400%) acquisition as well as glycinebetaine (270.31%) content were found in plants treated with PGPR consortium. On the other hand, in the salt sensitive variety (Galaxy-13), *P. fluorescens* treated plants showed significantly improved leaf-water relations, glycinebetaine (10.78%) content, shoot potassium (23.07%), root calcium (50%) uptake, and yield parameters, respectively. Plant root length (71.72%) and potassium content (113.39%), root and shoot fresh and dry biomass, turgor potential (231.02%) and free proline (317.2%) content were maximum upon PGPR inoculation in consortium. Overall, Aas-11 (salt tolerant variety) showed significantly better performance than Galaxy-13 (salt sensitive variety). This study recommends *B. pumilus* and *E. aurantiacum* for the salt tolerant (Aas-11) and *P. fluorescens* for the salt sensitive (Galaxy-13) varieties, as potential bioinoculants to augment their growth and yield through modulation of morpho-physiological and biochemical attributes under saline conditions.

Keywords: plant growth promoting rhizobacteria, salt tolerance, osmotically active compounds, salt stress, climate change

INTRODUCTION

Climate change, a hot topic of the current era, has affected planet earth in different ways and a rapid increase in saline landscapes is one of them that ultimately leads to global food insecurity and reduced agricultural productivity (Bharti et al., 2016). Around the globe, 20% of irrigated land is severely damaged by salt accumulation (Selvakumar et al., 2014). This land deterioration is expected to reach up to 50% by the year 2050 (Hossain, 2019). Almost 70% yield loss has been reported among cereal crops including wheat, rice, maize, and barley due to soil contamination by salinity and sodicity (Rajendran et al., 2009; Hussain et al., 2019).

So far, reclamation of such soils is being done by utilizing a variety of inorganic (gypsum, limestone, sulfuric acid and derivatives of sulfur, synthetic fertilizers), and organic (green and farm yard manure, industrial waste like press mud) measures (Qayyum et al., 2016). Similarly, Plant breeders and biotechnologists are in a constant struggle for the development of salt tolerant crop varieties either through natural selection, QTL mapping, marker assisted selection or by genetic manipulation via introduction of salt tolerant genes obtained from other organisms (Qadir et al., 2017). However, at field level, due to multiple factors, satisfactory outcomes have not been observed by such biological means for stress tolerance enhancement among the agro-economical significant crops (Khare et al., 2018).

Recently, exploitation of root adhering plant growth promoting rhizobacteria (PGPR) inhabiting hyper- saline conditions has gained attention as an alternative eco-friendly biological approach to get better crop productivity from salt deteriorating lands (Talaat, 2015). Improvement in plant growth aided by these microbes is well documented (Barnawal et al., 2012; Bharti et al., 2014). These halophilic/halotolerant plant growth-promoting rhizobacteria employ their key mechanisms by colonizing the plant rhizosphere to combat brutal environmental stresses and subsequent ruinous yield penalties. Strategies adopted by these microbes include *de novo* synthesis of osmolytes for cellular osmotic adjustment, regulation of ionic transporters and maintenance of homeostasis to reduce toxic effects of Na^+ and Cl^- ions, activation of reactive oxygen species scavenging defense system of plants to cope with deleterious effects of oxidative stress, respectively (Munns and Tester, 2008). Moreover, these microbes synthesize phytohormones, ACC deaminase, biological nitrogen fixation, siderophores, exopolysaccharides, volatile compounds and antifungal or antibacterial metabolites, mobilization of mineral ions, enhancement of photosynthesis, osmotic adjustment through accumulation of osmotically active metabolites like amino acids, sugars, polyols and betaines and detoxification of reactive oxygen species by antioxidants (Talaat and Shawky, 2013; Shabani and Sabzalian, 2016). Hence, these tiny creatures, by using different direct and indirect mechanisms, support plants to combat many biotic and abiotic challenges (Talaat, 2015; Subramanian et al., 2016).

By simple definition, salinity is a form of chemical (abiotic) factor that causes accumulation of soluble salts in the rhizospheric system. This condition adversely affects plant metabolism in two ways. Initially, high concentration of salts induces hyperosmotic and hyperionic situations which damage root architecture consequently leading to impaired water and nutritional acquisition. This eventually triggers secondary stress, i.e., oxidative stress, ultimately resulting in denaturation of DNA and proteins, and membrane instability due to lipid peroxidation. All these phenomena lead to programmed cell death and the collapse of the entire plant (Meloni et al., 2003; Kim et al., 2014).

Wheat (*Triticum aestivum* L.) is a staple food for 35% of the human population (Agriculture statistics of Pakistan 2017–19). Different plant species have acquired various ranges of stress tolerance. Some wheat varieties, for example, can sustain up to 10 dS/cm salinity level with minor yield losses and fall under the salt tolerant category (Munns et al., 2006). However, there is an immense need to enhance crop productivity up to 57% to meet ever increasing food demands by the year 2050 in parallel with continuous 1 to 2% land loss caused by salinity per year (Hossain, 2019).

It was hypothesized that PGPR residing in hyper-saline ecological conditions have the potential to modulate plant physiology by induced systemic tolerance to promote growth in salt degraded lands. Therefore, the present study was designed to evaluate the role of halotolerant PGP microbes by using two commonly used strategies (single strains and consortium) on two contrasting genotypes of wheat plant (salt tolerant and salt susceptible) and parameters used to investigate their role in plant growth and yield improvement include morpho-physiological and biochemical characteristics. This study will be helpful to explore the potential of native salt tolerant strains of PGPR and further their utilization as biofertilizer for wheat crop to minimize yield losses due to salt stress. In the future, this could lead to developing an effective bioformulation for such problematic soils.

MATERIALS AND METHODS

Experimental Area and Materials

This experimental work was conducted in the greenhouse, located at University of Agriculture, Faisalabad, Pakistan during wheat growing season November 2015–April 2016. Seeds of wheat (salt tolerant variety; Aas-11 and salt susceptible variety; Galaxy-13) were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan. Three pre-characterized halotolerant PGPR strains, *Pseudomonas fluorescens* (Accession # KX644132), *Bacillus pumilus* (Accession # KX580768) and *Exiguobacterium aurantiacum* (Accession # KX580769) were collected from NBRC, Microbial Physiology Lab, SEBD, NIBGE, Faisalabad. The salt tolerance profiling on the basis of minimal inhibitory concentration and PGP characteristics (phosphate solubilization, IAA production and ACC metabolism) of these strains were assessed (qualitatively and quantitatively) based on selective media in a previous study (Ullah, 2019). Ullah and Bano (2019) demonstrated that these PGPR have significantly improved the growth and yield of maize grown in saline sodic soil as well physico-chemical properties of soil. Compatibility of these strains was also assessed using a cross streak method (Semenov et al., 2007) on NaCl supplemented LB-medium prior

to seed inoculation. Pure saline soil used for the experiment was brought from field area of Biosaline Research Station, Pakka Anna, Faisalabad, Pakistan. The soil contained EC_e: 13.41, pH: 9.1, organic matter: 1.39%, available nitrogen: 1.4 mg kg^{-1}, available phosphorus: 19.6 mg kg^{-1}, extractable potassium: 2.1 mg kg^{-1}, sodium: 55 mg kg^{-1}, chloride: 999.96 mg kg^{-1} and soil texture was clay loam.

Seed Sterilization and Inoculation

Seeds were surface sterilized with 10% sodium hypochlorite solution and subsequent washing with autoclaved distilled water prior to inoculation with PGPR (Cheng et al., 1997). Inocula were prepared by transferring an 24 h old bacterial colony into Luria Bertani broth, kept on shaker at 120 rpm overnight at 28°C, centrifuged to get pellets, re-suspended in distilled water and optical density (at 660 nm) was adjusted to be 1, which was equal to 10^{-6} cells/ml. Then, surface sterilized wheat seeds were soaked in bacterial inocula containing single PGPR strains, and the consortium of three bacterial cultures for 2 h, while for control treatment seeds were soaked in autoclaved distilled water, respectively. Seeds were sown in plastic pots each containing 8.5 kg sterilized soil. Sowing was done at the rate of 20 seeds pot^{-1} at the depth of 1.5 inch. Thinning was done at the two-leaf stage up to 10 plants per pot^{-1}. Irrigation was carried out with tap water (pH 7) following sufficient intervals. The experiment was based on completely randomized factorial design comprised of five treatments for each variety with five replications (**Table 1**). Data was recorded 70 days after sowing at vegetative stage to evaluate the impact of PGPR on growth and physiochemical attributes. Yield data was collected at crop maturity level.

Morpho-Physiological and Biochemical Analysis of Plants

Morphological Parameters of Plant

Data regarding root and shoot length of randomly selected plants was recorded right after sampling by using a field meter rod. Fresh weight of root and shoot was measured by electric balance. Dry biomass of 7 days oven dried samples at 115°C was recorded using the same electric balance.

Physiological Parameters of Plant

Water relation attributes

Water potential. Leaf-water potential was measured according to the method of Scholander et al. (1964). For that purpose, a fully expanded young leaf was excised from each plant. At dawn leaf water potential was measured by using Scholander type pressure chamber (Arimad-2-Japan).

Osmotic potential. The same leaves were used for osmotic potential determination and stored in a freezer at −20°C for at least 7 days. After 7 days, the sap was extracted by pressing them with glass rod. The sap was placed on an osmometer [Wescor Vapor Pressure Osmometer (Model VAPRO 5520, El Cajon, CA, United States)] for the measurement of solute potential (Ball and Oosterhuis, 2005).

Turgor potential. Turgor potential was calculated as the difference between water potential and osmotic potential by following the equation as cited by Nobel (1999).

$$\Psi p = \Psi w - \Psi s$$

TABLE 1 | Description of experimental components (treatments).

Notations	Treatments
T_1	Seed soaked in autoclaves distilled water (control or uninoculated)
T_2	Seeds inoculated with *P. fluorescens*
T_3	Seeds inoculated with *B. pumilus*
T_4	Seeds inoculated with *E. aurantiacum*
T_5	Seeds inoculated with consortium (3 bacterial strains used in T_2, T_3, and T_4)

Biochemical Parameters of Plant

Estimation of Total Soluble Proteins

The soluble proteins of the samples were determined by the Bradford method (Bradford, 1976). To extract protein, 0.25 g fresh leaves were grinded using a tissue grinder in 5 ml of 50 mM cooled phosphate buffer (pH 7.8) placed in an ice bath. The homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant was used for protein determination. Each sample, 100 μl, was taken in an Eppendorf tube and mixed with 1.0 ml of Bradford reagent. These sample solutions were incubated at 37°C for 10–15 min along with the blank and absorbance was noted at 595 nm using a spectrophotometer (IRMECO U2020).

Determination of Antioxidant Enzymatic Activity

Enzyme extraction

Enzymatic antioxidants of wheat plants were extracted by grinding 0.25 g of fresh leaf material in 5 ml of 50 mM cooled potassium phosphate buffer (pH 7.8). This homogenized material was centrifuged at 10000 × *g* for 22 min at 4°C. The pellet was discarded and the supernatant was used for the estimation of activities of different antioxidants enzymes.

Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) was determined by monitoring its potential to cause inhibition in the photo reduction of nitro blue tetrazolium chloride (NBT) by following the procedure given by Giannoplitis and Ries (1977). The reaction mixture (3 ml of 50 mM potassium phosphate buffer (pH 7.8), 75 mM MEDTA, 13 mM methionine, 50 μM NBT, riboflavin, 1.3 μM) and 50 μl enzyme extract for the detection of enzyme activity. The tubes with reaction mixture lacking enzyme extract were used as control. Then these tubes were placed under fluorescent lamp (30 W) for 10 min, lamp was turned off and absorbance of mixture was recorded at 560 nm using a spectrophotometer (IRMECO U2020). One unit of enzyme was taken as the amount of enzyme used to cause 50% inhibition in the photochemical reduction of NBT.

Catalase (CAT) and peroxidase (POD)

The activities of catalase (CAT) and peroxidase (POD) were evaluated according to the procedure given by

Chance and Maehly (1955) with some minor modifications. The reaction mixture (3 ml) for CAT contains 5.9 mM H_2O_2, 50 mM potassium phosphate buffer (pH 7.8). To initiate the reaction 0.1 ml of the enzyme extract was added the above prepared mixture. The decrease in absorbance was read at 240 nm at every 20 s interval. One unit of CAT was taken as absorbance change of 0.01 units per min. The POD reaction mixture (3 ml) contained 40 mM H_2O_2, 20 mM guaiacol, 50 mM potassium phosphate buffer (pH 7.8) and 100 µl of enzyme extract. Then the change in absorbance at 470 nm was monitored after every 20 s. One unit of POD activity was defined as the change of 0.01 absorbance unit per min per mg of protein.

Determination of Organic Compatible Solutes

Proline Determination

Free proline content was determined by using the protocol described by Bates et al. (1973). The third leaf from the top (0.25 g) was homogenized in 5 ml of 3% aqueous sulfosalicylic acid and homogenate was filtered through Whatman No. 2 filter paper. One ml of filtrate was taken and mixed with 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid) and 1 ml of glacial acetic acid in a test tube. The mixture was vortexed shortly and heated at 100°C in a water bath for 1 h and then the reaction was terminated in the ice bath. 2 ml of toluene was added to the solution and vortexed for 15–20 s while it cooled. The chromophore containing proline was extracted from the aqueous phase in a test tube and warmed to laboratory temperature. The absorbance was taken at 520 nm using spectrophotometer (U2020 IRMECO).

Glycine Betaine

Grieve and Grattan (1983) method was followed for determination of glycine betaine content in leaf tissues. Briefly, 0.25 g dry material was homogenized with 5 ml of 0.5% toluene solution. Extract was centrifuged. 1 ml extract and 1 ml of 2N H_2SO_4 was mixed and 0.5 ml of this extract was taken in a separate test tube. 200 µL potassium tri-iodide was added in this extract and the test tube left in ice for 90 min. 2.8 ml distilled water was added then followed by addition of 6 ml 1,2-Dichloroethane. Upper layer was discarded and red lower layer was taken for reading at 365 nm.

Nutrient Analysis of Plant Roots and Shoots

Digestion Method

To carry out plant mineral analysis, (which includes plant material digestion and mineral content determination) a method described by Allen et al. (1985) was followed, i.e., the dried (0.1 g) plant material was grinded well and placed in the 50ml flasks containingH_2SO_4. The mixture was boiled on a hot plate under a fume hood until digestion was completed which was indicated by the presence of white fumes in the flasks. Upon cooling, 50 ml distilled water was added and mixture was filtered by using Whatman paper # 42. Filtrate was further used for the determination of mineral nutrients.

Determination of Na^+, K^+, and Ca^{2+}

Root and shoot sodium (Na^+) potassium (K^+) and calcium (Ca^{2+}) were determined by using a flame photometer (Jenway, PFP-7, United Kingdom).

Yield Parameters

At maturity, crop was harvested and data regarding spikes length per plant, number of spikelets per spike and 100 grains weight was recorded.

Statistical Analysis

The data was analyzed using Statistix version. 8.1. An ANOVA (two-way) was performed to analyze the effect of treatments and errors associated with the experiment. Further, LSD ($p = 0.05$) test was used to identify significant difference among treatments means.

RESULTS

Morphological Plant Attributes

Effects of different treatments (single strains and consortium) on root length were recorded on both wheat varieties. However, the salt tolerant variety; Aas-11, showed substantial increase compared to the salt sensitive variety; Galaxy-13. Seeds of variety Aas-11, bio-primed with *P. fluorescens* exhibited significant increase (28.59%) in root length followed by *B. pumilus* (26.22%) as compared to un-inoculated control plants. Plants inoculated with *E. aurantiacum* (T_4) and consortium of PGPR (T_5) expressed a non-significant increase in root length compared to untreated control plants. On the other hand, in variety Galaxy-13, all treatments exhibited significant increase except T_2. The highest increase was recorded in T_5 (71.72%) followed by T_3 (67.35%) and T_4 (39.33%), respectively (**Figure 1A**).

Shoot length was significantly increased upon inoculation with PGPR in variety Aas-11; consortium of PGPR (T_5) represented maximum (14.36%) impact followed by T_3 = 13.94%, T_2 = 11.96% and T_4 = 10.44%. In contrast to it, variety Galaxy-13, experienced variable effects on length of plant shoot like T_4 (5.72%) followed by T_2 (2.90%) exhibited maximum increase but T_5 (consortium of PGPR) and T_3 (*B. pumilus*) showed reduced shoot length than control pants (**Figure 1B**).

Root fresh and dry weights were also significantly increased among all treatments in the salt tolerant variety where T_3 (0.704 and 0.386 g) showed highest increase followed by T_5 (0.584 and 0.342 g), T_4 (0.54 and 0.334 g) and T_2 (0.244 and 0.068 g), respectively, as compared to un-inoculated control plants (T_1 = 0.092 and 0.04 g). However, in Galaxy-13, only the combined application of PGPR manifested a significant increase in root fresh (30.72%) and dry (66.66%) weight than T_1 (control). All other treatments showed decreased values ranging between (6 to 15%) except T_3 (*B. pumilus*) where a slight increase was observed in root fresh weight. On the other hand, root dry weight was decreased in following manner $T_2 > T_4 > T_3$, in contrast with control plants (**Figures 1C,E**).

Just like root fresh weight, PGPR inoculation imposed a significant positive impact on shoot fresh weight in

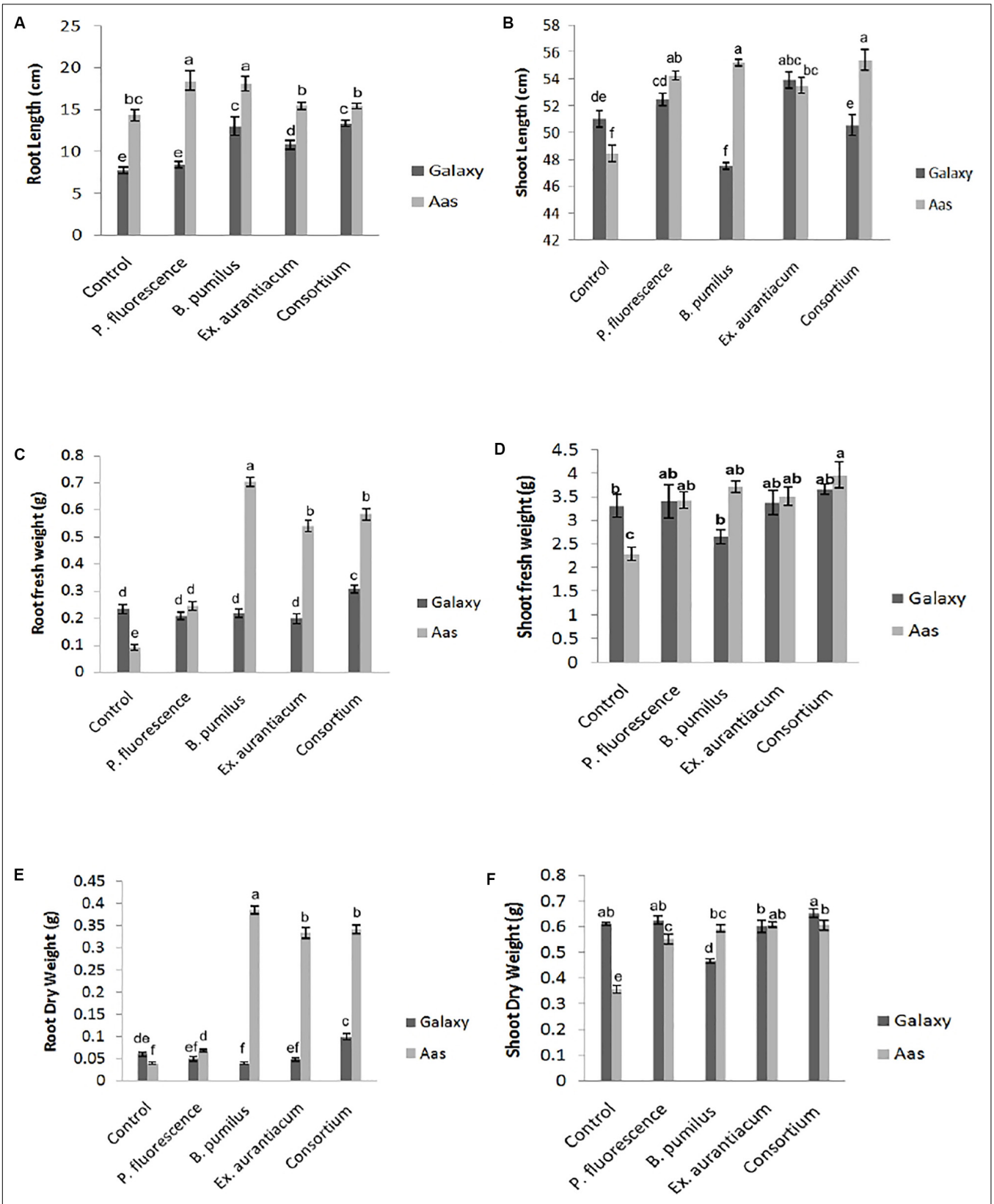

FIGURE 1 | Effect of salt tolerant PGPR on growth attributes of two contrasting wheat varieties under saline condition. **(A)** Root length. **(B)** Shoot length. **(C)** Root fresh weight. **(D)** Shoot fresh weight. **(E)** Root dry weight. **(F)** Shoot dry weight.

Aas-11. T_5 (72.73%) expressed highest values for shoot fresh weight followed by T_3 (*B. pumilus* = 61.84%) > T_4 (*E. aurantiacum* = 52.96%) > T_2. (*P. fluorescens* = 49.39%) In the case of Galaxy-13, none of the treatments showed a significant increase but T_5 (consortium of PGPR = 10.36%) showed maximum increase followed by T_2 (2.83%) and T_4 (1.80%) as compared to untreated control plants while T_3 (19.95% decrease) showed the least value (**Figure 1D**).

Shoot dry weight was significantly increased among all PGPR treated plants such as T_4 (*E. aurantiacum* = 70.39%) followed by T_5 (PGPR consortium = 69.83%), T_3 (*B. pumilus* = 65.92%) and T_4 = 54.74% in salt tolerant variety (Aas-11) but in case of salt sensitive variety, this improvement was observed in T_5 (6.86%) followed by T_2 (2.61%) then control. However, T_4 (*E. aurantiacum*) treated plants exhibited reduced value for shoot dry weight. *B. pumilus* (T_3) inoculated plants showed significant reduction in value (**Figure 1F**). The variations in dry and fresh weight of different treatments depend on many physiological and environmental conditions.

TABLE 2 | Mean values of physiological attributes of two contrasting wheat genotypes inoculated with salt tolerant PGPR under saline condition.

Galaxy-13 variety	WP	OP	TP
T_1	-1.57^{a}	-1.62^{a}	0.04^{cd}
T_2	-1.23^{de}	-1.26^{bc}	0.02^{d}
T_3	-1.28^{bcd}	-1.33^{b}	0.05^{cd}
T_4	-1.25^{cd}	-1.36^{b}	0.11^{ab}
T_5	-1.36^{bc}	-1.51^{a}	0.15^{a}
Aas-11 variety			
T_1	-1.38^{b}	-1.54^{a}	0.158^{a}
T_2	-1.24^{d}	-1.33^{b}	0.092^{bc}
T_3	-1.11^{ef}	-1.17^{cd}	0.059^{cd}
T_4	-1.06^{f}	-1.08^{d}	0.026^{d}
T_5	-1.12^{ef}	-1.1^{cd}	0.068^{cd}

WP, water potential (–MPa); OP, osmotic potential (–MPa), TP, turgor potential (MPa), T_1, control; T_2, P. fluorescens; T_3, B. pumilus; T_4, E. aurantiacum; T_5, consortium. Different letters followed by mean values are significant ($p < 0.05$). Values with same letters are non-significant ($p = 0.05$).

Physiological Plant Attributes

Water Relations in Plant

Water and osmotic potential were significantly improved in the salt tolerant variety (Aas-11). Maximum improvement was observed in plants inoculated with *E. aurantiacum* (T_4 = 29.43% decrease). Other treatments represented the improved trend (19.52 to 10.36%) as follows; T_3 > T_5 and T_2 in contrast with un-treated control plants. However, turgor potential was significantly lower among plants which received *P. fluorescens* (T_2) as inoculation with gradual increase in T_5, T_3, and T_4 as compared with un-inoculated plants. In the case of the salt sensitive variety (Galaxy-13), water and osmotic potential of PGPR treated plants were significantly improved except T_5 (6.84%) which showed non-significant improvement in osmotic potential. T_2 (*P. fluorescens*) treated plants exhibited the highest (22.09%) improved water potential followed by T_4 (20.82%), T_3 (18.925%), and T_5 (13.85%) compared to control. whereas osmotic potential was highly improved in T_2 followed by T_3 (*B. pumilus*) and T_4 (*E. aurantiacum*), respectively. Turgor potential was maximum in T_5 (231.01%) followed by T_4 while T_3 (141 to 43%) results were at par with the control value. However, T_2 showed a slight decrease compared to control (**Table 2**).

Biochemical Plant Attributes

Accumulation of Osmotically Active Metabolites

Information about the effects of PGPR application on different biochemical attributes is presented in **Figure 2**. PGPR application caused a significant impact on the accumulation of free proline, glycine betaine and total soluble contents in the salt tolerant variety, i.e., Aas-11. However, effect of *E. aurantiacum* (T_4) on glycine betaine accumulation remained non-significant. Free proline content was observed to be maximum among *B. pumilus* (T_3 = 73.78 μmol g^{-1}) treated plants followed by *E. aurantiacum* (T_4 = 62.29 μmol g^{-1}), *P. fluorescens* (T_2 = 40.25 μmol g^{-1}) and PGPR consortium (T_5 = 32.85 μmol g^{-1}), respectively (**Figure 2A**). The highest increase in glycine betaine content was recorded in T_5 (55.21 μmol g^{-1}) plants followed by T_3 (52.06 μmol g^{-1}) and T_2 (49.48 μmol g^{-1}) compared to the non-treated control (44.66 μmol g^{-1}) plants (**Figure 2B**). Moreover, the total soluble proteins were increased in T_3 (3.39 *unit*/mg protein) followed by T_4 (3.37 *unit*/mg protein), T_5 (3.27 *unit*/mg protein) and T_2 (2.89 *unit*/mg protein) as compared to un-inoculated control (1.32 *unit*/mg protein) plants (**Figure 2C**). Examining the salt sensitive variety, i.e., Galaxy-13, free proline content was significantly enhanced in all treatments such as T_5, T_3, and T_4 (63.81–48.89 μmol g^{-1}) but T_2 treated plants represented a decrease in its level. However, it differed non-significantly to the control plants. While the accumulation of glycine betaine was significantly high in T_5 (63.81 μmol g^{-1}) followed by T_3, T_2, and T_4 which ranged between 52.06 and 45.9 μmol g^{-1}, respectively (**Figure 2B**). However, only *B. pumilus* (T_3 = 3.68 *unit*/mg protein) manipulated a significant increase in total soluble proteins content in contrast with un-treated control plants while the rest of the treatments showed a non-significant increase (10.69 to 0.78%) in their content which is as follow, T_5 > T_4 and T_2 (**Figure 2C**).

Determination of Antioxidant Enzymatic Activity

All treatments imposed their substantial impact on activities of antioxidant enzymes in the salt tolerant variety (**Figure 3**). Significant reduction in activity of superoxide dismutase was observed among all treatments (**Figure 3A**). The least activity was recorded in T_3 (*B. pumilus* = 3.744 *unit*/mg protein) with gradual increase in T_4 (*E. aurantiacum* = 3.834 *unit*/mg protein) < T_5 (Consortium = 4.958 *unit*/mg protein) < T_2 (*P. fluorescens* = 5.070 *unit*/mg protein). A variable effect on the activity of peroxidase was observed among all PGPR treated plants. (**Figure 3B**) i.e., least activity was observed in T_4 (0.834 *unit*/mg protein) with gradual increase among T_3 < T_2 (1.314 and 1.826 *unit*/mg protein) which is lower than control (T_1 = 2.113 *unit*/mg protein) plants. However, peroxidase activity in T_5 (PGPR consortium = 2.574 *unit*/mg protein) inoculated plants, significantly higher than un-inoculated control. Catalase

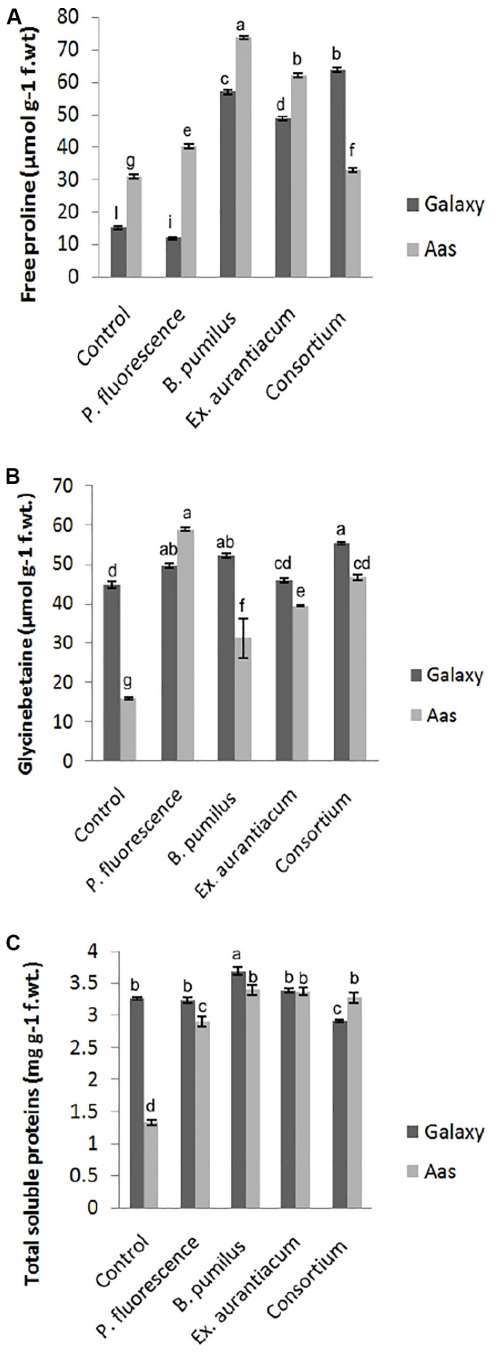

FIGURE 2 | Effect of salt tolerant PGPR on accumulation of osmotically active metabolites of two contrasting wheat varieties under saline conditions. **(A)** Free proline content. **(B)** Glycine betaine content. **(C)** Total soluble proteins content.

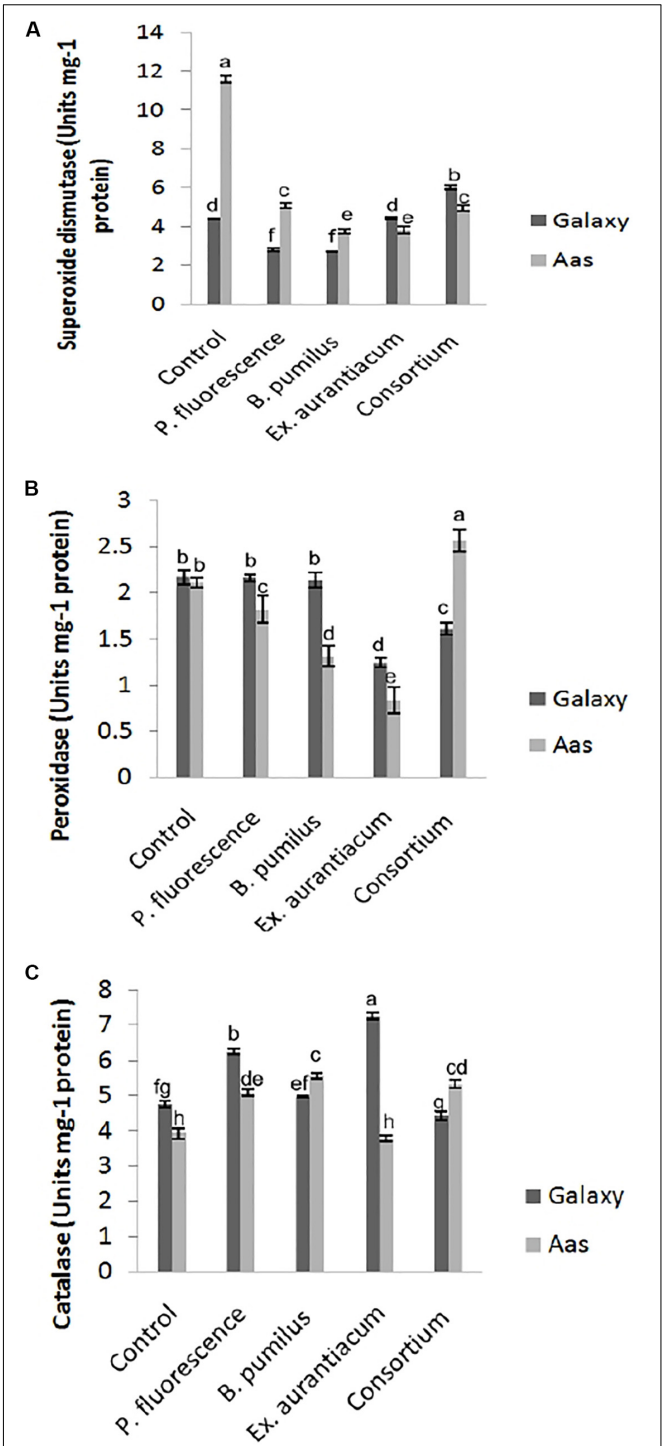

FIGURE 3 | Effect of salt tolerant PGPR on the activity of enzymatic antioxidants of two contrasting wheat varieties under saline conditions. **(A)** Superoxide dismutase activity. **(B)** Peroxidase activity. **(C)** Catalase activity.

activity was non-significantly increased in $T_3 > T_5 > T_2$. However, plants treated with *E. aurantiacum* ($T_4 = 3.799$ *unit*/mg protein) expressed reduced activity in comparison with un-inoculated control plants (**Figure 3C**).

On the other hand, in the case of the salt sensitive variety (Galaxy-13), a significant decrease in superoxide dismutase activity was recorded in *B. pumilus* ($T_3 = 2.695$ *unit*/mg protein) with a gradual increase in *P. fluorescens* ($T_2 = 2.79$ *unit*/mg protein) while no change was observed in *E. aurantiacum* ($T_4 = 4.42$ *unit*/mg protein) treated plants (**Figure 3A**). Enhanced

activity was found in only T_5 (PGPR consortium = 6.040 *unit*/mg protein) treated plants. Peroxidase activity was significantly lower in T_4 (1.242 *unit*/mg protein) treated with gradual increase in T_5 (1.609 *unit*/mg protein) plant leaves which differed significantly with other treatments. Non-significant reduction in peroxidase enzyme activity was observed among $T_2 > T_3$ as compared with control plants (**Figure 3B**). Catalase activity was significantly enhanced among *E. aurantiacum* inoculated plants T_4 = 7.262 *unit*/mg protein followed by T_2 = 6.24 *unit*/mg protein treated plants while T_3 treated plants exhibited non-significant increase in its value. However, T_5 plants, whose seeds were bioprimed with PGPR consortium, showed 6.86% reduced activity compared to control plants but the difference was not significant (**Figure 3C**).

Analysis of Mineral Nutrients in Plant Roots and Shoots

Variable results on nutrient acquisition were recorded upon PGPR application among both varieties (**Table 3**). Sodium content in plant roots remained non-significant in all treatments of the salt tolerant variety (Aas-11). All treatments showed a slightly reduced sodium uptake compared to the control except the T_5 (PGPR consortium) treated plant, which showed no change in its value. On the other hand, in the salt sensitive variety (Galaxy-13), a slight increase (11 and 29%) in its acquisition was observed in T_2 (*P. fluorescens*) and T_4 (*E. aurantiacum*) inoculated plants while the effect of rest of the treatments remained un-changed as compared to control plants.

Shoot sodium acquisition was significantly increased among both varieties upon PGPR inoculation. Where T_2 showed maximum sodium uptake followed by T_5, T_3, and T_4. Similarly, significant results were recorded in Galaxy-13, where the maximum value was represented in T_2 followed by T_4 and T_5 plants. However, T_3 (*B. pumilus*) showed slight low (8.16%) sodium uptake compared to control.

TABLE 3 | Mean values of mineral nutritional attributes of two contrasting wheat genotypes inoculated with salt tolerant PGPR under saline condition.

Galaxy-13 variety	R Na^+	S Na^+	R K^+	S K^+	R Ca^{2+}	S Ca^{2+}
T_1	0.60^{a}	9.8^{de}	3.37^{de}	2.60^{de}	0.6^{a}	2.25^{c}
T_2	0.90^{a}	12.5^{b}	4.70^{c}	3.20^{cd}	0.9^{a}	2.20^{c}
T_3	0.60^{a}	9.0^{ef}	4.50^{cd}	2.10^{e}	0.6^{a}	1.4^{d}
T_4	0.80^{a}	12.4^{b}	4.50^{cd}	2.60^{de}	0.8^{a}	2.62^{c}
T_5	0.60^{a}	11.4^{bc}	7.20^{b}	3.00^{cd}	0.6^{a}	1.40^{d}
Aas-11 variety						
T_1	0.80^{a}	7.6^{f}	2.20^{e}	3.70^{bc}	0.8^{a}	0.90^{d}
T_2	0.70^{a}	14.6^{a}	4.37^{cd}	4.30^{ab}	0.7^{a}	3.50^{b}
T_3	0.70^{a}	12.1^{b}	6.50^{b}	3.30^{cd}	0.7^{a}	3.75^{b}
T_4	0.70^{a}	10.6^{cd}	8.50^{a}	4.5^{a}	0.7^{a}	4.50^{a}
T_5	0.80^{a}	12.8^{b}	6.00^{b}	4.80^{a}	0.8^{a}	4.50^{a}

R Na^+, root sodium (mg g^{-1} dry wt.); S Na^+, shoot sodium (mg g^{-1} dry wt.); R K^+, root potassium (mg g^{-1} dry wt.); S K^+, shoot potassium (mg g^{-1} dry wt.); R Ca^{2+}, root calcium (mg g^{-1} dry wt.); S Ca^{2+}, shoot calcium (mg g^{-1} dry wt.); T_1, control; T_2, P. fluorescens; T_3, B. pumilus; T_4, E. aurantiacum; T_5, consortium. Different letters followed by mean values are significant ($p < 0.05$). Values with same letters are non-significant ($p < 0.05$).

In variety, Aas-11, maximum potassium content in root tissues was recorded in T_4 (286.36%) followed by $T_3 > T_5 > T_2$ (195.45–98.28%) treated plants. On the other hand, highest amount of potassium in plant shoots was found in T_5 (29.72%) followed by T_4 (21.62%) and T_2 (16.21%) as compared with un-treated control plants. Collectively, root and shoot potassium uptake was significantly increased in the salt tolerant variety upon PGPR inoculation except T_3 which exhibited only a slight decrease in shoot potassium content compared to the control plants. In the case of salt susceptible variety, T_5 (PGPR consortium = 113.3%) followed by T_2 (*P. fluorescens*) treated plants exhibit significant increase in root potassium content while this increase remained non-significant in T_4 and T_3 treated plants. Variable impact on potassium uptake by plant shoots was observed among all treatments where T_2 (23.07%) followed by T_5 (15.38%) showed a non-significant increase in value. While its content among *E. aurantiacum* inoculated plants remained similar to control, however, T_3 exhibited slight reduced potassium value.

PGPR imposed non-significant impact on root calcium content in both varieties. PGPR consortium showed similar values to control but rest of treatments exhibited slight (12.5%) decrease in root calcium pool in salt tolerant variety. However, in the salt sensitive variety, maximum root calcium was recorded in T_2 (50%) followed by T_4 (33%) treated plants than control but the rest of the two treatments showed no significant change in its value as compared with un-treated control plants.

Calcium content in plant shoots was substantially increased among all treatments in Aas-11 where values for T_5 and T_4 were equal (400% increase) and followed by T_3 and T_2 (316 and 288%). On the other hand, in Galaxy-13, only T_5 and T_3 (37.78% increase) treated plants showed a significant increase in calcium content while T_4 was at par with control plants. However, T_2 showed a slight decrease in its value compared to the control.

Yield Attributes of Plant

Yield contributing components including spike length, number of spikelets per spike and 100 grains weight were substantially increased upon PGPR seed inoculation either as single strain or consortium (**Table 4**). Maximum values were recorded in plants treated with *B. pumilus* followed by PGPR consortium (T_5) and *E. aurantiacum* (T_4) in salt tolerant variety. However, impact of *P. fluorescens* inoculation was only evident in increasing 100 grains weight. Whereas in salt susceptible variety; *Galaxy-13, P. fluorescens* inoculation exhibited maximum values among all yield contributing components, followed by T_5 and T_4. While the effect of *B. pumilus* inoculation was at par with un-inoculated control plants.

DISCUSSION

Soil salinity is a prevalent environmental restraint to agriculture productivity and food security. Salt stress is responsible for 20–50% yield losses of important agricultural commodities including wheat, rice and maize around the world (Subiramani et al., 2020). So, there is a pressing need to adapt new sustainable approaches in addition to the use of organic or inorganic soil

TABLE 4 | Mean values of yield attributes of two contrasting wheat genotypes inoculated with halophilic PGPR under saline condition.

Galaxy-13 variety	Spike length	No. of spikelets spike^{-1}	100 G. wt
T_1	6.84^{f}	21.4^{d}	3.46^{d}
T_2	12.72^{ab}	37.8^{ab}	4.6^{bc}
T_3	7.64^{ef}	24.6^{cd}	4.52^{c}
T_4	9.30^{d}	27.8^{C}	5.16^{ab}
T_5	11.02^{c}	34.8^{b}	5.30^{a}
Aas-11 variety			
T_1	8.28^{de}	25.8^{C}	3.56^{d}
T_2	8.72^{de}	26.8^{C}	4.38^{c}
T_3	13.60^{a}	40.2^{a}	5.46^{a}
T_4	12.28^{b}	34.8^{b}	5.24^{a}
T_5	13.46^{ab}	39.8^{a}	5.28^{a}

Spike length (cm), No. of spikelets spike^{-1}; 100 G. wt, 100 grains weight (g); T_1, control; T_2, P. fluorescens; T_3, B. pumilus; T_4, E. aurantiacum; T_5, consortium. Different letters followed by mean values are significant ($p < 0.05$). Values with same letters are non-significant ($p < 0.05$).

amendments along with salt resistant crop varieties to improve the productivity of such problematic soils (Egamberdieva et al., 2019). Exploitation of salt tolerant PGPR has recently emerged as an effective strategy to handle aforesaid situation (Grover et al., 2011). These halophilic and halotolerant microorganisms are capable of being sustained in hyper-saline habitats (Talaat, 2018). Main mechanisms responsible for their survival under stressful environment include *de novo* synthesis or uptake of osmoprotectants and specialized ion transport systems like Na^+/H^+ antiporters, respectively (Egamberdieva et al., 2019). Bacterial species belonging to genera Pseudomonas, *Bacillus, Enterobacter, Agrobacterium, Streptomyces, Klebsiella,* and *Ochromobacter* have been extensively reported as efficient bio-inoculants in saline agriculture (Sharma et al., 2016; Singh and Jha, 2016; Sarkar et al., 2018).

The main focus of this study was to investigate the potential impact of salt tolerant PGPR on wheat growth and yield enhancement through modulation of morpho-physiological and biochemical mechanisms. For this purpose, three PGPR strains were used. A strain of *P. fluorescens* was isolated from roots of maize plant grown in non-saline habitat. The other two microbes, *B. pumilus* and *E. aurantiacum* were isolated from roots of wheat, grown in Khewra salt range (Ullah, 2019). The salt tolerant potential of the latter two PGPR strains was higher than the first (Ullah, 2019).

Previous studies (Ansari et al., 2019; Xie et al., 2019) have demonstrated the significant contribution of several bacterial sp. belonging to *Pseudomonas*, *Bacillus*, and *Exiguobacterium* (Kasana and Pandey, 2018) genera in plant growth promotion under growth limiting conditions.

Safari et al. (2018) reported the enhanced salt tolerance index and substantial increase in germination percentage and seedling vigor in wheat upon inoculation with *P. fluorescens* under NaCl induced salinity. A similar response on barley growth and yield (spike length, fertility index and grains weight) parameters was described by Azadikhah et al. (2019) when treated with ACC deaminase producing *P. fluorescens* under salt stress. Nadeem et al. (2016) claimed that plant growth promotion via treatment with ACCD, IAA, and siderophore producing *P. fluorescens* is directly related to its better colonizing ability in plant rhizosphere. A number of studies on the potential of *Bacillus* sp. have also been documented in literature. Din et al. (2019) reported that bacterial strains belonging to *Bacillus* genus showed substantial role in salt stress alleviation in wheat due to their capability to produce EPS, ACCD and IAA production *in vitro*. Xie et al. (2019) and Ansari et al. (2019) documented that the supportive role in salt stress alleviation and wheat growth improvement revealed by *B. pumilus* inoculation was related to escalated levels of photosynthesis, transpiration and proline accumulation as well as reduced antioxidant levels. Bacillus strains resistant to salt stress contributed to improve K^+ and Ca^{2+} acquisition and enhancement of protein and nitrogen content in rice seedlings grown under salt stress (Khattak et al., 2019). However, only limited data is available regarding the PGP activities showed by *E. aurantiacum* (Ullah and Bano, 2019). Strahsburger et al. (2018) reported the draft genome of *E. aurantiacum* strain PN47, data obtained from this study confirmed its adaptive features in hyper-osmotic and alkaline environment.

Our findings are concomitant with the previously reported literature that the application of salt tolerant PGPR strains *P. fluorescens*, *B. pumilus*, and *E. aurantiacum* substantially increased the growth and yield of wheat crop grown under saline conditions. It may be due to the fact that these PGPR were able to metabolize ACC deaminase, solubilize insoluble phosphate minerals and produce a significant quantity of IAA (Ullah and Bano, 2019). However, in our study, *B. pumilus* (T_3) and *E. aurantiacum* (T_4) showed promising results in the case of the salt tolerant variety; Aas-11 while the salt susceptible variety; Galaxy-13 performed more effectively upon inoculation with *P. fluorescens* (T_2) and synergetic behavior of inoculated PGPR strains was quiet eminent too (T_5). This variant response of inocula can be regarded as the PGPR colonization potential in rhizosphere varies with plant genotype, species, and developmental stage etc. (Delaplace et al., 2015; Poli et al., 2016; Wintermans et al., 2016) as well as physio-chemical characteristics of surrounding soil and nutrition competition among microbial communities. Basically, phytomicrobiome development is recruited by the plant itself which excretes various type of root exudates (Chaparro et al., 2012; Trabelsi and Mhamdi, 2013) and this interaction is regulated at biochemical and genetic level through signal transduction (Nelson and Sadowsky, 2015; Massalha et al., 2017; Smith et al., 2017).

In the current experiment, *B. pumilus* (characterized for *in vitro* PGP potential) inoculated plants (variety; Aas-11) showed maximum root fresh and dry weight, accumulation of free proline and total soluble proteins contents in leaf tissues along with reduced activity of enzymatic antioxidants activity. However, reduced activity of antioxidant is an evident feature of PGPR induced modulation of plant physiology that resulted in reduced ROS contents (Singh et al., 2016). *E. aurantiacum* showed the highest shoot dry weight and improved water and osmotic potential. Maximum Ion (K^+ and Ca^{2+}) acquisition

by root tissues and decreased level of shoot sodium content suggest the efficacy of this PGPR strain in regulation of plant ion transporters to inhibit sodium uptake and promotion of potassium and calcium uptake by plant (Strahsburger et al., 2018). Combined application of PGPR (T_5) exhibited an increase in shoot length, fresh weight, K^+ and Ca^{2+} amount and glycine betaine content. Here, the synergetic role manipulated by PGPR was quite evident in the mitigation of ionic toxicity upon exposure to salt stress and resulted in increased growth and yield of wheat plants. A significant difference in shoot length and turgor potential was noted in *P. fluorescens* treated plants as compared to un-inoculated control plants.

On the other hand, if we look at salt sensitive variety; Galaxy-13, the effect of *P. fluorescens* on improved water relations, osmolyte (glycine betaine) accumulation, reduced activity of superoxide dismutase and elevated levels of shoot potassium and root calcium contents were recorded as compared to control plants. The implication of PGPR consortium (T_5) showed improved root/shoot growth parameter, increased root potassium content and maximum amount of proline content accumulation. While shoot length and calcium content were maximum in *E. aurantiacum* treated plants in comparison with un-treated control plants. Whereas total soluble protein content was highest in *B. pumilus* inoculated plant tissues.

The above-mentioned outcomes revealed the supportive role of inoculated PGPR strains in growth and yield enhancement of wheat crop under salt stressed conditions. It also suggests that IAA producing bacteria accelerated the modulation of the plant's morpho(growth parameters)-physiological (water relations) characteristics (Emami et al., 2019) and biochemical (osmolytes accumulation and reduced activity of enzymatic antioxidants) attributes. Moreover, inoculation assisted the plant to maintain nutrition balance via increased K^+ and Ca^{2+} uptake and reduced sodium ion acquisition. Hence, PGPR improved the yield of wheat crop planted under stressful conditions.

These results are consistent with the previous reports which showed increased plant growth and yield upon inoculation with salt tolerant PGPR under salt stress (Singh and Jha, 2016). Thus, PGPR strains, *P. fluorescens*, *B. pumilus* and *E. aurantiacum* can be regarded as promising microorganisms to formulate biofertilizer specific for saline soils to minimize wheat crop yield penalties caused by soil salinization. Many PGPR strains belonging to genera *Pseudomonas, Ochrobactrum, Bacillus, Azospirillum, Azotobacter, Rhizobium, Stenotrophomonas, Serratia*, and *Enterobacteria* (Maleki et al., 2011) have been extensively reported and exploited as effective candidates to be formulated as biofertilizers – green biotechnology, as an alternative sustainable approach in saline agriculture (Egamberdieva et al., 2019).

CONCLUSION

Outcomes of the present study inferred that PGPR employed beneficial impact on physio-chemical attributes of inoculated plants consequently leading toward alleviation of salinity induced damages through improved water relations, enhanced compatible solutes accumulation, stimulated potassium and calcium acquisition and reduced antioxidant enzymes activity. These alterations in cellular metabolism ultimately led to the improved growth and yield among both salt tolerant and susceptible varieties under salt stress. However, the salt tolerant variety showed far better growth and yield than the sensitive variety. *B. pumilus* and *E. Aurantiacum* single strains and consortium manifested a more evident impact on the salt resistant genotype while in the case of the salt sensitive genotype, *P. fluorescens* single strain and consortium played a pivotal role in growth and yield improvement. Further experimentation at multiple field locations and detailed investigation of molecular mechanisms in near future can lead toward application of these microbes as biofertilizer in salt affected soil for enhanced wheat crop production.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

AN conducted the experiments and sampling. AN and MS designed the project. AN, MS, and A carried out data analysis and manuscript preparation. MS, A, FM, AI, MI, and MM reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 Induces Salt Tolerance by Modulating Physiology and Biochemical Activities of *Arachis hypogaea*

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Arachis hypogaea (Peanut) is one of the most important cash crops grown for food and oil production. Salinity is a major constraint for loss of peanut productivity, and halotolerant plant growth promoting bacteria not only enhance plant-growth but also provide tolerance against salt stress. The potential of halotolerant bacterium *Stenotrophomonas maltophilia* BJ01 isolated from saline-soil was explored to enhance the growth of peanut plants under salt stress conditions. Interaction of *S. maltophilia* BJ01 enhances the growth of the peanut plants and protects photosynthetic pigments under salt stress. Lower electrolyte leakage (about 20%), lipid peroxidation (2.1 μmol g^{-1} Fw), proline (2.9 μg mg^{-1} Fw) content and H_2O_2 (55 μmol g^{-1} Fw) content were observed in plants, co-cultivated with PGPR compared to untreated plants under stress condition. The growth hormone auxin (0.4 mg g^{-1} Fw) and total amino acid content (0.3 mg g^{-1} Fw) were enhanced in plants co-cultivated with PGPR under stress conditions. Overall, these results indicate the beneficial effect of *S. maltophilia* BJ01 on peanut plants under salt (100 mM NaCl) stress conditions. In conclusion, bacterium *S. maltophilia* BJ01 could be explored further as an efficient PGPR for growing legumes especially peanuts under salt stress conditions. However, a detailed agronomic study would be needed to ascertain its commercial role.

Keywords: peanut, saline agriculture, halotolerant bacteria, salt stress, *Stenotrophomonas*, PGPR - plant growth-promoting rhizobacteria, *Arachis hypogaea*, plant microbe interaction

INTRODUCTION

Soil salinity adversely affects the system of the plants at the physiological, biochemical, and molecular levels (Roy et al., 2014). Salinity causes osmotic stress, nutrient imbalance/unavailability, reduction of photosynthesis, ion toxicity, generation of reactive oxygen species (ROS), and ethylene (stress hormone) (Alexander et al., 2019a). Around the world, approximately 77 million hectares

(Mha) of agricultural land is affected by salt (Arora, 2017). In India, a total of 9.38 Mha is affected by salinity, while specifically the Gujarat state has a significant share of the salinity-affected area of about 2.23 Mha (Srivastava et al., 2019). Arid and semiarid regions of the world are more affected by salinity due to inadequate rain and agricultural practices (Glick et al., 2007). Among crop plants, cereals and legumes are the most sensitive to salt. In legumes, salt affects the nodulation process and finally the nitrogen fixation (Ramana et al., 2012). Even 100 mM of salt is enough to inhibit nodule formation (Dardanelli et al., 2009). Salt creates a hindrance in Ca absorption, which in turn affects the growth of roots and root hair, hence providing an additional mechanism to hinder nodule formation (Bouhmouch et al., 2005). Peanut (*Arachis hypogaea* L.) is an important oil crop that is used for food, fodder, and industrial raw material. India in particular has been reported to have the second largest peanut production after China (Fabra et al., 2010).

To tackle the effects of salinity on crops and enhance productivity, several methods are employed, including but not limited to good agricultural practices, genetic manipulation of crops to make them resistant to salt, improvement of the agricultural soil, and irrigation water use. Application of halophilic/halotolerant plant growth-promoting bacteria in stressed soil is the most useful and environmentally friendly approach to increase the productivity and health of crops as well as enhance the soil system in the long term (Alexander et al., 2019a; Fazeli-Nasab and Sayyed, 2019). Plant roots secrete various nutrient substances (∼40% of photosynthetic products) known as root exudates, that play a significant role in the attachment and growth of various endophytic and free-living bacteria (Wang et al., 2016). Some of these bacteria enhance the plant growth and health even under stress conditions. These bacteria are known as plant growth-promoting rhizobacteria (PGPR) (Cook et al., 1995; Alexander et al., 2019b). PGPR enhance the plant growth and development in several ways, including via nitrogen fixation, phosphate solubilization, production of phytohormones, ACC deaminase activity, production of exopolysaccharide (EPS), priming the plant immunity (induced systemic resistance; ISR), acting as a biocontrol agent, and increasing the plant antioxidant enzymes that are produced under stress conditions, such as the ascorbate peroxidase (APX), the catalase (CAT), and the glutathione reductase (GR) (Kloepper et al., 2004; Arevalo-Ferro et al., 2005; Yang et al., 2009: Upadhyay et al., 2012; Bal et al., 2013; Bhargava et al., 2017; Sarkar et al., 2018).

Stenotrophomonas is a gram-negative, yellow-pigmented bacillus, which is a member of the gamma-Proteobacteria class (Moore et al., 1997). It is either free-living or endophytic and is associated with many plant species (Egamberdieva et al., 2016). Different species of *Stenotrophomonas* have previously been reported for their ability to promote plant growth (Ryan et al., 2009; Berg et al., 2010; Alavi et al., 2013; Singh and Jha, 2017). Egamberdieva et al. (2011) and Singh et al. (2013) have isolated *Stenotrophomonas* stains from high salinity soils. Members of this species can survive in high salt concentrations because of the production of compatible solutes, especially glucosyl glycerol (GG) and trehalose, which also help the plant to survive in harsh environmental conditions (Alavi et al., 2013). *Stenotrophomonas maltophilia* BJ01 was isolated from the rhizosphere of *Cyperus laevigatus* L., which was grown at the coastal region of Dwarka, India and submitted to the Indian marine microbial culture collection of CSMCRI, Bhavnagar with culture collection number IMMCC255 *S. maltophilia* BJ01 grew in an environment that contained up to 4% NaCl (unpublished data) and it was shown to possess the *nifH* gene (Singh et al., 2013). Quorum quenching (QQ) and antibiofilm activity of the strain has been reported, hence further supporting its ability to promote plant growth and biocontrol against various plant pathogenic bacteria. It can therefore be employed as part of a strategy that enhances plant survival under harsh growth conditions (Singh et al., 2013). We have also previously reported the nitrogen fixing ability of *S. maltophilia* BJ01 and its potential to promote plant growth and specifically support peanut plant growth under conditions where N_2 is lacking (Alexander et al., 2019b).

The nitrogen fixing ability of legumes is adversely affected by soil salinity that hinders nodule formation, low microbial diversity around root which maintains the holobiome of plant hence halotolerant rhizobacterial species which can naturally survive in saline soils can be useful in agriculture especially in saline soils (Hamaoui et al., 2001; Dardanelli et al., 2008; Egamberdieva, 2011; Patel et al., 2012; Etesami and Beattie, 2018). Recently there are a few studies which shows the positive effect of PGPR on legumes under salt stress. The salt tolerance capacity of soybean was elevated when plants co-cultivated with halotolerant bacteria under 200 mM NaCl stress (Khan et al., 2019). The bacteria *Bacillus megaterium* NRCB001, *B. subtilis* subsp. *subtilis* NRCB002 and *B. subtilis* NRCB003 isolated from rice rhizosphere showed the plant growth promoting potential under salt stress (130 mM NaCl) when co-cultivated with *Medicago sativa* (alfalfa) (Zhu et al., 2020). *Bacillus megaterium* AL-18, *B. cereus* AL-19 (PGPR isolated from *Tamarix ramosissima*) improved the growth of *Phaseolus vulgaris* under salt stress (Abdelmoteleb and Gonzalez-Mendoza, 2020). This study aims to assess the plant growth-promoting attributes of a halophytic bacterium, namely *S. maltophilia* BJ01, and how these affect the growth of peanut plants under salt stress conditions.

MATERIALS AND METHODS

Plant Material and Bacterial Interaction

Seeds of *Arachis hypogaea* cv. GG 20 were collected from the Gujarat Seed Corporation, Sihor, Gujarat, India. The seeds were surface sterilized according to the previously optimized protocol (Alexander et al., 2019b). In brief, the seeds were washed in 70% ethanol for 2 min and submerged in 0.1% $HgCl_2$ for 10 min followed by washing with double autoclaved Milli-Q water 4-5 times to remove any traces of $HgCl_2$. Sterilized seeds were placed in small tissue culture bottles (50 mL) containing sterilized cotton soaked with 1/2 MS (Murashige & Skoog) media in the bottom and kept in the dark for 2-3 days for germination. After 7 days of germination, seedlings of equal

size were transferred to the hydroponics culture with the help of floating thermocol disks in 500 mL beaker containing 1/2 MS media. The plantlets were allowed to acclimatize for seven days. The bacterial inoculum was prepared according to the previously reported protocol Alexander et al. (2019b). In brief, For the bacterial inoculum preparation, the bacterial strain was streaked on DYGS agar plate (dextrose 1.0 g L^{-1}; malate 1.0 g L^{-1}; peptone 1.5 g L^{-1}; yeast extract 2.0 g L^{-1}; $MgSO_4.7H_2O$ 0.5 g L^{-1}; L-glutamic acid 1.5 g L^{-1}; pH 6.0) from glycerol stock stored in -80°C and incubated for 16 hrs at 30°C, followed by subculture in 5 mL DYGS broth media overnight at 30°C and 180 rpm in an incubator shaker. The overnight grown culture was reinoculated in 150 mL of DYGS medium, and the culture was centrifuged at 4000 × *g* for 10 min once the bacterial growth reached the OD600 nm = 0.6. The supernatant was discarded, and the pellet was re-suspended in 1/2 MS media before co-cultivation with the plant.

Following acclimatization, plants were divided into four groups: (1) without bacterial inoculum and salt stress; (2) with bacterial inoculum and without salt stress; (3) without bacterial inoculum and with 100 mM salt stress; (4) with bacterial inoculum and 100 mM salt stress. All 4 sets were supplemented with 300 mL of 1/2 MS media and were grown in a culture room at 25 ± 2°C under a 16-h/8-h light/dark cycle (light intensity 170 ± 25 μmol m^{-2} s^{-1}) for another 14 days. The media and inoculum in each plant were changed every seven days, and changes in morphology were recorded. The day that the plants were inoculated with the bacterium was considered to be day zero. After 14 days of stress, the root length, the shoot length, the fresh weight and the dry weight of the plants were recorded and samples were harvested for further analysis.

Chlorophyll Estimation

Total chlorophyll contents of leaf tissues were estimated according to the method given by Arnon (1949) in which leaf tissue (100 mg) was crushed with the help of mortar pestle in 80% acetone and incubated for 6 h in the dark. This was subsequently centrifuged at 10000 × *g* and the supernatant was pooled out. Absorbance was recorded at 663 and 645 nm. Total chlorophyll contents were calculated using the following equations:

$$\textit{Total Chlorophyll} = \frac{\left[\left(20.2 \times Abs_{645}\right) + \left(8.02 \times Abs_{663}\right)\right] \times \textit{vol of the sample in ml}}{\textit{weight of tissues}}$$

$$\textit{Chlorophyll a} = \frac{\left[\left(12.7 \times Abs_{663}\right) - \left(2.6 \times Abs_{645}\right)\right] \times \textit{vol of the sample in ml}}{\textit{weight of tissues}}$$

$$\textit{Chlorophyll b} = \frac{\left[\left(22.9 \times Abs_{645}\right) - \left(4.68 \times Abs_{663}\right)\right] \times \textit{vol of the sample in ml}}{\textit{weight of tissues}}$$

Electrolyte Leakage

Leaves from the distal end of the primary branch were harvested and washed thoroughly with deionized water to remove surface adhered electrolytes. Samples were kept in 10 mL falcons (Eppendorf, United States) containing double distilled water and kept at room temperature on a rotary shaker for 24 h and electrical conductivity (EC) of this water (L1) was measured in μScm^{-1} using a conductivity meter (Seven Easy, Mettler Toledo, United States). These samples were autoclaved at 120°C for 20 min, cooled at room temperature (RT), and electrical conductivity (L2) was determined (Lutts et al., 1996). The electrolyte leakage was calculated by the following equation:

$$EL\,(\%) = \frac{L1}{L2} \times 100$$

Membrane Stability Index

Leaves of the same age and size were harvested from the primary branch, they were washed adequately and they were kept in 10 mL vials that were placed on a shaker for 24 h. The EC was subsequently recorded. These samples were put in the water bath (Julabo) at 40°C for 30 min and cooled at RT, and the EC was measured (L_1). The same samples were boiled off at 100°C for 20 min, and the EC (L_2) of the cooled samples was recorded to calculate the MSI (Jha et al., 2013). The following equation was used for the calculation:

$$MSI(\%) = \left[1 - \frac{L_1}{L_2}\right] \times 100$$

Proline Content

The proline estimation was done as per Bates et al. (1973). 100 mg of plant leaf samples were crushed in liquid nitrogen and extracted in chilled sulphosalicylic acid (SSA). An equal volume of extract and ninhydrin reagent were mixed and incubated at 100°C for 1 h. After cooling the samples in ice bath, toluene was added in the reaction mixture, followed by vortexing and centrifugation. The upper phase was collected, and absorbance was taken at 520 nm. The proline content was calculated by using the standard curve of the known amount of proline.

Total Amino Acid Content

Plant leaf samples (100 mg) were extracted with 80% chilled ethanol. This extract was treated with an equal volume of 0.2 M citrate buffer (pH 5) and 1% ninhydrin reagent. The tubes containing reaction mixture were heated at 95°C in a water bath for 15 min. After cooling, the samples were centrifuged, and the absorbance was read at 570 nm (Patel et al., 2016).

Auxin Content

For the auxin estimation, the extract of leaf samples was prepared in 95% chilled ethanol, and the reaction was carried out further only in ice. The extract was mixed with a double amount of Salkowski reagent and was kept in the dark for 20 min. The absorbance was recorded at 535 nm (Andreae and Van Ysselstein, 1960). The total auxin amount was calculated by a standard curve drawn with the known concentration of indole acetic acid (IAA).

Total H_2O_2 Contents

Extract of 100 mg leaf samples was prepared in 80% ice-cold acetone, and hydrogen peroxide was quantified by the modified

method (Mukherjee and Choudhuri, 1983). The absorbance was measured at 415 nm. The total H_2O_2 content was calculated by a standard curve drawn with the known concentration of H_2O_2.

In vivo Localization of Hydrogen Peroxide and Superoxide Radicals

The hydrogen peroxide and the superoxide radicals in stressed and unstressed plant samples were determined *in vivo* using a histochemical stain of 3,3- diaminobenzidine (DAB) and nitro-blue tetrazolium (NBT) respectively (Singh et al., 2016). Solutions of DAB and NBT were prepared in 10 mM phosphate buffer (pH 7.8). Fresh leaves were immersed in the freshly prepared DAB or NBT, they were kept in the dark for 2 h, and they were illuminated in white light for DAB (8 h) and NBT (1 h). The blue and brown spots that appeared on the leaves indicated *in vivo* localization.

Lipid Peroxidation

Lipid peroxidation was estimated, according to Hodges et al., 1999 by quantifying the malondialdehyde (MDA) content. Leaf samples (100 mg) were homogenized in chilled 80% ethanol for extract preparation. The extract was divided into two sets; one set was mixed with an equal volume of thiobarbituric acid reagent (containing TBA; 1 mL of 0.5% w/v prepared in 20% w/v TCA); another set was mixed with an equal volume of TCA (20% w/v). Both sets were incubated at 95°C for 30 min, were cooled at RT, and were centrifuged at 10000 × *g* for 10 min. The optical density of the supernatant recorded at 440, 532, and 600 nm. MDA content was calculated according to the following equation:

$$A = \left[Abs_{532+TBA} - Abs_{600+TBA}\right] - \left[Abs_{532-TBA} - Abs_{600-TBA}\right]$$

$$B = \left[Abs_{440+TBA} - Abs_{600+TBA}\right] \times 0.0571$$

$$MDA\left(\mu molg^{-1}\right) = \frac{A-B}{15700} \times 10^6$$

Statistical Analysis

Each group contained five plants, and the experiment was performed three times. Statistical analysis was performed by GraphPad Prism software. All data were subjected to one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test. All values are expressed as the mean ± SE. '*' denotes $P < 0.05$; '**' denotes $P < 0.01$ and '***' denotes $P < 0.001$.

RESULTS AND DISCUSSION

Interaction of *S. maltophilia* BJ01 Enhances the Growth of the Plant Under Salt Stress

Previously we have reported the plant growth promoting potential of *S. maltophilia* BJ01 under N_2 starvation conditions (Alexander et al., 2019b). Here we are evaluating the potential of this bacterial strain under 100 mM salt stress condition. After the interaction of the PGPR strain, BJ01 with the plant under control condition (without salt stress) and stress condition (100 mM NaCl) plant were evaluated for their growth pattern for 14 days.

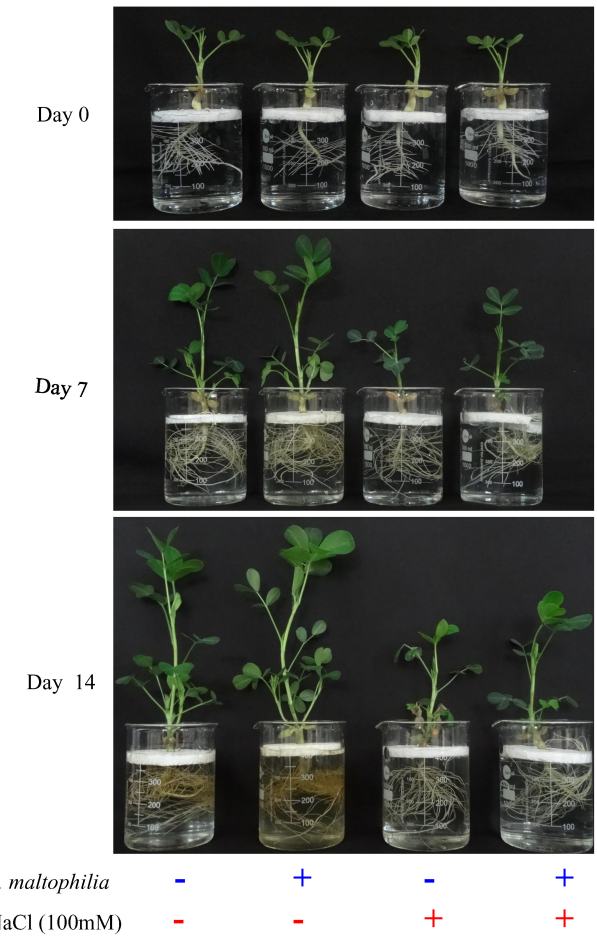

FIGURE 1 | Morphological difference in inoculated and uninoculated plants. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. " + " represents the presence of bacteria or NaCl, whereas "-" represents the absence of NaCl or bacteria.

Higher plant growth was observed in the plant treated with the bacteria under salt stress (**Figure 1**.). The shoot length of the treated plants under salt stress was significantly different from their untreated control. The shoot length of the untreated plant was about 13.4 cm whereas the shoot length of the treated plant was about 16 cm (**Figure 2A**). There was no significant difference in the root of the untreated and treated plants in salt stress conditions (**Figure 2B**). Enhanced production of auxin could be possible reason for the shoot elongation. The improved fresh weight (Fw) was observed when plants are grown with the bacteria. Under salt stress conditions, the fresh weight of the untreated plant was 5 g and the fresh weight of the treated plant was about 7 g (**Figure 2C**). Similarly, improved dry weight (Dw) was observed when the plant treated with bacteria. About 0.7 g and about 0.8 g of dry weight were observed in untreated and treated plants under stress conditions, respectively (**Figure 2D**).

For survival under abiotic stress, plants generally compromise their growth, physiology, and development because the resources like nutrients and photosynthetic byproducts are used in defense

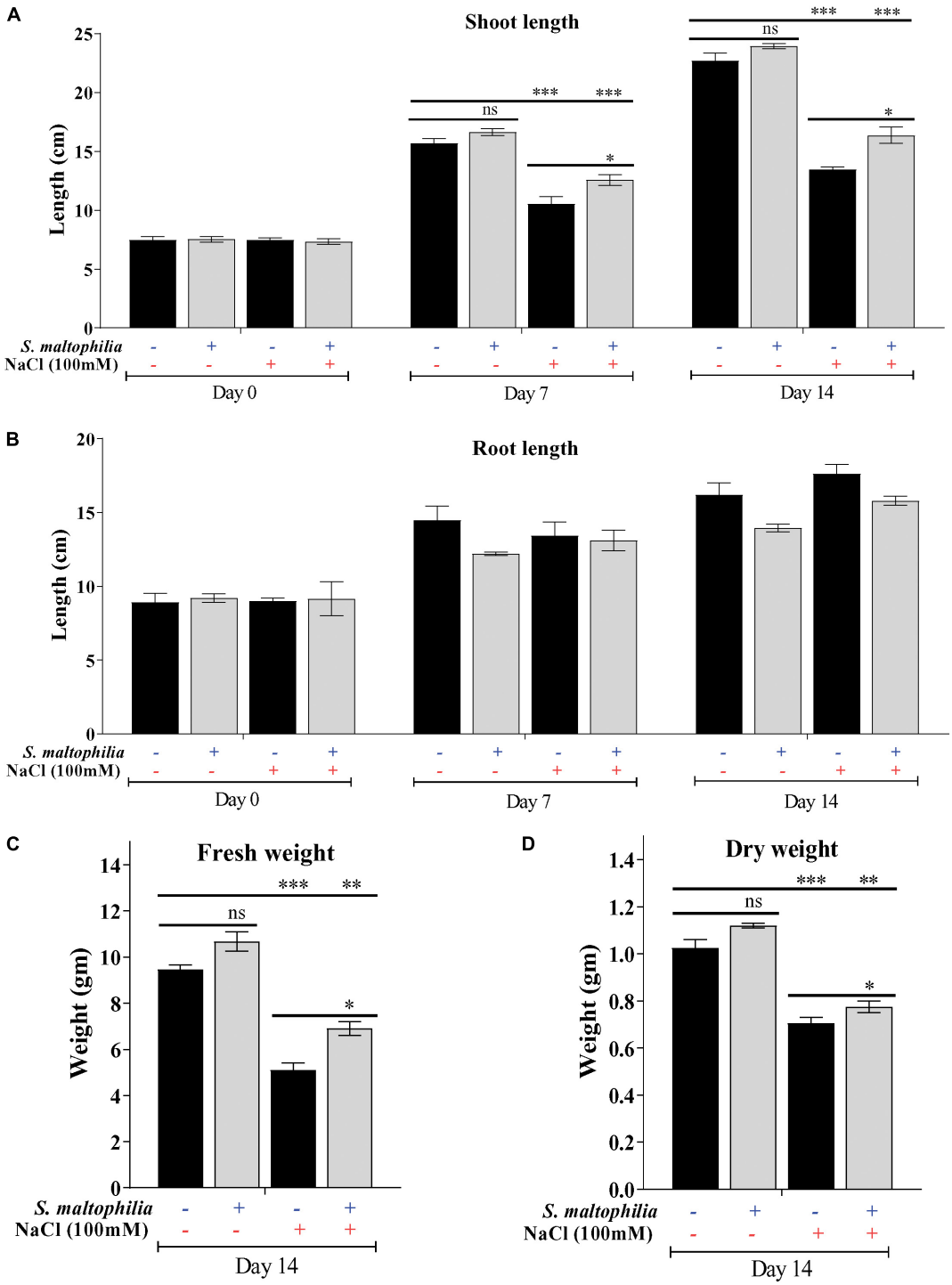

FIGURE 2 | Difference in various growth parameters and comparative analysis. Shoot length **(A)**, root length **(B)** fresh weight **(C)** dry weight **(D)** of control, and stressed plants. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. " + " represents the presence of bacteria or NaCl whereas "-" represents the absence of NaCl or bacteria. Bars denote means ± SE. '*,' '**,' '***' indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively and 'ns' represents no significant difference.

(Egamberdieva et al., 2019). In this study, we observed that the untreated plant (without bacterial interaction) under salt stress plant growth was stunned, shoot length, fresh weight, dry weight reduced drastically. On another set where plants were treated with bacterial inoculum under salt stress showed improved growth (shoot length, fresh weight and dry weight).

These observations showed the role of *S. maltophilia* BJ01 in growth and development under salt stress conditions. Similar results were also reported in which PGPR showed the improved growth in crop *Solanum melongena* L, *Triticum aestivum,* and *Chenopodium quinoa* under salt stress (Fu et al., 2010; Orhan, 2016; Yang et al., 2016). Bacterial inoculation reduces the salt stress and showed the improved plant growth and phosphate uptake in *Phaseolus vulgaris* (Abdelmoteleb and Gonzalez-Mendoza, 2020). The co-cultivation of plant growth-promoting rhizobacteria also showed the improved plant growth specially the dry weight of the *Medicago sativa* (alfalfa) under salinity stress (Zhu et al., 2020).

Photosynthetic Pigment of *Arachis hypogaea* Was Protected by *S. maltophilia* BJ01 Under Salt Stress

Salt stress affects the plant cells physiologically and due to osmotic pressure, cells get dehydrated which results in stomatal closure, reduced cell growth and reduced chlorophyll content in plants (Shannon and Grieve, 1998). The peanut plants were grown under salt stress (100 mM) for 14 days. The leaves turned pale and necrosis in leaves were observed which are the sign of chlorophyll degradation and senescence. When the plants are grown with the *S. maltophilia* BJ01 under salt stress, the plant have much healthy leaves and higher chlorophyll concentration. The chlorophyll a, chlorophyll b, and total chlorophyll contents were 0.2 mg g^{-1} Fw, 0.3 mg g^{-1} Fw, and 0.5 mg g^{-1} Fw respectively in the plant without bacteria (**Figures 3A–C**). The chlorophyll a, chlorophyll b and total chlorophyll contents were 0.4 mg g^{-1} Fw, 0.3 mg g^{-1} Fw, and 0.7 mg g^{-1} Fw respectively in a plant grown under salt stress with bacteria (**Figures 3A–C**). The positive effect of rhizospheric bacteria on chlorophyll content and photosynthetic ability of host plant under saline stress condition was also reported in *Zea mays* and *Oryza sativa* (Nadeem et al., 2007; Rojas-Tapias et al., 2012; Yoolong et al., 2019). The protection of the photosynthetic pigment under salt stress was also reported in common bean (*Phaseolus vulgaris*) by rhizospheric bacteria (Abdelmoteleb and Gonzalez-Mendoza, 2020).

S. maltophilia BJ01 Modulates the Plant Physiology After Interaction Under Salt Stress

The plant grown under salt stress with bacteria showed reduced electrolyte leakage and high membrane integrity compared to plants grown under salt stress without bacteria. About 42% of electrolyte leakage was found in the plant grown in salt stress without bacteria and about 19% electrolyte leakage was found in the plant grown in salt stress with bacteria (**Figure 4A**). The membrane stability of the plant under salt stress without bacteria was about 40% whereas plants grown under salt stress with bacteria were about 77% (**Figure 4B**). Under salinity stress plant cells has a higher concentration of Na^+ and Cl^- and low concentration of K^+; this ionic imbalance destabilizes/damages the cell membranes (due to Ca^{2+} displacement) and causes the leakage of electrolytes from cell sap (Hussain et al., 2008). The interaction between bacteria and plants attenuate the deleterious effect on plant cells which occurs due to high salt concentration and helps cells to maintain its structure and survival. Similar results were also obtained in *Cajanus cajan* (L.), where electrolyte leakage is higher in salt condition and it reduced by the application of arbuscular mycorrhiza (Garg and Manchanda, 2009). Reduction of the electrolyte leakage in chickpea (*Cicer arietinum* L.) under salt stress by *Azospirillum lipoferum* FK1 reported by El-Esawi et al. (2019). These results are suggesting that the *S. maltophilia* BJ01 reducing the salt stress on the plant which results the improved membrane stability.

Co-cultivation of *S. maltophilia* BJ01 Leads to the Better Biochemical Performance of *Arachis hypogaea* Under Salt Stress

The plant grown with the bacteria under salt stress showed lower proline content and higher total amino acid accumulation in the plant in comparison to the control counterpart. The proline content of the plant grown under salt stress without bacteria was about 3.2 μg mg^{-1} Fw and with bacteria was about 2.9 μg mg^{-1} Fw (**Figure 5A**). The total amino acid

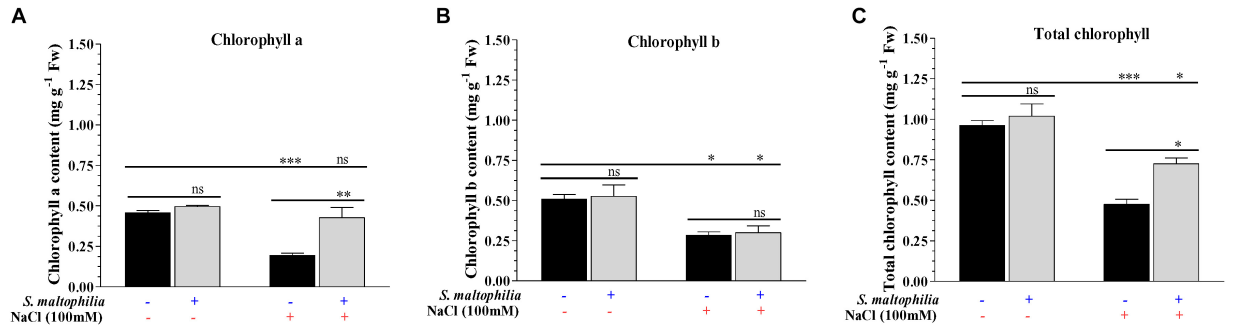

FIGURE 3 | Estimation of photosynthetic pigments. Chlorophyll a contents **(A)**, chlorophyll b contents **(B)** and total chlorophyll content **(C)** of inoculated and uninoculated plants. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. " + " represents the presence of bacteria or NaCl whereas "-" represents the absence of NaCl or bacteria. Bars denote means ± SE. '*,' '**,' '***' indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively and 'ns' represents no significant difference.

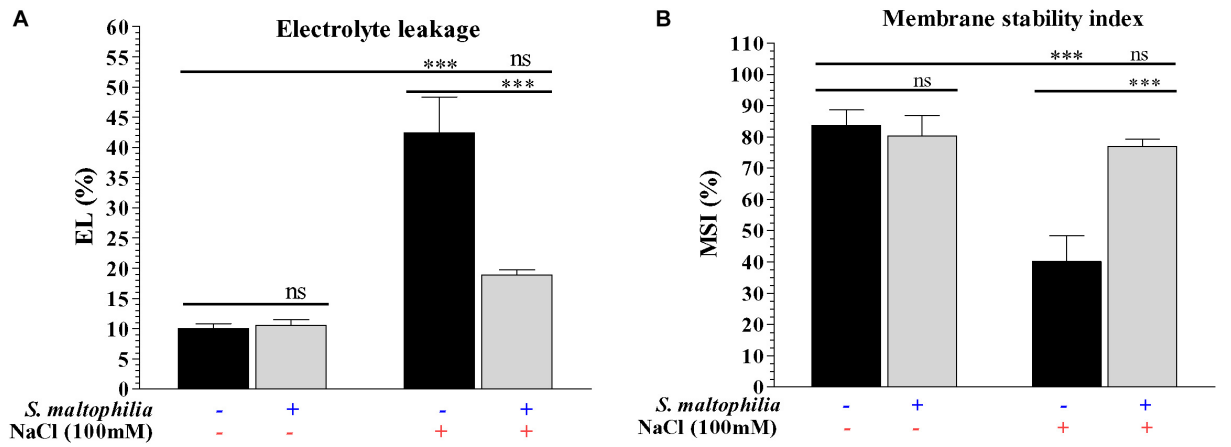

FIGURE 4 | Measurement of physiological parameters. Membrane Stability Index (MSI) **(A)** and electrolyte leakage (EL) of control and stressed plants **(B)**. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. " + " represents the presence of bacteria or NaCl whereas "-" represents the absence of NaCl or bacteria. Bars denote means ± SE. '*,' '**,' '***' indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively and 'ns' represents no significant difference.

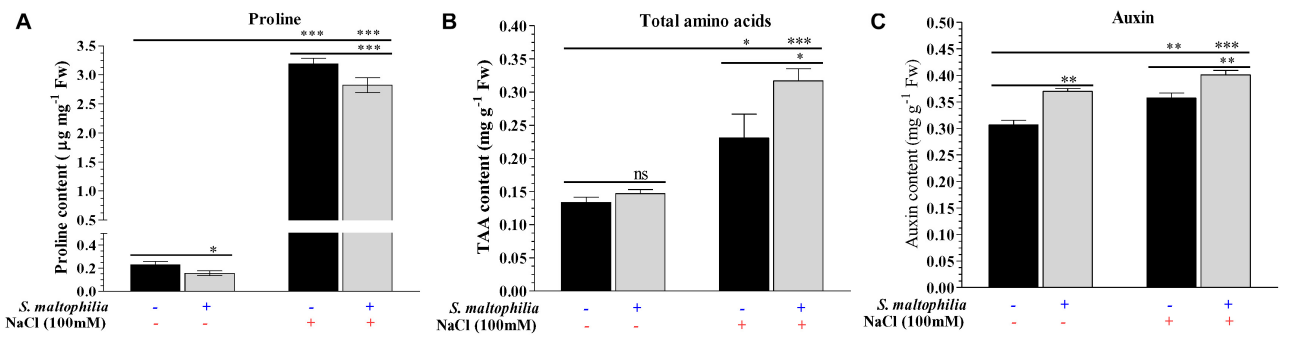

FIGURE 5 | Biochemical changes in plants due to bacterial interaction. Quantification of proline **(A)**, total amino acid (TAA) **(B)** and auxin **(C)** concentration of control and treated plants. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. **" + "** represents the presence of bacteria or NaCl whereas **"-"** represents the absence of NaCl or bacteria. Bars denote means ± SE. '*,' '**,' '***' indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively and 'ns' represents no significant difference.

concentration was quantified in plants. About 0.23 mg g^{-1} Fw was found to present in plant grown in salt stress without bacteria and about 0.31 mg g^{-1} Fw was present in the plant grown with bacteria (**Figure 5B**). Maintenance of turgidity and viscosity in cells is a significant challenge for plants under salt stress. To cop up with this condition plants synthesize osmolytes/osmoprotectants which help plant to survive under harsh conditions and maintain water retention inside the cells (Zulfiqar et al., 2020). Amino acids like valine, isoleucine, proline, aspartic acid, etc., act as osmoprotectants and generate in high concentration by plants under salt condition (Burg and Ferraris, 2008). Proline is one of the amino acids which acts as osmoprotectant under various abiotic stress conditions and scavenger for hydroxyl free radicles (Claussen, 2005; Peng et al., 2008). The presence of lower proline content in the plant with the bacteria reflects the role of *S. maltophilia* BJ01 to helping the plant to overcome with the salt stress. A higher amount of total amino acids (TAA) content in plants having salt stress and bacterial inoculation shows the role of bacteria further strengthen the plant system under a saline environment. Han and Lee (2005) also found that the interaction of *Glycine max* with *Bradyrhizobium japonicum* under salt stress leads to lower production of the proline. To further strengthen our finding that the plant growth promoting rhizobacteria reduces the salt stress on the plants leading the lower production of proline was also reported after interaction of halotolerant rhizobacterium *Pseudomonas koreensis* MU2 with Soybean (*Glycine max* L.) under salt stress by Adhikari et al. (2020).

The auxin production in the plant grown under salt stress without bacteria was 0.35 mg g^{-1} Fw, whereas in the plant treated with bacteria was 0.40 mg g^{-1} Fw (**Figure 5C**). Auxins are phytohormone which play a crucial role in the growth, development under stress conditions for plants (Egamberdieva et al., 2017). Indole-3-acetic acid (IAA) is the most common version of auxin found in plants and its concentration decreases

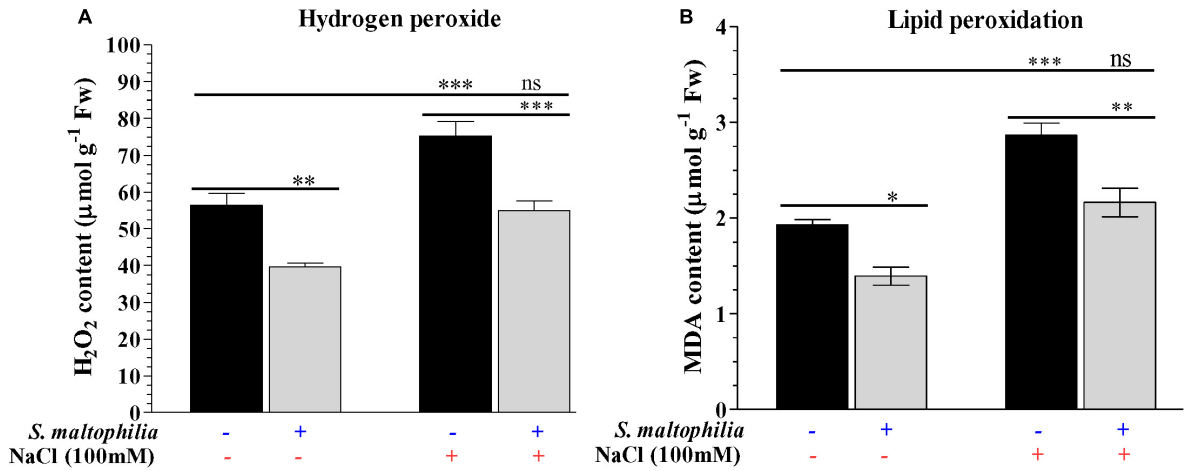

FIGURE 6 | Estimation of reactive oxygen species and lipid peroxidation of the plant. Quantification of hydrogen peroxide (H_2O_2) **(A)** and MDA contents **(B)**. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. **" + "** represents the presence of bacteria or NaCl, whereas **"-"** represents the absence of NaCl or bacteria. Bars denote means ± SE. '*,' '**,' '***' indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively and 'ns' represents no significant difference.

in salt stress results in decreased growth of plants (Albacete et al., 2008). In our study, we found that the concentration of auxin decreases in salt stress without bacteria however, in plants with bacterial inoculation concentration of auxin increases. Increment in auxin concentration revealed that the bacterial interaction enhances the auxin synthesis in plants which helps plant for survival and growth under salt stress. This result is in accordance with Tiwari et al. (2011) and Noori et al. (2018).

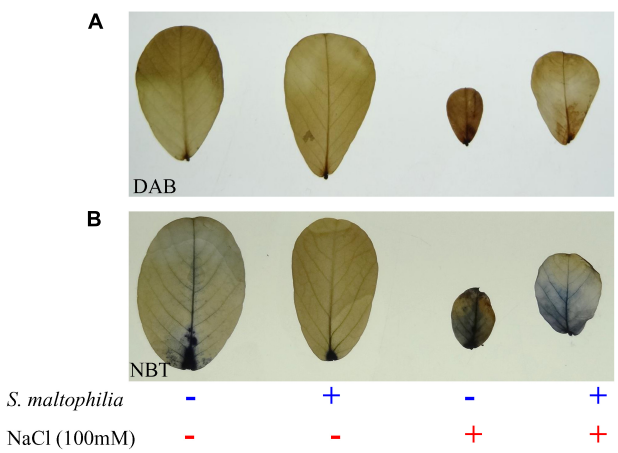

FIGURE 7 | *In vivo* localization of reactive oxygen species in plant leaves. Staining of peroxide and superoxide free radicals via DAB **(A)** and NBT **(B)**. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. " + " represents the presence of bacteria or NaCl, whereas "-" represents the absence of NaCl or bacteria. Bars denote means ± SE. '*,' '**,' '***' indicate significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively and 'ns' represents no significant difference.

Reactive Oxygen Species (ROS) Buildup in *Arachis hypogea* Protected by *S. maltophilia* BJ01 Interaction Under Salt Stress

Reduced production of hydrogen peroxide was observed in the plant grown with the bacterial in comparison to the plant grown without bacteria in the salt stress condition. About 75 µmol g^{-1} Fw H_2O_2 was found in the plant without bacteria under salt stress whereas 55 µmol g^{-1} Fw was measured in the plant with bacteria under stress condition (**Figure 6A**). These results were also supported by the *in vivo* localization of these ROS (superoxide and H_2O_2) in plant leaves (**Figures 7A,B**). In stress condition plants overproduce reactive oxygen species (ROS) which act as a signaling molecule for downstream regulation of defense mechanism; this situation called oxidative stress (Demidchik, 2015). Among the ROS, hydrogen peroxide (H_2O_2) is considered as the most stable molecule (Foyer and Noctor, 2009) and generate high concentration under salt stress (Singh et al., 2016). Lower H_2O_2 concentration in plants with bacterial inoculation in salt stress shows the beneficial effect of *S. maltophilia* BJ01 on peanut under stress conditions which reduces the oxidative stress on the plant system. Similar results were also obtained in strawberry plants by rhizobacterial treatment (Arıkan et al., 2020).

The lower production of malondialdehyde (MDA) in the plant grown with the bacteria was observed in the comparison of the plant without bacteria under salt stress. The MDA content 2.8 µmol g^{-1} Fw was measured in a plant grown without bacteria, whereas 2.1 µmol g^{-1} Fw was measured in a plant grown with the bacteria under salt stress (**Figure 6B**). Membrane lipids are highly reactive toward the ROS which results in lipid peroxidation and generates MDA, which is an indicator of membrane disintegration (Hodges et al., 1999; Catalá, 2006; Farmer and Mueller, 2013). More membrane damage causes

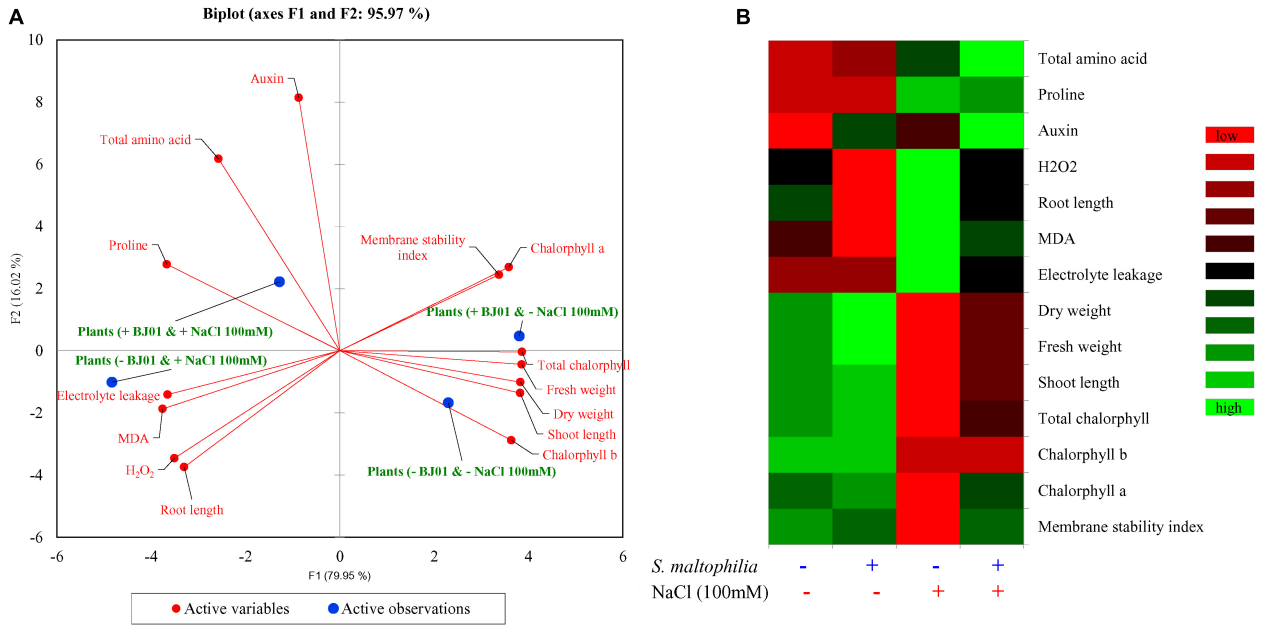

FIGURE 8 | Multivariate data analyses of plant grown with or without bacteria under control and stress conditions. Principal component analysis **(A)** and integrated heat map **(B)**. Plant grown without NaCl considered as control condition and plant under 100 mM salt considered as stressed conditions. " + " represents the presence of bacteria or NaCl, whereas "-" represents the absence of NaCl or bacteria.

more production of MDA molecules which in our case reduced by bacterial treatment in a plant under stress condition. Singh and Jha (2016) obtain similar results in wheat inoculated with halotolerant bacteria under stress conditions.

Morpho-Physio-Biochemical Response of Plant Grown With or Without Bacteria Under Control and Stress Conditions

Principal component analysis (PCA) was carried out to extricate the response of the peanut plants under different growth conditions. The bi-plot analysis reveals the differential response of the plant under control and stress conditions when co-cultured with bacteria and without bacteria (**Figure 8A**). Differential responses to the variables was also observed in the integrated heat-map in different conditions of the plant growth (**Figure 8B**). The multivariance analysis strongly suggests that the bacterial interaction highly influence the morphology, physiology and biochemistry of the plant.

CONCLUSION

In this study, the beneficial effects of *Stenotrophomonas maltophilia* BJ01 on *Arachis hypogaea* GG20 plants under 100 mM salt concentration were evaluated. Here we found that the plant growth promoting rhizobacteria isolated from halotolerant grass species can help the peanut plant to withstand the deleterious effect of salinity by supporting the plant at the morphological, physiological and biochemical level. Inhabitance in harsh conditions and nitrogen fixing ability of this bacterial strain help plants under direct salt stress. To meet up the demand for food for the growing population of the world under various abiotic stress, we need a more sustainable and environmentally friendly method. Thus, this study opens the door for the agricultural application of PGPR to overcome biotic and abiotic stress instead of chemical application. Further studies of genomic, proteomic, and metabolomics of holobiome (plant and associated microbiome) can be a beneficial intervention in this field to understand plant microbe interaction and uncover the mysteries of plant immunity and its survival.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AM conceived and designed the experiments. AA performed the experiments. AA, VS, and AM analyzed the data and wrote the manuscript.

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Soil Salinity Drives the Distribution Patterns and Ecological Functions of Fungi in Saline-Alkali Land in the Yellow River Delta, China

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High soil salinity is the main factor that limits soil microbial activity in the Yellow River Delta (YRD); however, its effects on fungal community and ecological function are unknown. Here, we comparatively investigated the diversity and structures of soil fungal communities targeting the internally transcribed fungal spacer gene using Illumina MiSeq sequencing methods under a salt gradient with five levels, namely, Low: low-salinity soil, Medium: medium-salinity soil, High: high-salinity soil, Extreme: extreme-salinity soil, and a non-salted site as the control (Non-saline). The results show that bulk density (BD) values significantly increased ($p < 0.05$), while significantly lower values of soil total carbon (TC), total nitrogen (TN), and fungal Shannon and Chao indexes were observed as the salinization gradient increased ($p < 0.05$). The relatively high levels of the families *Nectriaceae* and *Cladosporiaceae* distinguished two of the clusters, indicating two enterotypes of low (Non-saline and Low) and high (Medium, High, and Extreme) salinity soils, respectively. The family *Nectriaceae* was most abundant in the networks, and the positive correlations were more pronounced than negative correlations; however, *Cladosporiaceae* was the family most negatively correlated with others based on the network analysis. At the ecological function level, plant saprotrophs and litter saprotroph were significantly less abundant in extremely saline soil than non-saline soil. The change in soil properties (TC, TN, and BD) caused by soil salinization [salt and electrical conductivity (EC)] regulated the diversity of soil fungal communities, and ecological function, as indicated by Pearson correlation analyses. We suggest further investigation into the ecological functions of soil microorganisms in the extremely saline-alkaline soils of the YRD.

Keywords: soil salinity, soil pH, fungal community and diversity, salt tolerant fungi, yellow river delta

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INTRODUCTION

Salinization is one of the main problems causing land degradation and crop yield reduction throughout the world (Rath and Rousk, 2015). In the Yellow River Delta (YRD) in China, soil salinization has spread at an unprecedented rate from coastline to inland areas over the past two decades (Zhao et al., 2020) due to sea-level rise and increased groundwater

abstraction (Cubasch et al., 2014). High saline-alkali soils are the essential factor that not only negatively influences vegetation growth (Cui et al., 2009) but also affects soil respiration, soil microbial biomass, and the microbial growth rate (Campbell and Kirchman, 2013; Rath et al., 2019a). Hence, it is important to evaluate the effect of soil salinization on soil microbial community structure for the improvement of saline-alkali lands in the YRD.

Soil salinity has been shown to be the most important factor affecting the global distribution of soil microorganisms (Lozupone and Knight, 2007; Auguet et al., 2010). Salinity is a major factor shaping soil bacterial diversity and composition in many natural habitats (Campbell and Kirchman, 2013; Zhao et al., 2020). For this reason, a salinity gradient is likely to affect soil fungal patterns (Mohamed and Martiny, 2011). In the complex soil ecosystem, fungal diversity has important consequences for ecosystem functions (van der Heijden et al., 2008). For example, mycorrhizal fungi increases nutrient capture by expanding the surface area of plant roots (Kramer et al., 2012). Saprotrophic fungi are involved in organic matter decomposition, and greater saprotrophic fungal diversity increases organic matter decomposition (van der Wal et al., 2013; Schmidt et al., 2019). Pathotrophic fungi affect crop growth, but they also control other plant or fungal pest populations (Vega et al., 2009; Wang and Wang, 2017). Despite their importance to ecosystems, few studies have considered how salinity affects the ecological function of fungi. In particular, the fungal structure and function at different salinities and pH values in the YRD have not been investigated.

It is well known that environmental factors have significant effects on soil fungal communities (Bachelot et al., 2016). For example, soil pH is one of the most important factors affecting soil fungal communities (Geml et al., 2014; Hu et al., 2017), and Maestre et al. (2015) revealed that soil pH was negatively related to fungal diversity at the global scale. Additionally, Geml et al. (2014) observed that soil fungal communities were closely related to the soil carbon (C) and nitrogen (N) contents. In saline-alkali soils of temperate grassland in northern China, our findings suggest that soil pH was negatively correlated with fungal diversity compared with soil salinity and the C/N ratio (soil carbon to nitrogen ratio; Yang et al., 2020). As soil salinization and alkalization frequently co-occur, it is necessary to identify which factor has the greater influence on the composition and diversity of soil fungi in the severe salinization region of the YRD. Groups of fungi with different ecological functions had different relationships with soil properties (Schmidt et al., 2019). For instance, the relative proportion of mycorrhizal fungi was negatively correlated with soil pH whereas animal pathogens were positively correlated with soil organic matter in cropland (Schmidt et al., 2019). Far less is known about how those groups of fungi respond to the variation in soil properties caused by soil salinity.

Although the microbial responses to salinity in saline-alkali lands have become a hot issue (Hu et al., 2016), shifts in structure and function of fungi as determined using soil fungal internally transcribed spacer methods in saline-alkali soils of the YRD have rarely been reported. Traditionally, almost all biodiversity studies of fungal ecology only consider species composition and disregard the interactions among different fungi; however, network interactions could be important to ecosystem processes and functions than species diversity (Zhou et al., 2011). In this study, we measured the soil fungal composition and assessed networks of co-occurrence using high-throughput sequencing technology along a salinity gradient, and we also evaluated the effect of salinity on the ecological function of fungi using FUNGuild software (Nguyen et al., 2016). The aims of the present study were (1) to identify the community composition and the fungal diversity along the salinity gradient, (2) to determine the co-occurrence networks among soil fungi and identify their ecological functions in saline soils, and (3) to evaluate the key soil factors affecting the soil fungal community structure and ecological function.

MATERIALS AND METHODS

Study Sites and Soil Collection

The sampling area was a part of the YRD in northern Shandong on the southern shore of the Bohai Sea (37°54'60"N, 117°57'33"E, elevation 1 m). This area has a semi-humid continental climate characterized by a mean annual air temperature and rainfall of 12°C and 600 mm, respectively. The site has a coastal saline soil with a silt-sand texture. We selected five salinity levels from low to extreme salinization (**Figure 1**). In brief, land dominated by *Setaria viridis* and low-salt tolerant vegetation was selected as low-salinity soil (Low). *Suaeda salsa* and medium-salt tolerant vegetation dominated saline-alkali lands that were selected as medium-salinity soil (Medium). Saline-alkali lands without vegetation growth were selected as high-salinity soil (High), and Extreme-salinity soil (Extreme) was saline-alkali lands with salt crystallization. Maize (*Zea mays*) croplands with low salinity were selected as the control (Non-saline), and these are mainly affected by flooding freshwater. The mean soil electrical conductivity (EC) value ranged from 0.92 (Non-saline) to 1.78 ds/m (Low), to 3.16 ds/m (Medium), to 17.26 ds/m (High), and finally to 34.41 ds/m (Extreme; **Table 1**).

Four transects with a distance of about 3 km represented four repetitions, and five plots (Non-saline, Low, Medium, High, and Extreme) separated at least 500 m from each other were randomly established along each transect (**Figure 1**). To remove plant root disturbance, the bulk soil (0–15 cm) was collected using a five-spot sampling method in each plot (5×5 m^2) in October 2019, and we mixed the samples into one composite sample. In total, 20 samples (five salinity levels × four repetitions) were collected in plastic bags, and the samples were carefully sieved through a 2 mm mesh. Then, we divided the soil samples into two subsamples. One subsample was air-dried for the analysis of soil basic properties, and the other part was stored in a −80°C freezer for microbiological analysis. Soil total carbon (TC) and total nitrogen (TN) concentrations were measured using a CHNS

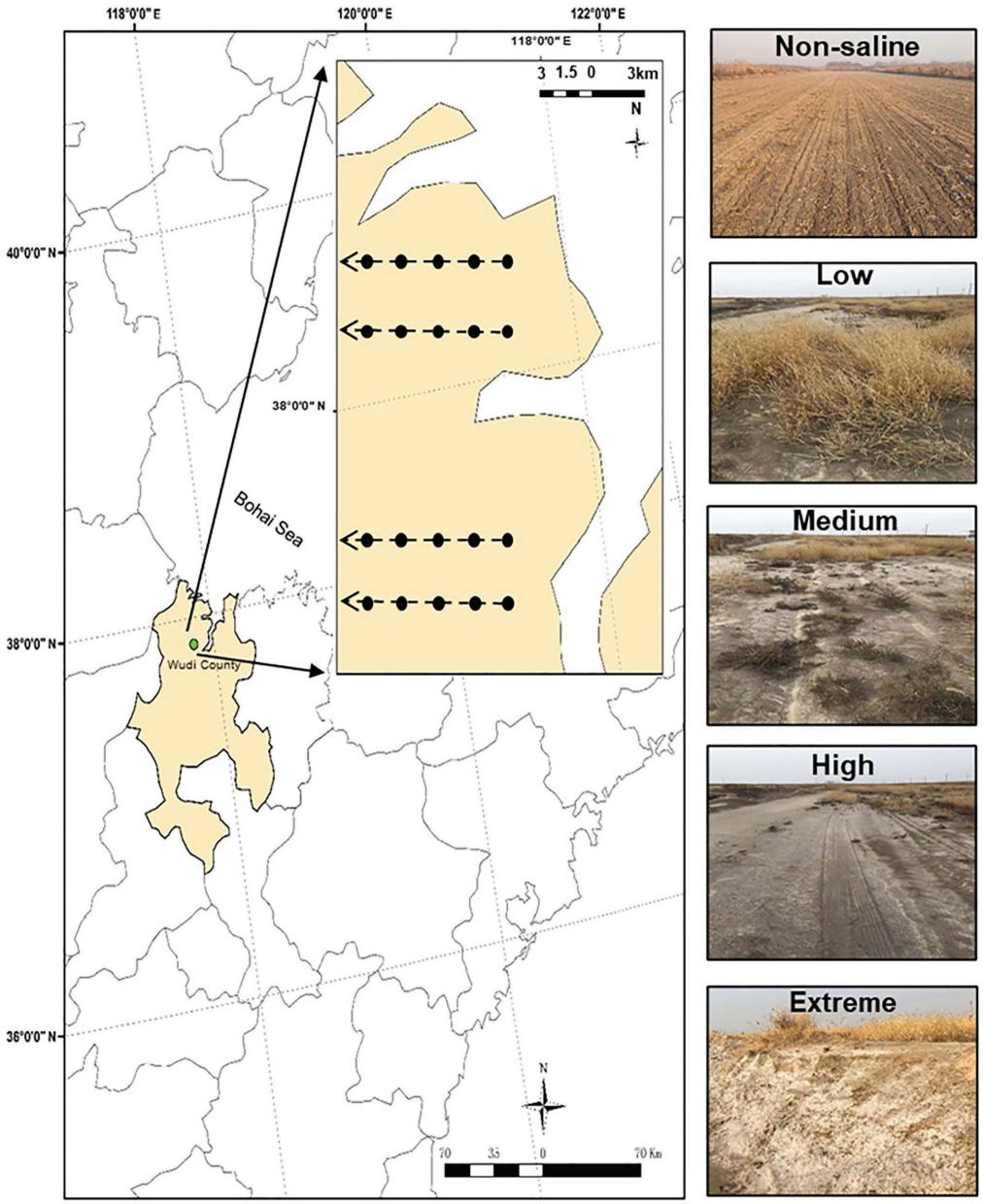

FIGURE 1 | The study region and five salinity gradients with different EC values. The map was created with ArcGIS v 10.2 (http://www.esri.com/arcgis/about-arcgis). Non-saline: non-salted, Low: low-salinity soil, Medium: medium-salinity soil, High: high-salinity soil, and Extreme: extreme-salinity soil.

TABLE 1 | One-way ANOVA of the soil properties of non-salted (Non-saline), low-salinity soil (Low), medium-salinity soil (Medium), high-salinity soil (High), and extreme-salinity soil (Extreme) sites. Values are mean ± SE.

	EC (ds m^{-1})	Salt (%)	pH	BD (g cm^{-3})	TC (g kg^{-1})	TN (g kg^{-1})	C/N ratio
Non-saline	0.92(0.10)e	0.04(0.01)d	8.70(0.05)a	0.96(0.02)d	21.10(1.13)a	0.63(0.05)b	34.70(4.59)d
Low	1.78(0.37)d	0.16(0.05)c	8.18(0.07)c	1.07(0.02)c	17.40(0.50)b	0.85(0.09)a	21.11(2.12)e
Medium	3.16(0.23)c	0.18(0.01)c	8.75(0.04)a	1.23(0.02)b	16.35(1.17)b	0.40(0.04)c	41.59(3.20c)
High	17.26(1.01)b	0.88(0.09)b	8.56(0.01)b	1.32(0.01)a	14.33(0.50)c	0.38(0.08)c	47.96(1.69)b
Extreme	34.41(0.63)a	3.58(0.13)a	8.49(0.01)b	1.34(0.01)a	10.78(0.11)d	0.20(0.01)d	53.88(0.54)a

EC, electrical conductivity; BD, bulk density; TC, soil total carbon; TN, soil total nitrogen; and C/N ratio, soil carbon to nitrogen ratios. In the list, different lowercase letters indicate the significant relationships ($p < 0.05$) among the five salinity gradients using the Duncan's test.

Element Analyzer (Elementar, Germany). Soil pH and EC were measured using a glass electrode in a 1:5 soil: water suspension. Soil bulk density (BD) was calculated using the ring knife method at a 0–15 cm depth. In brief, a foil sampler with a volume of 100 cm^{-3} was used to obtain the samples, which were then dried at 105°C for 24 h. The soil salt content was determined in a mixture with a soil: water ratio of 1:5, and the soil extract was then dried at 105°C for 24 h (Yang et al., 2020).

Fungal DNA Extraction and ITS Gene Sequencing Amplification

The fungal extraction and determination methods refer to our previous research (Li and Yang, 2019). In brief, the genomic DNA was extracted from each soil sample using a FastDNA®SPIN Kit for soil (MP Biomedicals, CA, United States). We accurately weighed 0.30 g soil sample from each treatment. Soil DNA integrity was then detected by 0.8% agarose gel electrophoresis. The non-coding region of fungal internally transcribed spacer (ITS) was amplified using ITS1 (5'-CTTGGTCATTTAGAG GAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') primers (White et al., 1990). The PCR analysis included pre-denaturation at 95°C for 3 min; 27 cycles at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 45 s, and an extension at 72°C for 10 min.

Illumina MiSeq sequencing produced double-ended sequence data (2 × 300) according to standard protocols performed by MajorBio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The obtained sequences were first filtered using the quantitative insights into microbial ecology. Raw FASTQ files were de-multiplexed and quality-filtered with the following criteria: (i) 300-bp reads were truncated at any site with an average quality score <20 over a 50-bp sliding window, and truncated reads shorter than 50 bp were discarded; (ii) exact barcode matching, less than two nucleotide mismatches in the primer, and no ambiguous characters in the read; and (iii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. UCLUST was used to sort the unique sequence set as an operational taxonomic unit with a 97% identity threshold.

Statistical Analysis

One-way ANOVA was used to identify the soil fungal Shannon diversity index, fungal Chao 1 richness index, and soil physicochemical properties of the five salinization levels. The level of significance was defined at $p < 0.05$ using Duncan's test in SPSS (ver. 19.0). Nonmetric multidimensional scaling (NMDS) analysis based on Bray-Curtis similarity matrices was performed to identify the total structural changes in soil fungi, and significance was tested by analysis of similarities (ANOSIM) in PAST (ver. 3.25). We calculated the Jensen Shannon distance (JSD) according to the abundance of fungi at the family level, and the maximization of the Calinski–Harabasz (CH) index was performed to select the optimal number of clusters using the *k*-medoids algorithm (PAM clustering) with R statistical software (ver. 3.6.3) using seven dissimilarity metrics (Tyakht et al., 2013). Redundancy analysis (RDA) was performed to analyze the relationship between the soil physicochemical properties and the whole fungal communities in terms of abundance at the family level. The significance of the effect of each variable was examined using Mantel tests (permutations = 999), and the resulting significance level was tested by the Mantel r statistic and *p* values. We used the Networkx software to establish the co-occurrence networks between families. The networks were constructed by calculating the correlation between families (coefficient was >0.5 and p was <0.01), and we evaluated the correlation information among families according to the transitivity, diameter, average shortest path length, degree and clustering of the networks. Using fungal ITS sequence data, we conducted both a phylogenetic and functional group analysis based on FunGuild (Nguyen et al., 2016) to assign fungal taxa into three nutrition modes – saprotrophy, symbiotrophy, and pathotrophy. The correlations between soil property parameters and the abundances and function of fungi were assessed by Pearson analyses in PAST (ver. 3.25).

RESULTS

Soil Physiochemical Property Responses to Different Salinity Levels

The soil salt, EC, and BD values significantly increased, while significantly lower values of TC were observed as the salinization level increased (**Table 1**; $p < 0.05$). In particular, soil pH was highest in medium salinity soil ($p < 0.05$). Soil TN decreased by 25.9, 52.9, 55.3, and 76.5% in non-saline, medium, high, and extreme salinity soils, respectively, compared with that in low salinity soil ($p < 0.05$). Non-saline, medium, high, and extreme salinity soils exhibited an increased soil C/N ratio of 0.64, 0.97, 1.27, and 1.55 times, respectively, compared with that in low salinity ($p < 0.05$).

Responses of Fungal Communities and Functions to Soil Salinity

The Shannon diversity of soil fungi in extreme salinity soil was significantly lower than that in low salinity soil (**Figure 2A**). In addition, significantly lower values of the soil fungal Chao richness index were observed in extremely saline soil (**Figure 2B**; $p < 0.05$). The NMDS and ANOSIM tests are shown in **Figures 3A**,**B**. NMDS showed that the fungal compositions in low, medium, high, and extreme salinity soils significantly differed from those in non-saline soil (stress = 0.104), and ANOSIM further confirmed that the Bray–Curtis distance between soil samples was greater than that within soil samples ($R = 0.755$, $p = 0.001$). The *Nectriaceae* and *Plectosphaerellaceae* families were the main microflora in the non-saline and low salinity samples (**Figure 4**); however, soil salinization dramatically increased the relative abundance of *Cladosporiaceae* from non-saline to extreme salinity soil. Additionally, the CH index showed that the data naturally separated into two clusters based on the JSD method (**Supplementary Figure S1**), and non-saline

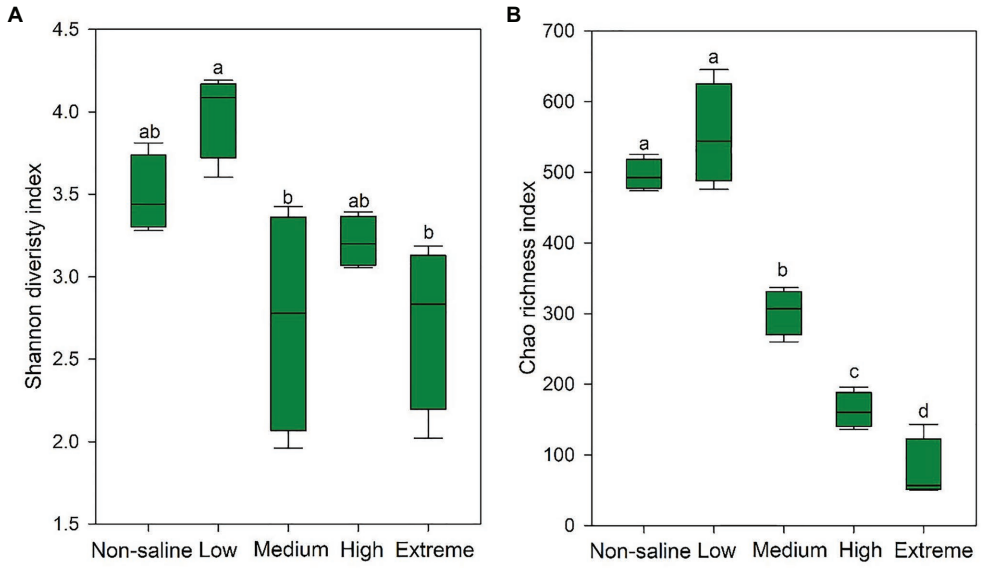

FIGURE 2 | The Shannon diversity **(A)** and Chao richness **(B)** indexes of the soil fungi in five different salinized soils, and the significant relationships at $p < 0.05$ were indicated by different letters using the Duncan's test.

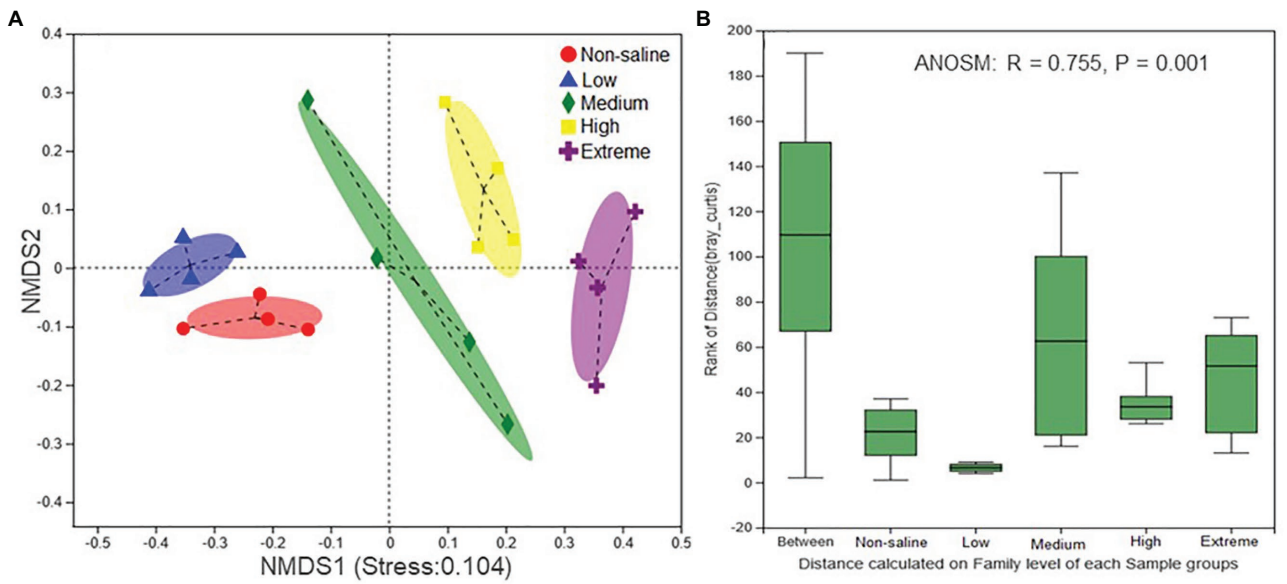

FIGURE 3 | The nonmetric multidimensional scaling (NMDS) ordinations based on the relative abundance of the fungal communities in five different salinized soils **(A)** and the significant differences between the community structures of each salinity level were evaluated using an analysis of similarities (ANOSIM; **B**).

and low salinity belonged to the *Nectriaceae* enterotype based on the CH index (**Figure 5A**), which was significantly higher than that in medium, high, and extreme salinity soils (**Figure 5B**). In contrast, medium, high, and extreme salinity soils belonged to the *Cladosporiaceae* enterotype based on the CH index (**Figure 5A**), which was significantly higher than that in non-saline and low salinity soil (**Figure 5C**). Our network results showed a high level of connectivity within the saline soils with transitivity, diameter, and average shortest path length was 0.57, 5, and 2.39, respectively (**Figure 6**). The degree of family *Nectriaceae* was highest in the networks (**Supplementary Table S1**), and the positive correlations were higher than negative correlations; however, *Cladosporiaceae* was the family most negatively correlated with others. The clustering of *Leptosphaeriaceae* was highest in the networks (**Supplementary Table S1**), indicating it is highly important in saline soils. At the ecological function

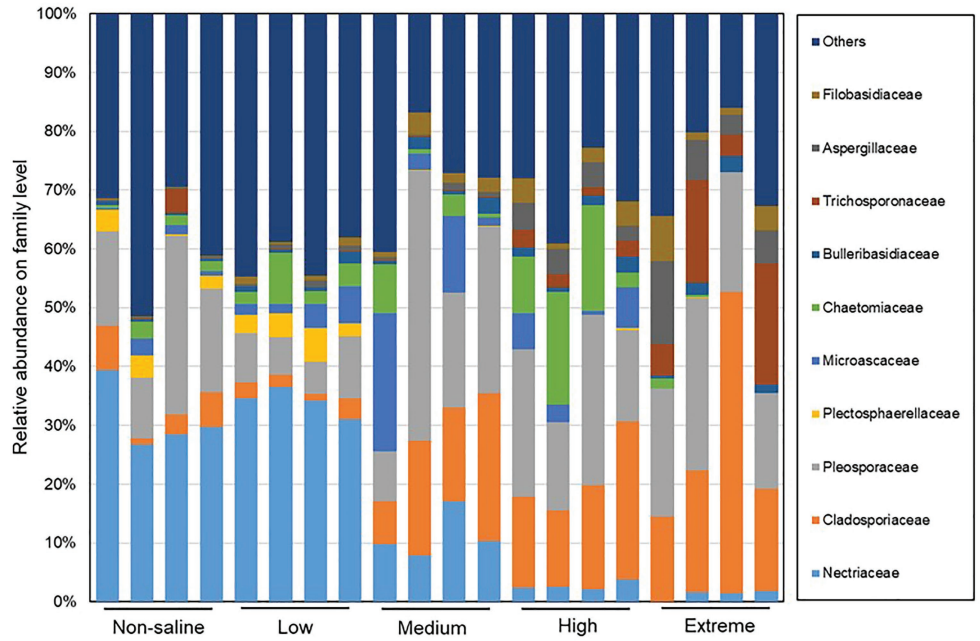

FIGURE 4 | The distribution patterns of the soil fungal family compositions in five different salinized soils.

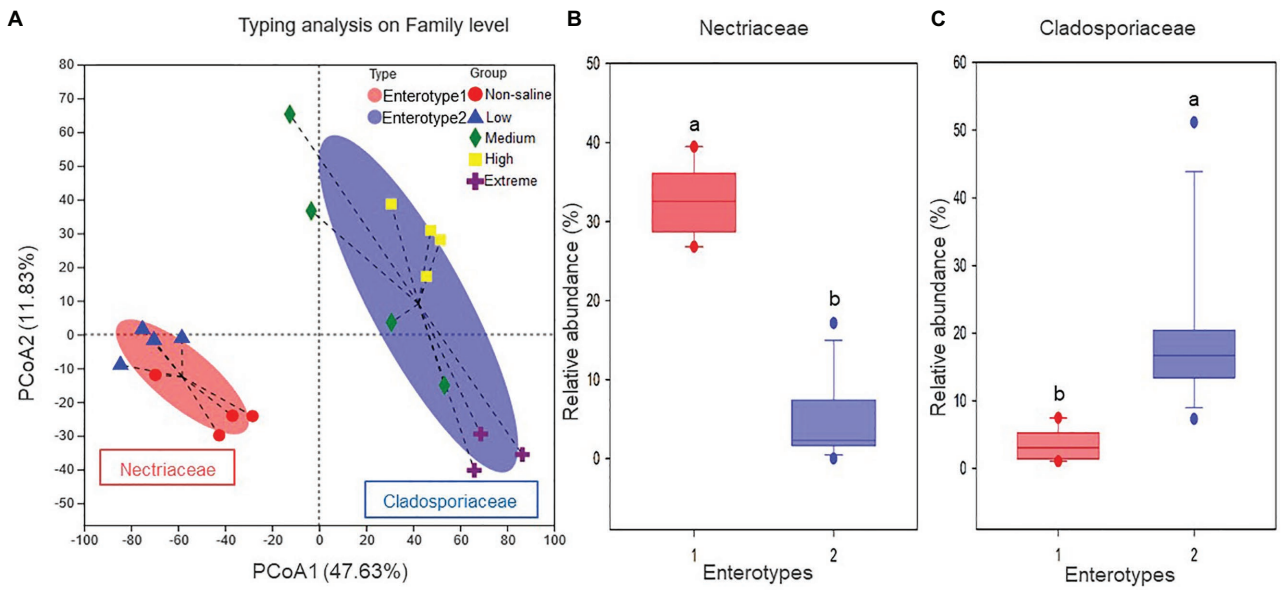

FIGURE 5 | The enterotype analysis for five different salinized soils: **(A)** the clustering on the first two principal components, and the proportions of fungal taxa characteristic of **(B)** *Nectriaceae* and **(C)** *Cladosporiaceae*.

level, plant pathogens had significantly lower numbers under medium salinity soils, and plant saprotrophs and litter saprotrophs were significantly lower in extremely saline soil than in non-saline soil. There were no significant differences in the numbers of animal pathogens and arbuscular mycorrhizae along the salinity gradient (**Figure 7**).

Soil Properties With Different Salinities Regulate Fungal Diversity, Communities, and Functions

A combination of variables explained 59.46% of the variance of the fungal communities, shown in the RDA biplots (**Figure 8**). The Partial Mantel test showed that the soil EC (Mantel $r = 0.61$,

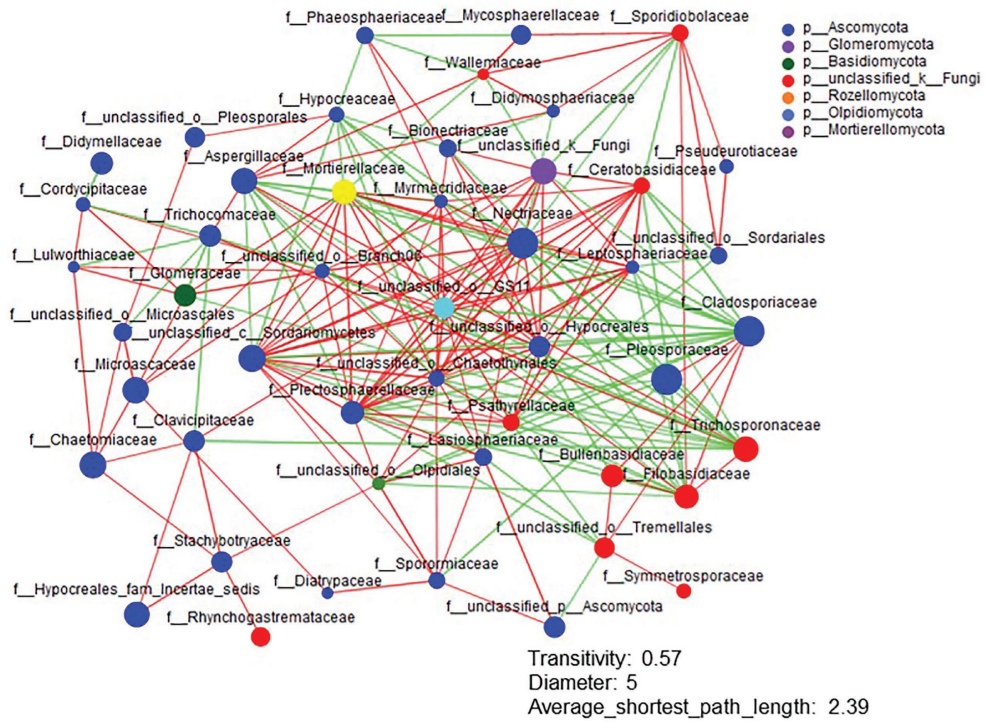

FIGURE 6 | The network analysis of the fungal interactions from non-saline soils to extreme salinity soils. Colors of nodes represent different major phyla, and the node representing the family is shown inside. A red line indicates a positive interaction (coefficient was >0.5 and *p* was <0.01), whereas a green line indicates a negative interaction (coefficient was <–0.5 and *p* was <0.01) between two individual nodes.

p = 0.001), BD (Mantel r = 0.54, p = 0.001), and pH (Mantel r = 0.21, p = 0.021) were positively and negatively correlated with soil salt (Mantel r = 0.48, p = 0.001) and soil TC (Mantel r = 0.55, p = 0.001), respectively, which significantly influenced the fungal communities (**Supplementary Table S2**).

Pearson correlation analyses showed that soil salt, EC, and BD had a weakly significant negative correlation with Shannon diversity ($p < 0.05$). In contrast, an extremely significant negative correlation was observed between soil salt, EC, and BD and Chao richness ($p < 0.001$); however, soil salt, EC, and BD had an extremely significant positive correlation with β diversity ($p < 0.001$; **Figure 9**). Soil pH had a significant negative correlation with the relative abundance of *Plectosphaerellaceae* ($p < 0.05$). The relative abundances of *Nectriaceae* and *Plectosphaerellaceae* were decreased as soil salt, EC, BD, and the C/N ratio increased ($p < 0.05$). By contrast, the relative abundances of *Cladosporiaceae*, *Bulleribasidiaceae*, and *Aspergillaceae* were increased with increases in soil salt, EC, BD, and the C/N ratio ($p < 0.05$). The relative proportion of animal pathogens was positively correlated with salt and EC and negatively correlated with C and N concentrations, and plant pathogens were negatively correlated with soil BD ($p < 0.05$). There was a positive correlation between litter saprotrophs and soil C and N concentrations; however, litter saprotrophs were also negatively correlated with soil salt, EC, and BD ($p < 0.05$; **Figure 9**).

DISCUSSION

Salinity Effect on Soil Properties, Fungal Communities, and Functions

Generally, the soil pH, salt content, and EC exhibit collinearity in saline-alkali soils (Zhao et al., 2018). Here, the soil salt content and EC significantly increased as the salinization level increased, which is in line with our previous study (Yang et al., 2020); however, soil pH showed no significant increase in the salinization level increased in this study, indicating the correlation between soil pH and salt is limited by the range of salt value, especially in the extremely saline soils of the YRD, China (Hu et al., 2016; Zhao et al., 2020). The negative relationship between soil TC and the salt content in the present study is consistent with the results reported by Morrissey et al. (2014) and is mainly due to the poor growth of plants affected by salinity, resulting in a low amount of organic carbon in the soil (Wong et al., 2010). Additionally, in the present study, the soil C/N ratio was higher in saline-alkali soils than in low salinity soils because the decrease in soil TN was higher than that in soil TC. As salinity increased, the soil BD increased significantly, which is consistent with the findings of Zhao et al. (2017), who reported that the soil BD increased along a salinity gradient in a drained coastal wetland, the YRD, China.

In agreement with other studies (Chowdhury et al., 2011; Elmajdoub and Marschner, 2015), salinity changed the microbial

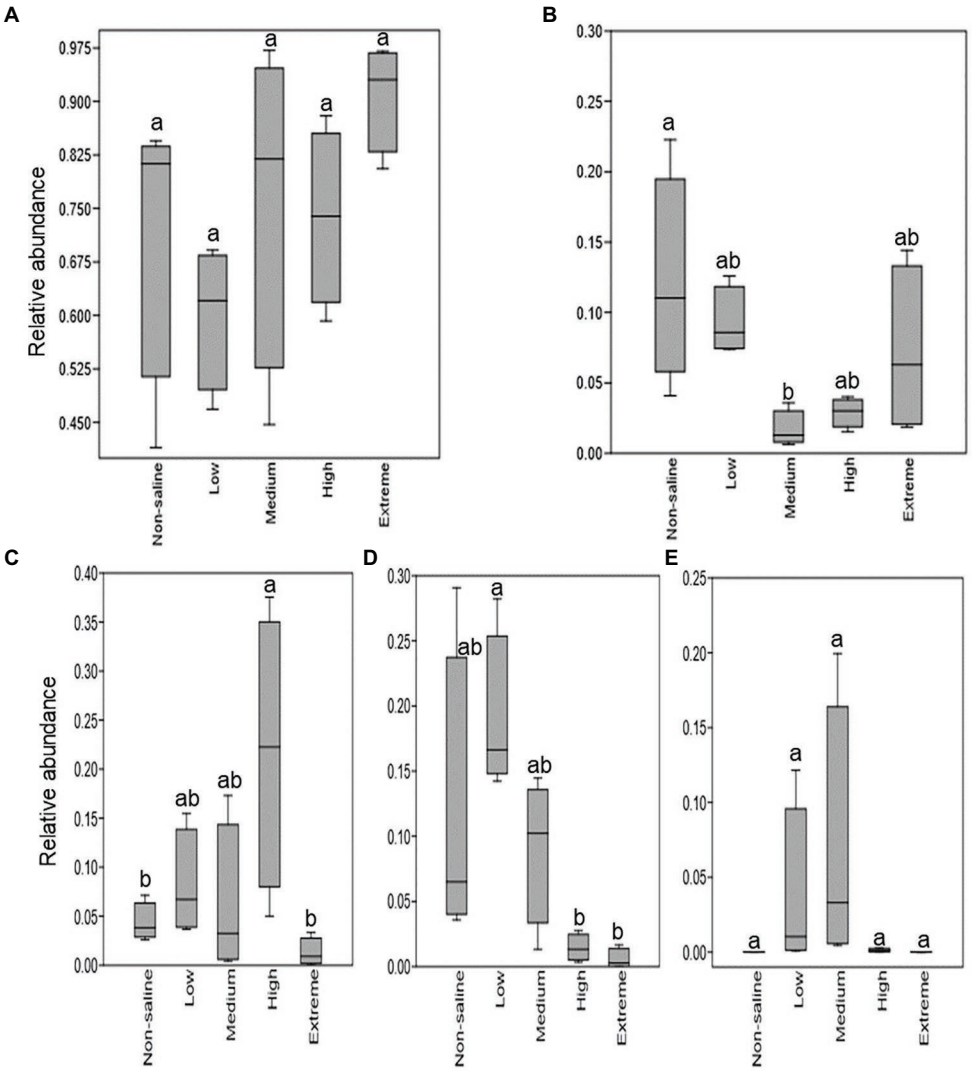

FIGURE 7 | The relative fraction of fungal guilds that show significant differences with five different salinized soils. **(A)** Animal pathogens; **(B)** plant pathogens; **(C)** plant saprotrophs; **(D)** litter saprotrophs; and **(E)** arbuscular mycorrhizal. Different letters indicate statistically significant differences between gradients ($p < 0.05$).

community structure because the difference in their tolerance to salinity. Our previous study found that Shannon diversity values in saline-alkali soils from grassland were significantly lower than those in low salinity soils (Yang et al., 2020), which was also confirmed in the present study. There was a weakly significant correlation between Shannon diversity and the soil salt content, and the similar salt content in low and medium salinity soils may not have been sufficient to cause detectable Shannon diversity in the present study. In addition, significantly lower values of the Chao richness index were observed in extreme salinity soil, which showed an extremely significant negative correlation with the soil salt content. Numerous studies have shown that fungal communities are influenced by soil salinity (Mohamed and Martiny, 2011; Krishnamoorthy et al., 2014). High soil salinity increased the relative abundance of the fungal phylum *Ascomycota* (Kim et al., 2019; Yang et al., 2020). Our results confirmed this: the fungal phylum *Ascomycota* and the related families *Cladosporiaceae* were significantly more abundant in high-saline-alkali soils, whereas, *Nectriaceae* and *Plectosphaerellaceae*, assigned to *Ascomycota*, were significantly less abundant in high saline-alkali soils, indicating an inconsistent response within the phylum *Ascomycota*, suggesting that shifts in community composition were mostly driven by shifts in soil salinity, with more salt-tolerant species (*Cladosporiaceae*) replacing less salt-tolerant ones (*Nectriaceae* and *Plectosphaerellaceae*; Rath et al., 2019b). It is difficult to explain those inconsistent responses, and there are few studies on the mechanism and function of salt tolerance of soil fungi. We here report that many species that form endospores and are thus able to survive in extreme environments and many of these species are known to be salt tolerant (Takami, 2011). We speculate that the ability to form spores might give *Cladosporiaceae* an advantage and allow them

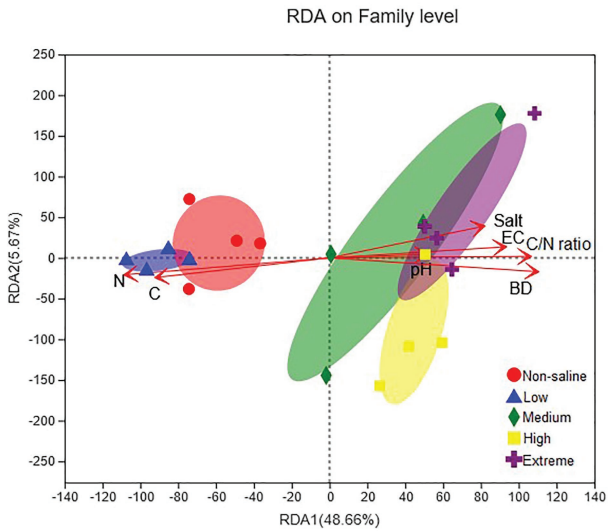

FIGURE 8 | Redundancy analysis (RDA) showing the impact of soil physiochemical properties (pH, EC, salt content, BD, TC, TN, and C/N ratio) on the fungal community structure. The significance of the effect of each property was assessed using Partial Mantel tests (permutations = 999).

to survive the acute effects of salt exposure and grow more abundant after other fungi have died off. This must be studied in detail.

The recent report by Wang et al. (2019) indicated that the adjustment of microbial interactions could be a strategy by which microbes cope with intense salinity and alkalinity stresses. Generally, positive links imply cross feeding and niche overlap, while negative relationships represent competition in the network (Zheng et al., 2017). In the present study, more positive links in family *Nectriaceae* were observed in the co-occurrence networks between species; however, *Cladosporiaceae* was the family most negatively correlated with others, indicating their crucial roles in competition for nutrients, water, and dissolved oxygen under restricted resource conditions in saline alkaline soils (Wang et al., 2019). In soil ecosystems, fungi comprise various ecological guilds (Nguyen et al., 2016). We conducted a functional group analysis based on the recently developed open annotation tool FunGuild (Nguyen et al., 2016) to assign fungal taxa to three ecologically functional groups–saprotrophy, pathotrophy, and symbiotrophy. Saprotrophic fungi (e.g., plant and litter saprotrophs) grow throughout the soil–litter interface, serve as the primary agents of plant litter decomposition (Crowther et al., 2012). Specifically, plant saprotrophs and litter saprotrophs were significantly less abundant along a salinity gradient, which was conducive to the non-saline (*Zea mays*) and low salinity soils (*Setaria viridis*) with high plant and litter biomass. Thus, we inferred that constantly reduced saprotrophic fungi in saline soils would decrease the rate of decomposition of plant litter and old soil C, which would eventually affect soil organic C and N turnover and accumulation (Yang et al., 2017). However, no significant differences in the numbers of animal pathogens were observed along a naturally inhomogeneous salinity gradient in this study. The decrease in soil nutrients (TC and TN) caused by soil salinization indirectly increased the number of animal pathogens. These reports were further supported by Chen et al. (2019), who showed that the relative abundance of animal pathogens was negatively associated with nutrition substrates.

Key Properties Affecting Soil Fungal Communities and Functions

Soil fungal communities are significantly affected by environmental factors (Leff et al., 2015; Bachelot et al., 2016). Our previous study suggested that the best indicator of soil structure quality (Pagliai and Vignozzi, 2002), soil total porosity (calculated from soil BD), can influence soil fungal communities (Yang et al., 2019). Soil salinization can significantly increase soil BD, reduce soil porosity, and indirectly regulate soil microbial structure (Zhao et al., 2017). We observed a stronger positive correlation between soil salinity and soil BD, indicating that salinization changes soil from an aerobic environment (more oxygen) to anaerobic environment (less oxygen). Under aerobic conditions, *Nectriaceae* and *Plectosphaerellaceae* were characterized by high abundance, which indicated a high demand for oxygen; however, anaerobic conditions were conducive to the growth of *Cladosporiaceae*, *Bulleribasidiaceae*, and *Aspergillaceae*. In addition to soil BD, many studies have implied that soil pH is one of the most important factors affecting soil fungal communities (Maestre et al., 2015; Hu et al., 2017). Zhao et al. (2018) suggested that pH is an equally important environmental factor controlling the bacterial community structure as salinity in northwestern China. In saline-alkali soils of the temperate grasslands in northeastern China, our previous findings suggested that soil pH was negatively correlated with fungal diversity compared with soil salinity and the C/N ratio (Yang et al., 2020). However, in the present study, soil salinity (EC and salt content) had a stronger effect on the soil fungal communities than the soil pH and soil TC according to the Mantel tests test and the Pearson correlation analyses. Results obtained from 16S rRNA high-throughput sequencing further strengthened our observations in the YRD. Zhao et al. (2020) reported a slight influence of pH on bacterial community compositions and diversities. Soil pH showed a significant correlation only with the abundance of *Cytophagia*. Our observations are in line with the above, where soil pH significantly negatively correlated with only the relative abundances of *Plectosphaerellaceae*. We speculate that soil microorganisms in northeastern China are mainly affected by soil pH (Li and Yang, 2019; Yang et al., 2020), compared with soil pH and salinity in northwestern China (Zhao et al., 2018), and salinity (soil EC and salt content) in the YRD (Zhao et al., 2020).

Different fungi with different ecological functions had different relationships with soil properties, and Schmidt et al. (2019) reported that the relative proportion of arbuscular mycorrhizae was negatively correlated with soil pH (neutral soil) in agroecosystems; however, in this study, soil pH (highly alkaline

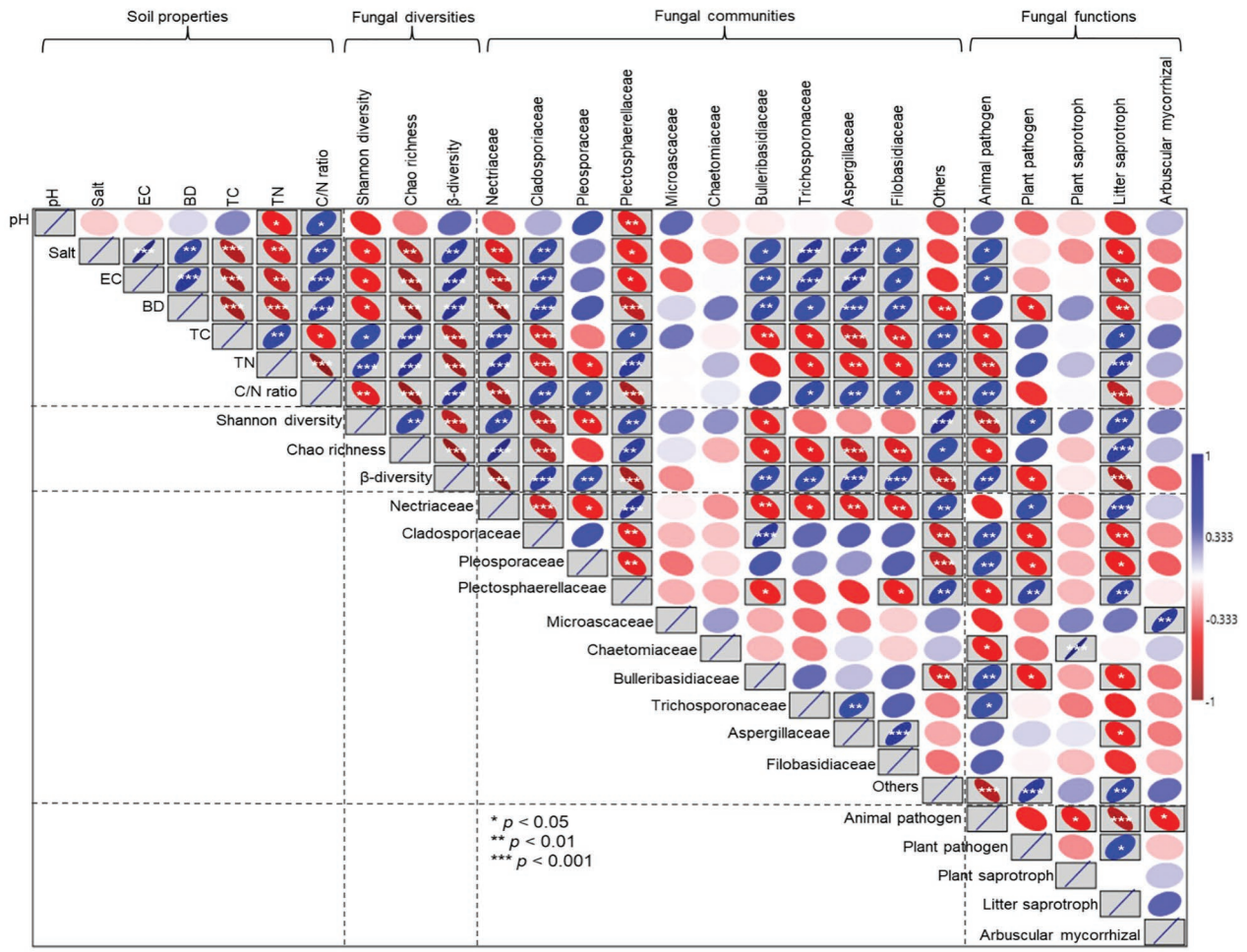

FIGURE 9 | Pearson correlation analyses between soil property parameters and fungal diversity, the relative abundances of specific fungal families, and ecological functions. EC, electrical conductivity; TC, total carbon; TN, total nitrogen; and C/N (soil total carbon/nitrogen). The blue upward sloping ellipse indicates the positive correlation, whereas the red downward sloping ellipse indicates negative correlation. The width of the ellipse represents the level of correlation. *, **, and *** in the box indicate the significance along the paths at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively.

soil) had no significant correlation with the relative proportion of arbuscular mycorrhizae, indicating that different range of pH values had different effects on soil arbuscular mycorrhizae. A study by Dighton (2003) showed that the soil carbon decomposition rates were primarily regulated by fungal saprotrophs, and the relative increase in saprotrophs was associated with increased nutrient content (Schmidt et al., 2019). Our results showed that the relative proportion of litter saprotrophs was positively correlated with soil C and N concentrations in salinity soils of YRD. In particular, the gradient of saline alkali in the experimental plot increased unevenly, and the soil salt content in low and medium saline soils showed no significant difference. In this way, animal pathogens showed no significant difference among gradients, but they were significantly positively correlated with soil salt content. Our results suggest that soil salinity decreased the abundance of litter saprotrophs and increased the abundance of animal pathogens, which increased our understanding of the impact of salinization on soil health. We recommend further investigation into the ecosystem functions of soil fungi in the extremely saline-alkaline soils.

CONCLUSION

Our study explored the distribution patterns of soil fungal communities and diversities in the extremely saline-alkaline soils of the YRD. The soil salt, EC, and BD values significantly increased, while significantly lower values of soil TC and TN were observed as salinization increased. Significantly lower values of the Shannon and Chao indexes were observed in extremely saline soil. Additionally, the CH index showed that the data naturally separated into two clusters based on the JSD method, and the relatively high levels of the families *Nectriaceae* and *Cladosporiaceae* distinguished two of the clusters, indicating two enterotypes of low and high salinity soils,

respectively. The *Nectriaceae* and *Cladosporiaceae* were the families most positively and negatively correlated with others, respectively, based on the network analysis. Plant saprotrophs and litter saprotrophs were significantly lower in extremely saline soil than in non-saline soil. Our results suggest that soil salinity is a primary factor that shapes soil fungal communities and provides a framework for future research to deeply analyze the mechanism and function of salt tolerance of soil fungi in saline-alkaline environments.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, SRP269059.

AUTHOR CONTRIBUTIONS

CY and JS designed the study. CY participated in sample collection, performed the experiment, and wrote the manuscript with the help of JS. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.594284/full#supplementary-material

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Protection of Photosynthesis by Halotolerant *Staphylococcus sciuri* ET101 in Tomato (*Lycopersicon esculentum*) and Rice (*Oryza sativa*) Plants During Salinity Stress: Possible Interplay Between Carboxylation and Oxygenation in Stress Mitigation

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Taj Z and Challabathula D (2021) Protection of Photosynthesis by Halotolerant Staphylococcus sciuri ET101 in Tomato (Lycopersicon esculentum) and Rice (Oryza sativa) Plants During Salinity Stress: Possible Interplay Between Carboxylation and Oxygenation in Stress Mitigation. Front. Microbiol. 11:547750. doi: 10.3389/fmicb.2020.547750

Tomato (*Lycopersicon esculentum*) and rice (*Oryza sativa*) are the two most important agricultural crops whose productivity is severely impacted by salinity stress. Soil salinity causes an irreversible damage to the photosynthetic apparatus in plants at all developmental stages leading to significant reduction in agricultural productivity. Reduction in photosynthesis is the primary response that is observed in all glycophytic plants during salt stress. Employment of salt-tolerant plant growth-promoting bacteria (PGPB) is an economical and viable approach for the remediation of saline soils and improvement of plant growth. The current study is aimed towards investigating the growth patterns and photosynthetic responses of rice and tomato plants upon inoculation with halotolerant PGPB *Staphylococcus sciuri* ET101 under salt stress conditions. Tomato and rice plants inoculated with PGPB showed increased growth rate and stimulated root growth, along with higher transpiration rates (E), stomatal conductance (g_s), and intracellular CO_2 accumulation (Ci). Additionally, correlation of relative water content (RWC) to electrolyte leakage (EL) in tomato and rice plants showed decreased EL in inoculated plants during salt stress conditions, along with higher proline and glycine betaine content. Energy dissipation by non-photochemical quenching (NPQ) and increased photorespiration of 179.47% in tomato and 264.14% in rice plants were observed in uninoculated plants subjected to salinity stress. Furthermore, reduced photorespiration with improved salinity tolerance is observed in inoculated plants. The higher rates of photosynthesis in inoculated plants during salt stress were accompanied by increased quantum efficiency (ΦPSII) and maximum quantum yield (F_v/F_m) of photosystem II. Furthermore, inoculated plants showed increased carboxylation efficiency of RuBisCO, along with higher photosynthetic electron transport rate (ETR) (J) during salinity stress. Although the total cellular ATP levels are drastically

affected by salt stress in tomato and rice plants along with increased reactive oxygen species (ROS) accumulation, the restoration of cellular ATP levels in leaves of inoculated plants along with decreased ROS accumulation suggests the protective role of PGPB. Our results reveal the beneficial role of *S. sciuri* ET101 in protection of photosynthesis and amelioration of salinity stress responses in rice and tomato plants.

Keywords: plant growth-promoting bacteria, salinity stress, *Oryza sativa*, *Lycoperiscon esculentum*, photosynthesis, photorespiration

INTRODUCTION

Salinity stress is the major environmental problem all over the world due to which the cultivable land area is decreased, and drastic reduction in root length, biomass, and growth is observed causing a decline in crop yields (Deinlein et al., 2014; Janda et al., 2016; Kumar et al., 2020). The limitation of plant growth under salinity conditions is primarily due to reduction in photosynthesis rate and high intracellular accumulation of Na^+ ions, which interfere with various physiological processes (Assaha et al., 2017; Analin et al., 2020). Decrease in net photosynthetic rate is associated with decreased availability of CO_2 as a result of diffusion limitations and decrease in the contents of photosynthetic pigments (Tholen and Zhu, 2011). Decrease in intracellular CO_2 concentration and photosynthesis during salinity stress due to stomatal closure leads to lesser availability of CO_2 to the RuBisCO enzyme binding, thereby enhancing the rate of photorespiration (Igamberdiev, 2015). Salinity stress-induced accumulation of excess salts is known to affect the function of photosystems by modulating the photosynthetic proteins in chloroplasts causing irreversible damage to the photosynthetic apparatus at all developmental stages in glycophytic plants (Sun et al., 2016; Li et al., 2019; Rodríguez-Ortega et al., 2019; Zhang et al., 2020). Adaptation of plants to salinity stress involves modification of complex physiological traits, metabolic pathways, and gene networks (Nounjan et al., 2012; Gupta and Huang, 2014; Ghorbani et al., 2018; Kumar et al., 2020). The key photosynthetic processes including RuBisCO enzyme activity, ATP generation, electron transport rate (ETR), and efficiency of light capture in the photosystems are seriously affected by salt stress (Chaves et al., 2009). Soil oxygen deficiency due to osmotic effect and Na^+/Cl^- toxicity is also observed in plants exposed to salinity stress (Barrett-Lennard and Shabala, 2013). The prevalence of hypoxia/anoxia conditions in root zone due to hindrance of O_2 diffusion in soil leads to reduction of ATP formation and diminished growth (Kozlowski, 1997; Barrett-Lennard, 2003). The damage to the photosynthetic apparatus caused by salinity is reflected by change in chlorophyll a (Chl a) fluorescence parameters, photorespiration, and carboxylation/oxygenation efficiency (Pieters et al., 2003). Plants protect themselves against photodamage by increasing the non-photochemical quenching (NPQ) energy dissipation, thereby reducing the relative quantum efficiency of PSII (ΦPSII) to maintain adequate balance between the photosynthetic electron transport and carbon metabolism. The downregulation of the photochemical linear electron transport during salt stress conditions limits the oxidative stress, and increased cyclic electron flow increases the photoprotective energy dissipation (Stepien and Johnson, 2009).

A potential and conventional strategy to minimize the soil salinization is microbial-assisted amelioration of salt-induced damage (Prittesh et al., 2020). Among the microbiota, a promising perspective to improve plant salinity tolerance is involvement of halotolerant plant growth-promoting bacteria (PGPB) on to salt-sensitive plant species (Numan et al., 2018; Bakka and Challabathula, 2020). The main objective of using halotolerant PGPB is to increase plant growth, development rate, yield, and increased tolerance toward salinity. Many reports suggested the involvement of PGPB for ameliorating the abiotic stress-induced damages in plants. Isolation and characterization of potential bacteria with inherent capability of salt tolerance will be useful for inoculation to plants to increase crop productivity in saline regions (Ahmad et al., 2013; Kang et al., 2019; Bakka and Challabathula, 2020; Prittesh et al., 2020). The genus *Staphylococcus* has many species, which have been isolated from diverse environments and characterized as effective halotolerant and plant growth-promoting rhizobacteria (Nanjani and Soni, 2014; Shahid et al., 2019).

Tomato and rice are the two most important crops worldwide that serve as excellent model systems to understand their physiological changes when exposed to abiotic stress factors. The adjustment in photosynthetic process is a protective mechanism adapted by plants to tolerate salt stress (Parida and Das, 2015). However, the adjustment of plant photosynthetic processes due to the inoculation of bacteria under salt stress is rarely known. Whether the inoculation of halotolerant bacteria modulates the sensitivity of photosynthesis process to salt stress aiding in the improvement of plant stress tolerance is still unclear. Thus, the situation warrants for better understanding of photosynthesis process during salinity stress with bacterial inoculation.

In this article, we present data on the effects of salinity stress on photosynthesis in tomato (*Lycoperiscon esculentum* L.) and rice (*Oryza sativa* L.) under the influence of halotolerant bacteria, *Staphylococcus sciuri* ET101. Our study is focused on the physiological changes at the level of photosynthesis and photorespiration processes affected by different levels of salinity stress. We used a moderately salt-sensitive tomato and salt-sensitive rice plants to observe the physiological changes that can be attributed to acclimation by salinity stress in the presence of halotolerant, plant growth-promoting bacteria, *S. sciuri* ET101. Based on the results, we discuss the role of interplay between the carboxylation and oxygenation efficiency of RuBisCO as

a possible factor for stress mitigation by the influence of bacterial inoculation.

MATERIALS AND METHODS

Isolation and Characterization of Halotolerant Bacterial Isolate ET101

The bacterial isolate ET101 was isolated from the rhizosphere of common glasswort (*Salicornia europaea* L.) grown in the sandy soil of salt pan areas of Tuticorin district, Tamil Nadu, India. The 15-cm deeper bulk soil from the soil surface and soil associated with plant roots was used for the isolation of halotolerant bacteria. The isolation was carried by serial dilution plate technique followed by pure culture technique in high salt (2.5 M NaCl) supplemented LB agar medium. The preliminary characterization of the bacterial isolate ET101 based on physiological and biochemical characteristics was carried out according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The molecular characterization of the ET101 isolate was carried out using colony polymerase chain reaction (PCR). Amplification of 16S rDNA gene was performed by using universal primers, 27F (5′-AGAGTTTGATCCTGGTCAGAACGCT-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTTCACCCC-3′) (Weisburg et al., 1991). The PCR products were purified (PCR purification kit, Qiagen), and further sequencing of the amplicons was performed (Xcelris Genomic Services Pvt. Ltd., India). The bacterial isolate ET101 was screened for a wide array of PGP traits growing at different NaCl concentrations (0.08, 1.35, 1.7, 2.05, and 2.5 M). The production of indole-3-acetic acid (IAA) supplemented with or without 0.05% L-tryptophan was determined using the method of Gordon and Weber (1951). The quantitative estimation of gibberellic acid (GA) production was carried out by the method of Borrow et al. (1955). The assay of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase) activity was determined by estimating the release α-ketobutyrate by the method of Honma and Shimomura (1978) and ammonia production by the method of Cappuccino and Sherman (1992).

Plant Material, Bacterial Inoculation, Salt Stress Treatment, and Root Colonization

Tomato (*L. esculentum* cv. PKM-1*)* and rice (*O. sativa* cv. Aiswarya) seeds procured from Sri Venkateswara Agro Store, Tiruchirappalli, Tamil Nadu, India, and Regional Agricultural Research Station, Pattambi, Kerala, India, were surface sterilized using 0.4% sodium hypochlorite for 2 min followed by 70% ethanol for 2 min and thoroughly washed with sterile deionized water. For inoculum preparation, the bacterial isolate was grown in LB broth for 24 h at 30°C. After centrifugation, the obtained biomass was washed with phosphate-buffered saline (PBS) for three times and finally adjusted the absorbance of 0.8 at 600 nm corresponding to 10^8 colony-forming units (CFU) mL^{-1} with sterile water. To ensure inoculation of sufficient bacterial cells per plant, bacterial inoculation is done to seeds and the plants germinated from the inoculated seeds. For seed inoculation, the surface sterilized seeds were kept immersed in the isolated bacterial suspension (10^8 CFU mL^{-1}) overnight with mild shaking using bacteriological shaker incubator. The bacterial-treated seeds were sown for germination in sterile soil-containing pots to obtain plantlets of 15 days. Equally grown plantlets were transferred to the plastic pots (500-mL capacity for tomato and 200 mL for rice) containing sterile potted soil mixture. The potted soil mixture consisted of three parts of red soil, one part of soilrite, one part of vermiculite, and one part of perlite for growing tomato and rice plants. While the asbestos-free vermiculite is composed of SiO_2 (40%), AlO_3 (18%), FeO_3 (11%), K_2O (5%), MgO (13%), and Na_2O (1%) and trace amounts of P_2O_5, C_4O_3, MgO_4, the soilrite mix TC is composed of 75% Irish peat moss and 25% horticulture grade expanded perlite having pH range between 5.0 and 6.5. Perlite is primarily composed of silicon (Si) and does not have significant nutrient content. The red soil was purchased from the local vendor mainly used for growing horticultural plants and soilrite, vermiculite, and perlite were purchased from Keltech Energies Ltd., India. The texture of the red soil is fine loamy with dark red to brown color and with moderate porous nature. The soil mixtures procured from the same vendors were used throughout the study. The transferred plantlets were kept in observation for healthy growth up to 7 days. For bacterial inoculation in plants, the tomato and rice plants were inoculated with ET101 culture using the method of soil drenching, and the plant growth promotion by bacterial inoculation was evaluated. Each treated pot was inoculated with 5 mL of bacterial inoculum for 3 days once, whereas the control plants were treated with sterile water. For imposing salinity stress to plants, the initial soil water content in pots was equally maintained at uniform levels in both the plants with or without ET101 inoculation. The effect of salinity stress in uninoculated and inoculated plants was evaluated by irrigating the plants with different concentrations of NaCl solution (0 mM [S0], 200 mM [S2], and 400 mM [S4]) with 1/10th volume (50 mL for tomato plants and 20 mL for rice plants) of respective pots for every 2 days. The stress treatments were conducted for 5 days to rice plants and 10 days to tomato plants. Plants were grown in a growth chamber with artificial light provided by fluorescent tubes at 12:12-h light–dark period with the incident Photosynthetically Active Radiation (PAR) at leaf level of 140 μmol photons m^{-2} s^{-1} light intensity in controlled laboratory conditions. The plants were assessed based on phenotypic and physiological changes caused by the bacterial inoculation upon salinity stress. Temperature ranged between 22°C at night and 28°C during the light period. The changes in growth and stress tolerance were measured after the salinity stress treatment of 5 days to rice plants and 10 days to tomato plants. Root colonization by bacterial isolate ET101 was determined according to the protocol of Hossain et al. (2008). The root-associated soil, the surface of plants roots, and the homogenized root tissues were used as sources for enumeration of bacterial concentration in inoculated plants. The roots were thoroughly washed with running tap water to remove adhering soil particles and then were rinsed with sterile distilled water and surface sterilized using 70% ethanol and blotted to dryness. Roots were homogenized in PBS

using sterile mortar pestle. Serial dilutions were prepared on LB plates containing 8% NaCl concentration, and the number of CFUs was determined after 24 h of incubation at 28 ± 2°C (**Supplementary Figure S6**).

Measurement of Leaf Water Status, Electrolyte Leakage, and Osmolyte Production

The relative water content (RWC) and electrolyte leakage (EL) were measured in the fresh, fully developed leaves of the plant. RWC was determined using the equation previously described by Teulat et al. (2003). RWC (%) = (FW – DW)/(TW – DW) × 100, where FW is fresh weight; TW, turgid weight; and DW, dry weight of the leaf used. The leaves were harvested after prior treatments from the plants, and the fresh weights were measured using an electronic balance. After the fresh weight measurements, the leaves were immersed in Petri dishes containing sterile distilled water for overnight at room temperature. The turgid weight was determined by surface drying of the turgid leaves by using absorbent paper and measuring the leaf weight. For measuring the dry weight, the leaves were oven dried at 80°C overnight and weighed. For the measurement of EL, the leaves were cut, and discs were transferred into test tubes containing 25 mL of distilled water. The test tubes were incubated for 8 h and vortexed occasionally for 10 s, and the electrical conductivity (EC_1) of the solution was measured using Systronics conductivity meter 304 at temperature of 25 ± 2°C in 2-μS range. Finally, the solution inside the test tubes was boiled for 20 min, and the electrical conductivity (EC_2) was measured again. Electrolytic leakage percentage was calculated by the equation of Dionisio-Sese and Tobita (1998). Distilled water served as blank. EL (%) = EC_1/EC_2 × 100. Ninhydrin was used to determine the proline contents of plants (Bates et al., 1973). The proline content in each treatment samples was determined by measuring the absorbance at 520 nm. The amount of proline was extrapolated using a standard curve prepared with L-proline and expressed in μg g^{-1}. The production of glycine betaine by the plants during different salt stress was estimated using the method of Grieve and Grattan (1983). The values were extrapolated using betaine hydrochloride as standard and expressed as μ g L^{-1}.

Measurement Photosynthetic Pigments

The chlorophyll was extracted from the fresh and fully expanded leaf samples (100 mg) with 80% acetone at 4°C under dark (Moran and Porath, 1980). Chlorophyll was estimated spectrophotometrically by measuring the absorbance of Chl a, chlorophyll b (Chl b), and carotenoid at 663, 645, and 480 nm, respectively, by using the equation of Arnon (1949): Chl a (mg/g^{-1}) = [12.7 (Ab663) – 2.69 (Ab645)] * V/1,000 * W; Chl b (mg/g^{-1}) = [22.9 (Ab645) – 4.68 (Ab663)] * V/1,000 * W; Total Chlorophyll (mg/g^{-1}) = [Chl a + Chl b]; Carotenoid (mg/g^{-1}) = [Ab480 + (0.114 * Ab663) – (0.638 * Ab645)] * V/1,000 * W, where V is final volume of chlorophyll extracted in 80% acetone; and W, fresh weight of the leaf used.

Measurement of Gas Exchange Parameters

The *in vivo* measurements of net photosynthesis rate (P_N), stomatal conductance (g_s), and transpiration (E) of the plants subjected to salt treatment with or without bacterial inoculation were done as light-response curves using a portable open-flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE, United States) at the levels of photosynthetic photon flux density (PPFD) of 30, 50, 75, 100, 150, 200, 500, 750, 1,000, and 1,500 μmol photons m^{-2} s^{-1} in a decreasing order at 25°C and the CO_2 concentration of 400 μmol CO_2 mol^{-1} air, ambient air humidity, and leaf temperature of 25°C. In addition to CO_2 assimilation rate, the value of ratio of CO_2 assimilation (P_N) to substomatal internal CO_2 content (Ci) is shown to recognize whether stomatal or non-stomatal limitation of photosynthesis is present.

Measurement of Chlorophyll Fluorescence Parameters

The Chl *a* fluorescence parameters were determined by using a fluorescence chamber head (LI-6400-40, LI-6400XT, LI-COR, Lincoln, NE, United States) integrated with the portable open-flow gas exchange system. While weak modulated measuring beams (0.03 μmol m^{-2} s^{-1}) were used for illuminating the dark-adapted leaves (20 min) to obtain the initial fluorescence (F_0), the saturating white light pulses of 8,000 μmol photons m^{-2} s^{-1} were applied for 0.8 s to ensure maximum fluorescence emissions (F_m). In light-adapted leaves, the steady-state fluorescence yield (F_s) was measured following a saturating white light pulse (8,000 μmol m^{-2} s^{-1}, 0.8 s) that was applied to achieve the light-adapted maximum fluorescence (F'_m). The actinic light was then turned off, and far-red illumination was applied (2 μmol m^{-2} s^{-1}) to measure the light-adapted initial fluorescence (F'_0). The NPQ was calculated as NPQ = (F_m/F_m') – 1. The actual PSII quantum yield was computed as Φ_{PSII} = (F'_m – F_s)/F'_m from which the apparent ETR (J) was calculated as J = Φ_{PSII} * PPFD * f * α, where PPFD was 1,500 μmol m^{-2} s^{-1}, f is a factor that accounts for the partitioning of energy between the PSI and PSII and is assumed to be 0.5 (indicating that the excitation energy is distributed equally between the two photosystems), and α is the leaf absorbance by the photosynthetic tissues and is assumed to be 0.87 (Maxwell and Johnson, 2000). All the measurements were determined on the middle leaf attached to each plant (three leaves per treatment). Photorespiration (P_R) was estimated as 1/12 [J – 4 × (P_N + R_D)] according to Valentini et al. (1995), where P_N is net photosynthesis rate and R_D is respiration rate in dark. Gas exchange was also measured in dark-adapted leaves after overnight dark incubation to obtain dark respiration rate (R_D) (μmol CO_2 released m^{-2} s^{-1}). The leaves were dark adapted for 20 min in the chamber head for measuring the dark respiratory rates (CO_2 efflux) using the portable photosynthesis system (LI-6400 XT; LI-COR Inc., Lincoln, NE, United States) in darkness. The CO_2 efflux rates were measured for 20 min with 1-min interval and were calculated as the difference between the ambient CO_2 and sample

CO_2. The CO_2 concentration in the chamber ambient air (Ca) was maintained at 400 μmol mol^{-1}.

According to Epron et al. (1995), J was divided into two components: $J_f = J_c + J_o$. J_c is the fraction of J_f used for CO_2 assimilation ($J_c = 1/3\ [J + 8(P_N + R_L)]$), and J_o is the fraction of J_f used for photorespiration ($J_o = 2/3\ [J - 4\ (P_N + R_L)]$). The J_o/J_c indicates the ratio of linear electron transport involved in oxygenation to the carboxylation. The calculated values of J_o and J_c were used to depict the changes in switching over of linear ETR from carboxylation to oxygenation. This approach assumes that all the reducing power generated by electron transport chain is used for photosynthesis and photorespiration, and Chl fluorescence gives reliable estimation of the quantum yield of electron transport. The rate of linear transport of electron involved in both carboxylation and oxygenation of RuBisCO was calculated according to Harley et al. (1992) as follows: $J_g = 4(P_N + R_L)(\mathrm{Ci} + 2I^{-*})(\mathrm{Ci} - I^{-*})$, where Ci represents intercellular content of CO_2, R_L represents mitochondrial respiration in light (Yin et al., 2009), and I^{-*} represents CO_2 compensation point measured in the absence of respiration using A-Ci curve. The rates of carboxylation (V_c) and oxygenation (V_o) of RuBisCO were calculated as follows: $V_c = 1/6[J_f/2 + 4(P_N + R_L)]$ and $V_o = 1/6[J_f - 4\ (P_N + R_L)]$, respectively (von Caemmerer, 2000).

Estimation of Adenylates

The estimation of total cellular adenylates was done by the method of Padmasree and Raghavendra (1999) and Dinakar et al. (2016). After the salt stress treatment, the 100 mg of fresh leaf samples was ground in liquid nitrogen using 3% $HClO_4$ (vol/vol) and centrifuged at 7,000 *g* for 10 min. The supernatants were neutralized using 150 mM triethanolamine (TEA) and incubated on ice for 30 min prior to centrifugation of 7,000 *g* for 10 min. The clear supernatant was used for estimation of ATP and ADP. The assay medium for ATP contained 150 mM TEA buffer (pH 7.5), 10 mM $MgCl_2$, 0.5 mM NADP, and 100 μL of neutralized sample. Glucose-6-phosphate dehydrogenase (0.023 μkat) (E.C. 1.1.1.49) was added to consume internal Glc-6-P levels. ATP levels were monitored by following the net increase in absorbance at 340 nm after addition of 10 mM glucose and 0.047 μkat hexokinase (E.C. 2.7.1.1). The assay medium for the assay of ADP contained 150 mM Tris-HCl pH 8.1, 7.5 mM $MgCl_2$, 0.08 mM NADH, 2 mM phosphoenol pyruvic acid, 100 μL of neutralized sample, 0.046 μkat lactate dehydrogenase (E.C. 1.1.1.27), and 0.067 μkat pyruvate kinase (PK, E.C. 2.7.1.40). The content of ADP was calculated from the net decrease in absorbance at 340 nm after the addition of PK.

In vivo Localization of Reactive Oxygen Species and Cell Damage in Leaf Tissues

Histochemical staining using 3,3′-diaminobenzidine (DAB) tetrahydrochloride and nitroblue tetrazolium (NBT, Sigma) was used to study *in vivo* localization of reactive oxygen species (ROS) (H_2O_2 and superoxide) in uninoculated and inoculated leaves of control and NaCl-treated plants. The leaves of both the plants were vacuum infiltrated by freshly prepared DAB solution (1 mg/mL) and NBT (1 mg/mL) in 10 mM potassium phosphate buffer (pH 7.8). The infiltrated leaves were kept in the dark overnight and placed under continuous light (300 μmol m^{-2} s^{-1}) at 25°C for 8 h. The stained leaves were bleached in a warm destaining solution [methanol: acetic acid: glycerol (3:2:1)] and fixed using a fixative reagent [methanol: deionized water: glycerol (5:4:1)] (Rangani et al., 2016). The tissue damage in leaves was observed by trypan blue staining method. The freshly harvested leaves were incubated overnight in lacto phenol-trypan blue solution (10 mL lactic acid, 10 mL glycerol, 10 g phenol, and 10 mg trypan blue dissolved in 10 mL distilled water) (Koch and Slusarenko, 1990). Stained leaves were then boiled for 1 min and then decolorized in 95% hot ethanol solution. The images of leaf tissues were obtained using a Canon Lide 120 Scanner under uniform white background.

Statistical Analysis

The statistical analysis was carried out using Sigmaplot v. 14.0 (Systat Software Inc.). The values obtained from three biological and three technical replicates were used for calculating the mean. The difference between the means of rates in the leaves of uninoculated and inoculated plants subjected to salinity stress was made by using analysis of variance. Multiple pairwise comparisons between different samples using Holm–Sidak test at a significance level of ($P < 0.001$) were used.

RESULTS

Characterization of ET101 Isolate

The physiological, biochemical, and molecular analysis revealed the isolated halophilic isolate ET101 as *S. sciuri*. The sequence of the isolate ET101 was submitted in GenBank with accession number MN960659. Phylogenetic tree analysis showed *S. sciuri* strain Dc-04 as the nearest homolog with 99% sequence similarity (**Supplementary Figure S1**). *S. sciuri* ET101 was further characterized for the production of various plant growth-promoting substances in the absence and presence of NaCl. Isolate ET101 produced substantial amount of IAA under *in vitro* conditions in the presence and absence of tryptophan, suggesting that it could synthesize IAA through tryptophan independent and dependent pathways. However, IAA accumulation is decreased in the absence of tryptophan. Additionally, upon treatment with increasing NaCl concentrations of 2.05 and 2.5 M, a noticeable decrease in IAA production (54.36%) was observed (**Figure 1A**). High amount of GA is produced by ET101 isolate irrespective of salt stress treatment. An increase in GA production (64.84–98.36%) was observed in bacteria treated with higher concentrations of NaCl (2.05 and 2.5 M) (**Figure 1B**). The isolate ET101 also exhibited ACC deaminase activity by producing α-ketobutyrate (26.74 ± 0.36 nmol/mg protein) after 96 h of incubation in the presence of 2.05 M NaCl (**Figure 1C**). However, at higher concentrations of NaCl (2.5 M), the amount of α-ketobutyrate production decreased by > 81.93%. Although ET101 produced higher amount of ammonia, 2.26 ± 0.113 μM/10^8 CFU after 96 h of incubation

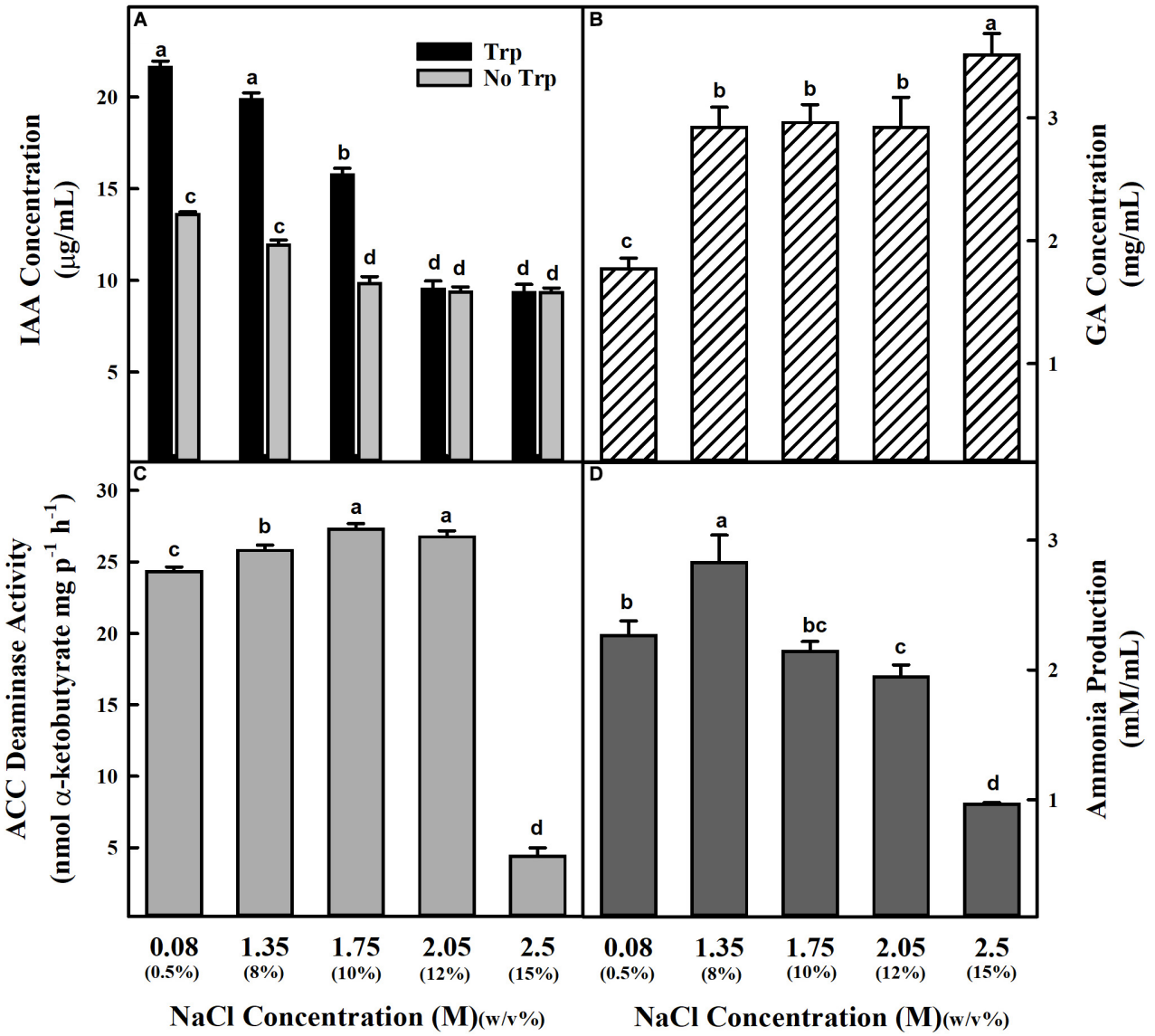

FIGURE 1 | Estimation of IAA, GA, ACC deaminase activity, and ammonia production by *Staphylococcus sciuri* ET101 isolate at different concentrations of NaCl. **(A)** The IAA production by the *S. sciuri* isolate ET101 at different NaCl concentrations with or without 0.5% L-tryptophan supplementation. **(B)** The quantitative estimation of GA production by the *S. sciuri* isolate ET101 at different NaCl concentrations. **(C)** The ACC deaminase activity by the *S. sciuri* isolate ET101 at different NaCl concentrations with 3 mM ACC. **(D)** The ammonia production by the *S. sciuri* isolate ET101 at different NaCl concentrations. Bars represent mean ± SE of three independent replicates. Different letters on bars indicate significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

(**Figure 1D**), with the increase in concentration of NaCl, a progressive decrease in ammonia production was observed.

Effect of Salt Stress, Water Relations, and Osmolytes Production in Uninoculated and Inoculated Tomato and Rice Plants

The uninoculated tomato and rice plants grown under different concentrations of NaCl (S0-0 mM NaCl, S2-200 mM NaCl, and S4-400 mM NaCl) exhibited a noticeable growth inhibition. The shoot length, root length, and biomass of plants decreased with increasing NaCl concentrations (**Supplementary Figures S2,S3**). In uninoculated rice plants treated with 200 mM NaCl (C2), while the percent decrease was 21.94% for shoot length, 8.62% for root length, and 11.19% for biomass; the plants treated with 400 mM NaCl (C4) showed decreases of 31.98, 32.45, and 15.59%, respectively (**Supplementary Figure S5**). Compared to uninoculated rice plants, increases in shoot length, root length, and biomass by 30.64, 16.44, and 1.48% were observed in ET101-inoculated rice plants (E0) without salinity stress treatment. Similarly, increases in shoot length (8.75%), root length (73.02%),

and biomass (114.79%) were observed in inoculated tomato plants under control conditions (E0 treatment). Isolate ET101 induced remarkable increases in plant biomass under 200 mM NaCl (E2) and 400 mM NaCl (E4) treatments by 69.11 and 76.44%, respectively (**Supplementary Figure S5**).

Significant increase in EL was observed in both uninoculated and inoculated plants of tomato and rice plants subjected to S2 and S4 salt stress. However, compared to uninoculated plants, the EL in inoculated plants was significantly lower (**Figure 2A**). While the RWCs in uninoculated tomato and rice plants were 89.10 and 96.20%, salinity stress led to a remarkable decrease in leaf water status in S2 and S4 stress-treated plants. The correlation of RWC to EL in tomato and rice plants shows decreased EL in inoculated plants during S2 and S4 stress conditions (**Figure 2A**). Remarkable increase in proline accumulation was observed in uninoculated and inoculated tomato and rice plants during S2 and S4 stress conditions. However, ET101-inoculated tomato and rice plants showed better RWC under control conditions and salt stress conditions (**Figure 2B**). Compared to uninoculated salt-stressed tomato plants, increases in RWC by 10.68 and 20.16% were observed in ET101-inoculated plants subjected to S2 and S4 salt stress conditions. Similarly, the ET101-inoculated rice plants also showed the lesser loss of water from leaf tissues and showed an increase in RWC by 14.91 and 9.12% at S2 and S4 salt stress treatments (**Figure 2B**). When compared to uninoculated tomato plants, although marginal non-significant decrease in proline content was observed in inoculated tomato plants subjected to S4 salt stress. While the ET101-inoculated rice plants showed marginal increase in proline content in S2 stress condition, a significant increase by 18.11% was observed under S4 stress condition. In ET101-inoculated tomato plants, compared to uninoculated plants at S2 stress condition, the increase in proline content was observed only under S2 stress condition (**Figure 2C**). Accumulation of glycine betaine was also observed in both tomato and rice plants in S2 and S4 salt stress condition. Compared to uninoculated plants, the amounts of glycine betaine were significantly increased, in tomato plants (33.52, 12.16, and 27.80%, respectively), whereas a marginal decrease (11.20 and 1.17%) was observed in rice plants inoculated with ET101 (**Figure 2D**).

Measurement of Photosynthetic Pigments and Gas Exchange Parameters

Remarkable decreases in Chl a, Chl b, total chlorophyll, and carotenoid contents were observed in leaves of uninoculated tomato and rice plants during salt stress conditions (C0, C2, and C4). However, plants inoculated with ET101 isolate (E0, E2, and E4) showed significant increase in Chl a, Chl b, total chlorophyll, and carotenoid content under different salt stress conditions (**Figures 3A–D**). The salinity stress has considerable influence on the photosynthetic parameters such as net photosynthetic rate (P_N), transpiration (E), and stomatal conductance (g_s) (**Figures 4A–C**). Under all tested light conditions, salinity stress severely impacted the photosynthetic parameters. Drastic decrease in net photosynthetic rate, transpiration, and stomatal conductance was observed in both tomato and rice leaves subjected to S2 and S4 salinity stress. In uninoculated plants, the P_N rates gradually increased with increasing light intensities; however, upon imposition of salinity stress, the P_N rates were decreased to a greater extent in both C2 and C4 conditions at all given light intensities. However, inoculation of tomato and rice plants with ET101 isolate resulted in higher P_N rates than uninoculated plants grown under C0 (76.08% in tomato and 20.14% in rice), C2 (13.11 and 76.25%), and C4 (347.11 and 220.119%) conditions (**Figure 4A**). Similar trend has been observed in the transpiration rates (E) and stomatal conductance (g_s). The decrease in transpiration rate (E) and stomatal conductance (g_s) was higher in uninoculated plants than inoculated plants at all light intensities. The stomatal conductance (g_s) in tomato E0 plants was significantly higher than in C0, whereas marginal difference was observed in the rice C0 and E0 plants. However, significant increase was attained between E2, E4, and C2, C4 plants of both tomato and rice varieties (**Figures 4B,C**). While the intercellular CO_2 rates were significantly higher in uninoculated tomato and rice plants subjected to S2 and S4 salt stress conditions than inoculated plants, the ratio of intercellular CO_2 to ambient (Ci/Ca), reflecting the relationship between g_s and non-stomatal capacity for photosynthesis was also significantly higher in uninoculated S2 and S4 treatments than ET101-inoculated tomato and rice plants (**Figures 5A,B**).

Measurement Chlorophyll Fluorescence Parameters

The photosynthetic induction in dark-adapted leaves was monitored by saturation pulse method of chlorophyll fluorescence measured together with gaseous exchange. These measurements were used for the calculation of quenching parameters (**Table 1**). The maximum quantum efficiency of PSII photochemistry (F_v/F_m) in uninoculated tomato and rice plants at S2 and S4 stress treatments showed decreases of 6.40 and 10.8%, and 17.12 and 24.50% (**Figure 5C**). Although marginal increase in F_v/F_m ratio was observed in inoculated plants than uninoculated plants at S0 (Control) in both tomato and rice plants, significant increase was observed at S2 and S4 stress treatments in ET101-inoculated plants. The ET101-inoculated plants exhibited marginal decrease in the F_v/F_m ratio of approximately 1.12 and 3.63% in S2 and 9.17 and 3.65% in S4 salt stress treatment in tomato and rice plants, respectively (**Figure 5C**). Although ETRs (J) were significantly higher in ET101-inoculated tomato and rice leaves than uninoculated leaves at S0 treatment, the rates gradually declined in tomato and rice leaves under S2 and S4 salinity stress. However, under control and salinity stress conditions, the leaves of ET101-inoculated plants had higher ETR (J) than uninoculated control plants (**Figure 5D**).

The estimated effective quantum yield of PSII (ΦPSII), although was higher in leaves of ET101-inoculated tomato and rice plants under control and salinity stress conditions, decreased with increasing salinity stress in both uninoculated and ET101-inoculated tomato and rice plants (**Figure 6A**). The inoculated tomato and rice plants showed higher ΦPSII rates

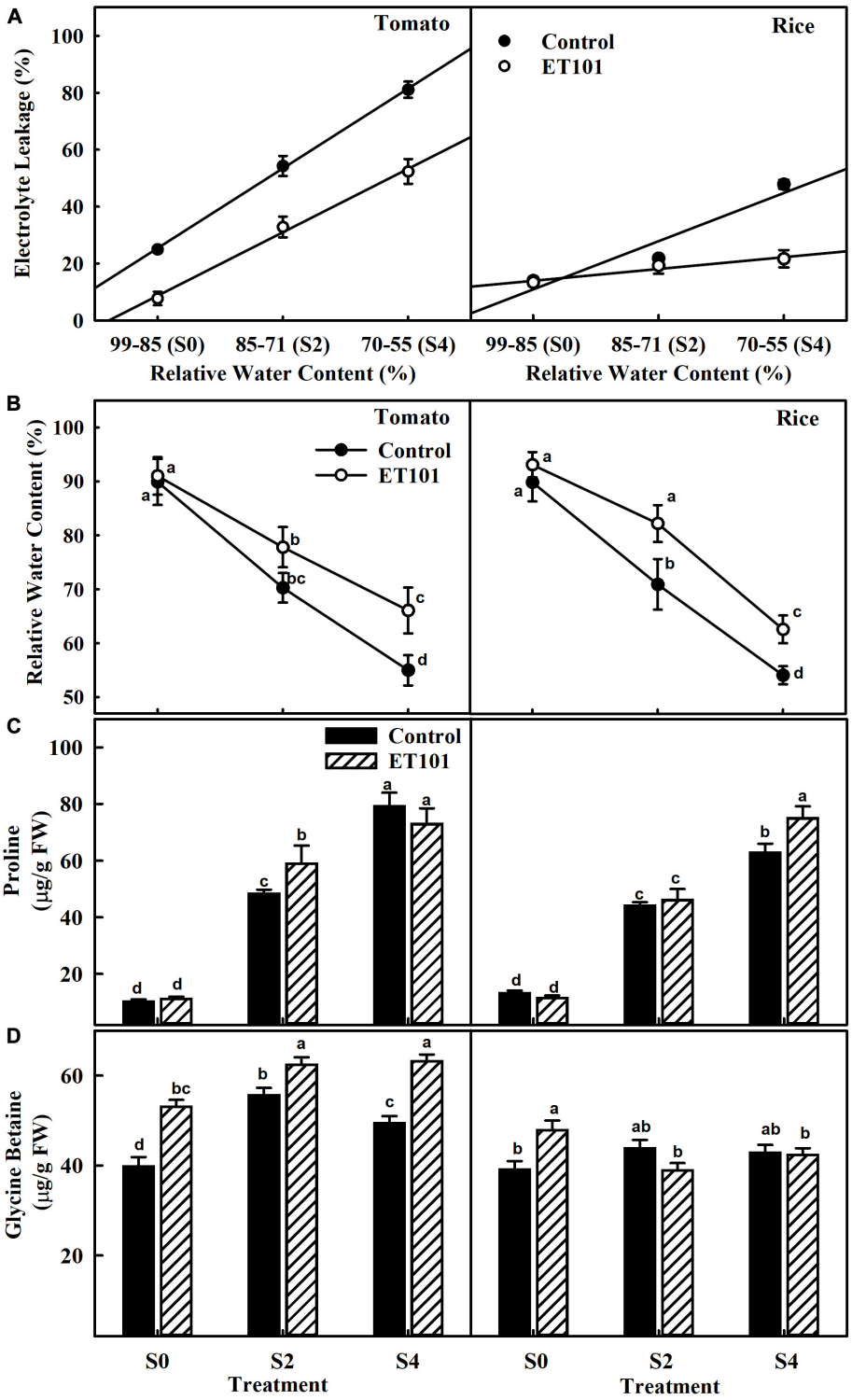

FIGURE 2 | Changes in relative water content, electrolyte leakage, and osmolyte production in leaves of uninoculated and inoculated tomato and rice plants subjected to salinity stress conditions. **(A)** Effect of *Staphylococcus sciuri* isolate ET101 inoculation on leaf relative water content and electrolyte leakage of tomato and rice plants subjected to different salt stress treatments (S0: 0 mM NaCl, S2: 200 mM NaCl, S4: 400 mM NaCl). **(B)** Leaf relative water content of tomato and rice plants. **(C)** Effect of ET101 inoculation on proline accumulation of leaves of tomato and rice plants. **(D)** Effect of ET101 inoculation on glycine betaine accumulation in leaves of tomato and rice plants. The open symbols denote ET101-inoculated plants, whereas the closed symbols represent uninoculated tomato and rice plants. Different letters on bars indicate significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

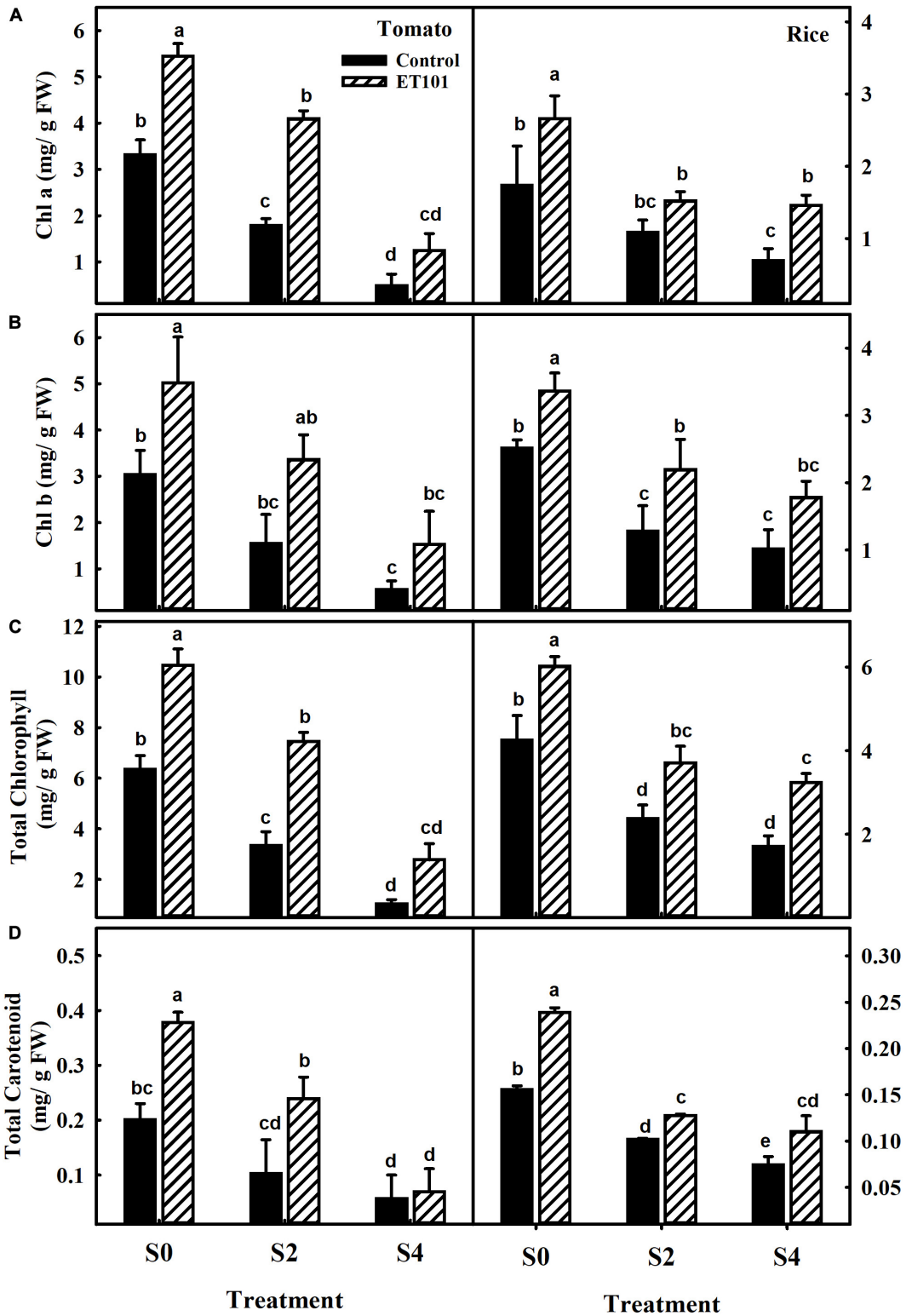

FIGURE 3 | Changes in photosynthetic pigments and carotenoid content in leaves of uninoculated and inoculated tomato and rice plants. Changes in **(A)** chlorophyll a (Chl a), **(B)** chlorophyll b (Chl b), **(C)** total chlorophyll content, and **(D)** total carotenoid content in leaves of tomato and rice plants subjected to different salt treatments (S0: 0 mM NaCl, S2: 200 mM NaCl, and S4: 400 mM NaCl). Data represent the mean ± SE of triplicates. Different letters on bars indicate a significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

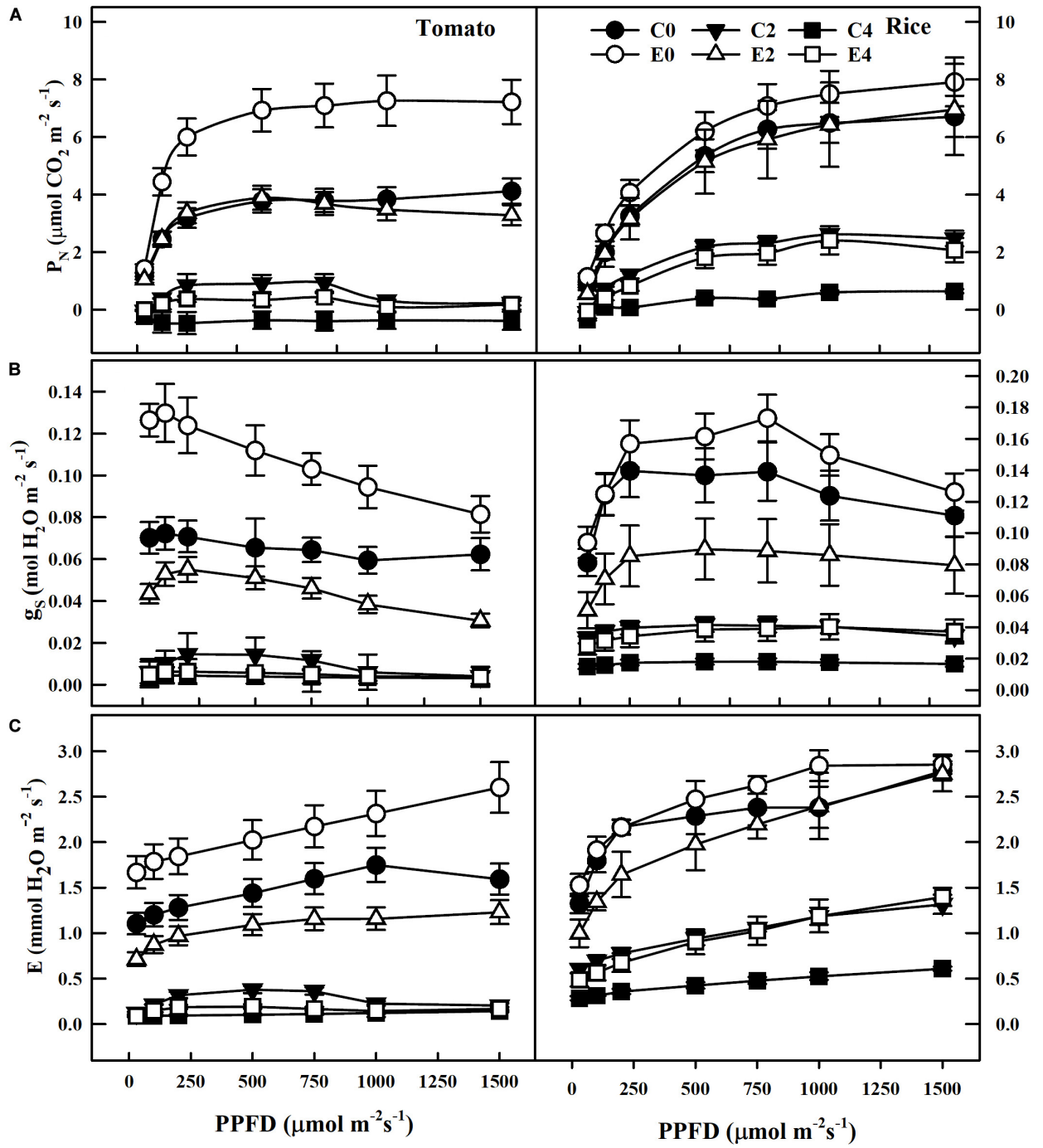

FIGURE 4 | Changes in CO_2 assimilation and gas exchange parameters in tomato and rice plants under different PPFD. **(A)** Effect of inoculation of ET101 isolate on CO_2 assimilation (A_{CO2}). **(B)** Effect of inoculation of ET101 isolate on stomatal conductance (g_s). **(C)** Effect of inoculation of ET101 isolate on transpiration (*E*) in leaves of tomato and rice plants subjected to different salt stress treatments. Values represent mean of triplicates from sample size $n = 6$. The open symbols denote ET101-inoculated plants, whereas the closed symbols represent uninoculated tomato and rice plants (C0: control + 0 mM NaCl, C2: control + 200 mM NaCl, C4: control + 400 mM NaCl; E0: ET101 + 0 mM NaCl, E2: ET101 + 200 mM NaCl, E4: ET101 + 400 mM NaCl).

than uninoculated plants. Compared to uninoculated plants, while the increase in ΦPSII rates in ET101-inoculated control plants was 14.46% in tomato and 39.89% in rice, the pronounced increase was observed in the leaves of inoculated plants under S2 (130.77% in tomato and 16.74% in rice) and S4 (65.49% in tomato and 77.38% in rice) stress conditions (**Figure 6A**). Although significant differences were not observed in the photochemical quenching (*qP*) rates between ET101-inoculated

TABLE 1 | The chlorophyll fluorescence parameters derived from the saturation pulse analysis.

Chlorophyll fluorescence parameters derived from the saturation pulse analysis		
F, F'	Fluorescence emission from dark- or light-adapted leaf, respectively	
F_0	Minimum fluorescence from dark-adapted leaf (PS II centers open)	
F_m, F'_m	Maximum fluorescence from dark- or light-adapted leaf, respectively (PS II centers closed)	
$F_V = F_m - F_0$	Maximum variable fluorescence from dark-adapted leaf	
F'_0	Minimum fluorescence from light-adapted leaf	
F_s	Steady-state fluorescence at any light level	
$F_V/F_M = 1 - (F_o/F_m)$	Estimated maximum quantum efficiency of PSII photochemistry	Kitajima and Butler, 1975; Krause and Weis, 1991; Schreiber et al., 1995
$\Phi_{PSII} = (F'_m - F_s)/F'_m$	Estimated effective quantum yield of PSII photochemistry at given PAR	Genty et al., 1989
$J_{PSII} = \alpha_{II} * \text{PAR} * \Phi_{PSII}$	Rate of linear electron transport in PSII at given PAR and portion of PAR absorbed by PSII (α_{II})	Bjorkman and Demmig, 1987; Genty et al., 1989
$\text{NPQ} = (F_m - F'_m)/F'_m$	Non-photochemical quenching of F_m	Schreiber et al., 1988; Walters and Horton, 1991
$qP = (F'_m - F_s)/(F'_m - F'_0)$	Coefficient of photochemical quenching based on the "puddle" model (i.e., unconnected PS II units)	Schreiber, 1986; Bjorkman and Demmig, 1987; Bilger and Bjorkman, 1990

and uninoculated tomato and rice plants in S0 treatment, leaves of ET101-inoculated plants showed higher qP values of approximately 47.22 and 19.28%, and 24.26 and 21.44% in tomato and rice plants during S2 and S4 salinity stress conditions (**Figure 6B**). The uninoculated tomato and rice plants exposed to salinity stress conditions (S4) showed a significant increase in NPQ (**Figure 6C**). However, the higher increase was observed in tomato and rice plants grown under S2 and S4 salinity stress condition with ET101 inoculation. Inoculation with ET101 increased NPQ of plants exposed to S2 and S4 salinity conditions by 11.10 to 110.4% compared with respective controls (**Figure 6C**).

Net photosynthetic rates (P_N) measured in leaves of ET101-inoculated tomato and rice plants were significantly higher than that of uninoculated plants in all treatments (**Supplementary Figure S4**). The apparent RuBisCO efficiency (P_N/Ci) measured in uninoculated plants showed a significant reduction in S2 and S4 treatments in tomato and rice plants. The leaves of uninoculated plants showed decreases of 93.58 and 95.48% in tomato and 48.74 and 86.15% in rice during S2 and S4 salinity treatments. However, the leaves of ET101-inoculated plants showed lesser reduction of about 46.78 and 94.26% in tomato and 17.15 and 76.35% in rice plants, respectively (**Figure 7A**). The ratio of ETR and net photosynthesis (J/P_N) ratio measured in uninoculated tomato and rice plants showed incremental increase in S2 and S4 treatments (**Figure 7B**). The J/P_N ratio measured in ET101-inoculated tomato and rice plants did not show any significant differences between the S0 and S2 treatments. However, a significant increase of 174.32% in tomato and 71.83% in rice compared with respective controls (C0) was observed in leaves of ET101-inoculated plants subjected to S4 stress treatment (**Figure 7B**).

At photosynthetically active radiation of 1,500 μmol m^{-2} s^{-1}, the rate of electron transport of PSII (J_f) calculated using chlorophyll fluorescence data decreased with increasing salinity stress in both tomato and rice plants. Although the ET101-inoculated tomato and rice plants E0 had higher electron transport (J_f), the leaves of the plants exposed to E2 and E4 stress showed drastic decrease in electron transport (J_f) but still had higher rates than that of C2 and C4 uninoculated salt-stressed plants (**Figure 8A**). Compared with the ET101-inoculated tomato and rice plants, the fraction of electron transport consumed by carboxylation plus oxygenation of RuBP (J_g) calculated by the data from gas exchange measurements decreased in C0, C2, and C4 plants. While the ETR (J_g) to support photosynthesis process followed the trend of PSII ETR (J_f), the J_g values of each treatment in both uninoculated and ET101-inoculated plants were substantially lower under salt stress than the J_f (**Figure 8B**). The higher values of J_f may indicate the presence of alternative electron flow. The leaves of ET101-inoculated plants showed considerable increase in ETR J_f and J_g in the respective treatment controls; however, at stress conditions, the rates are decreased (**Figure 8B**). The distribution of electrons within linear electron transport between photorespiration and RuBP carboxylation estimated as J_o/J_c decreased in S0 and remained higher in S2 and S4 treatment of both plants. The J_o/J_c ratio of distribution of electrons between carboxylation and oxygenation indicates the steady state of electron flow in uninoculated (C0) and ET101-inoculated (E0) plants in both the tomato and rice plants even at 30 min of photosynthetic induction at high light. Compared to the ET101-inoculated E2 and E4 plants, the C2- and C4-treated tomato and rice plants exhibited higher electron flow toward oxygenation rather than carboxylation. In severely stressed control plants C4, the proportion of electrons consumed in oxygenation was 130.74% higher in tomato and 86.24% in rice plants (**Figure 8C**).

The carboxylation rate (V_C) in uninoculated and ET101-inoculated tomato and rice plants was significantly decreased in C2, C4, E2, and E4 plants in a stress-dependent manner. However, leaves of ET101-inoculated tomato and rice plants showed higher V_C rates in control and stress conditions than leaves of uninoculated plants (**Figure 9A**). The C4 stress-treated uninoculated tomato and rice plants showed lowest V_C rates with 26.88% decrease in tomato plants and 12.40% decrease in rice plants compared to uninoculated controls at S0. Contrary to the observations with regard to V_C, the oxygenation rate (V_o) was lower in leaves of ET101-inoculated tomato and rice plants at E0 condition than in uninoculated plants (C0). The V_o

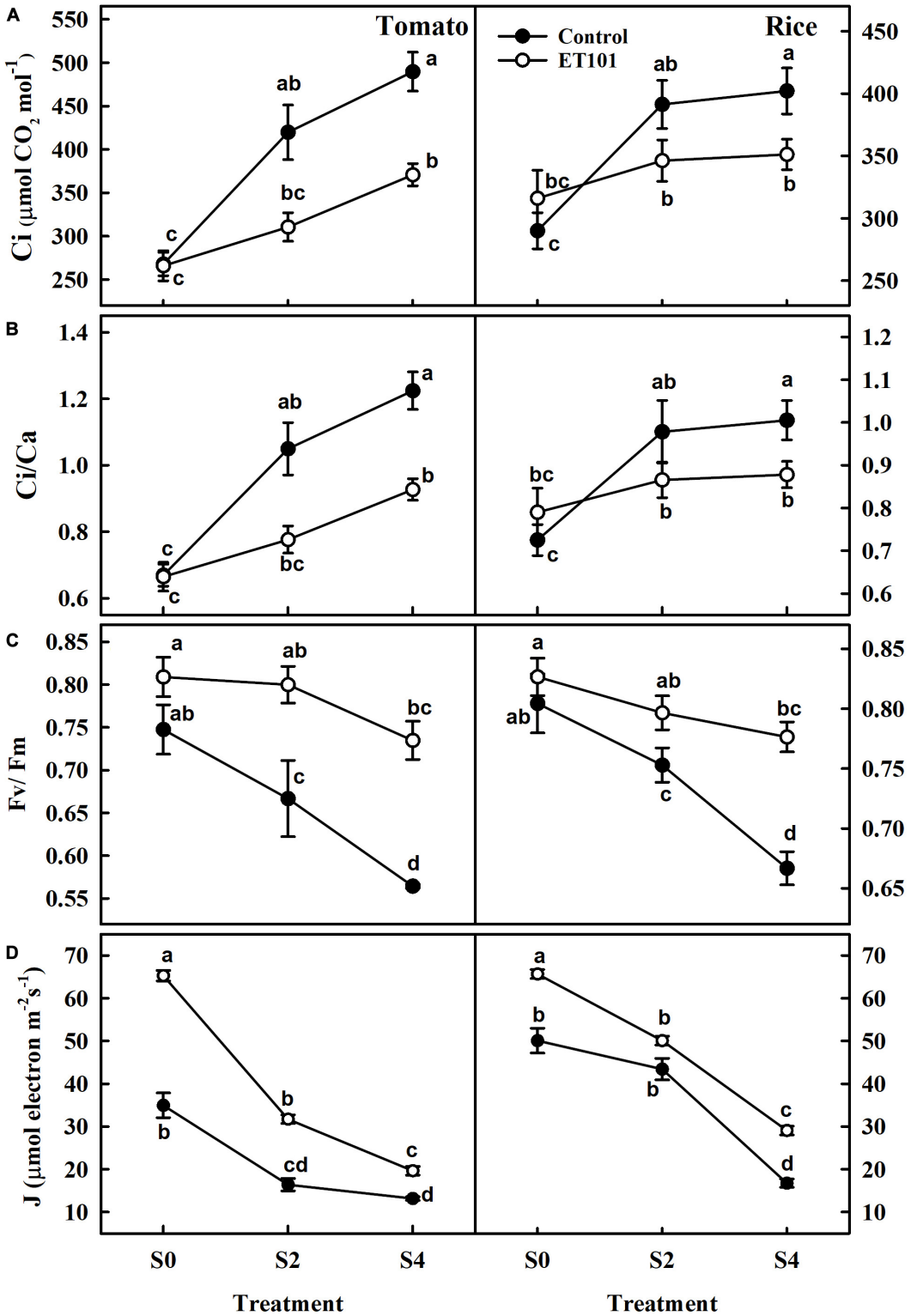

FIGURE 5 | Changes in **(A)** intracellular CO_2 concentration (Ci), **(B)** ratio of intercellular CO_2 to ambient CO_2 concentration (Ci/Ca), **(C)** maximum quantum yield (F_v/F_m), and **(D)** rate of electron transport rate in PSII (J) in tomato and rice plants subjected to different salinity treatments (S0: 0 mM NaCl, S2: 200 mM NaCl, and S4: 400 mM NaCl). The open symbols denote ET101-inoculated plants, whereas the closed symbols represent uninoculated tomato and rice plants. Values represent mean of triplicates from sample size $n = 6$. Different letters on bars indicate a significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

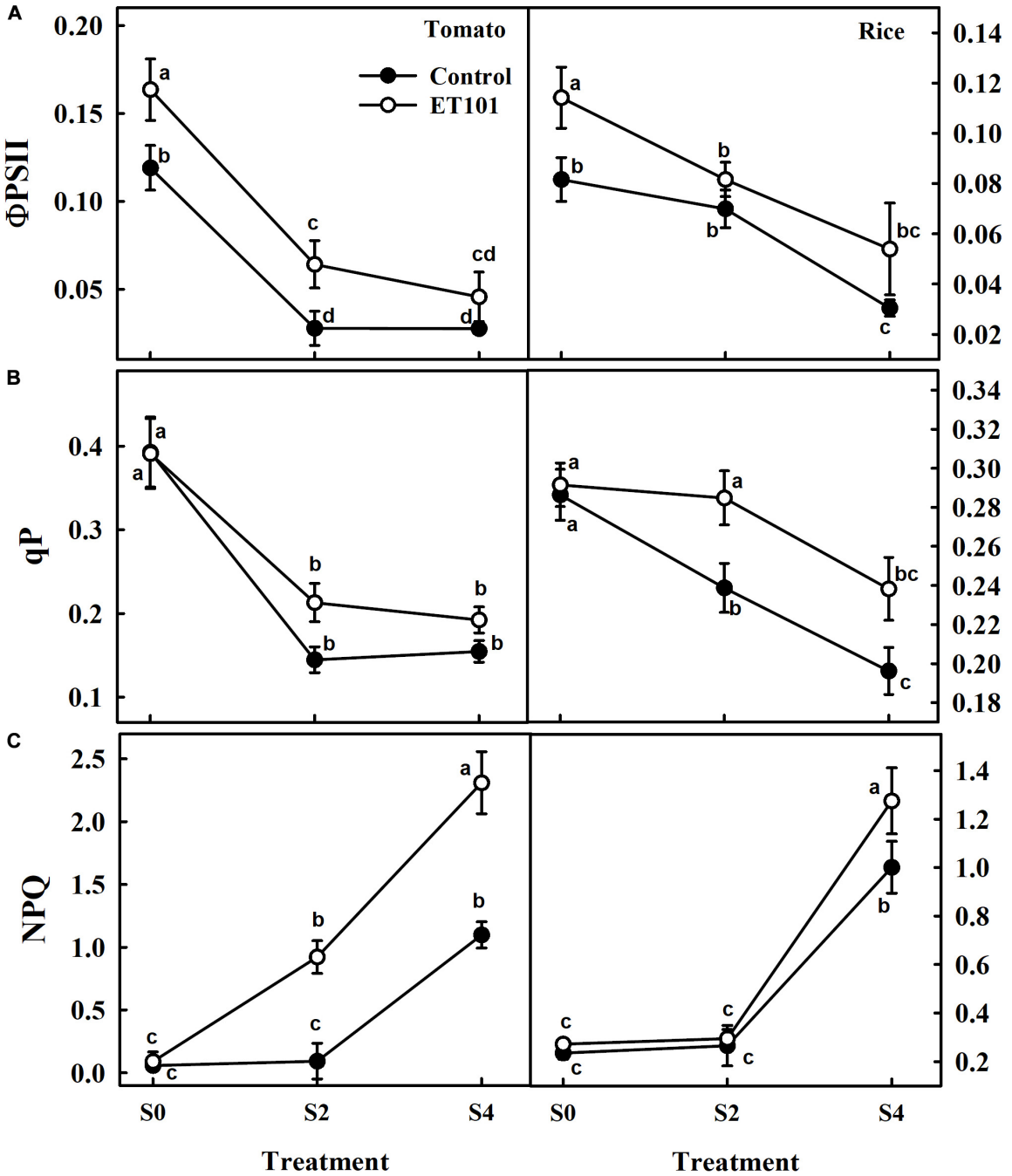

FIGURE 6 | Changes in **(A)** quantum efficiency (ΦPSII), **(B)** photochemical quenching (*qP*), and **(C)** non-photochemical quenching (NPQ) in leaves of tomato and rice plants subjected to salinity stress (S0: 0 mM NaCl, S2: 200 mM NaCl, and S4: 400 mM NaCl). Values represent the mean of triplicates from sample size ($n = 6$). The open symbols denote ET101-inoculated plants, whereas the closed symbols represent uninoculated tomato and rice plants. Different letters indicate significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

rates were maximum in leaves of uninoculated tomato (39.25% increase from C0) and rice plants (15.87% increase from C0) subjected to C4 stress conditions (**Figure 9A**). Compared to uninoculated plants, leaves of ET101-inoculated tomato plants showed increased P_N rates by 270.82 and 264.14% under S2 and S4 salinity stress conditions and 48.87 and 48.84% in rice plants, respectively (**Figure 9B**). The P_R rates were significantly lower in E2 and E4 stress conditions in both tomato and rice plants compared to the controls and C2 and C4 plants (**Figure 9B**). Leaves of uninoculated tomato and rice plants under high salinity stress (C4) showed increased P_R of 179.47% in tomato and 76.87% in rice compared to respective controls. However, in case

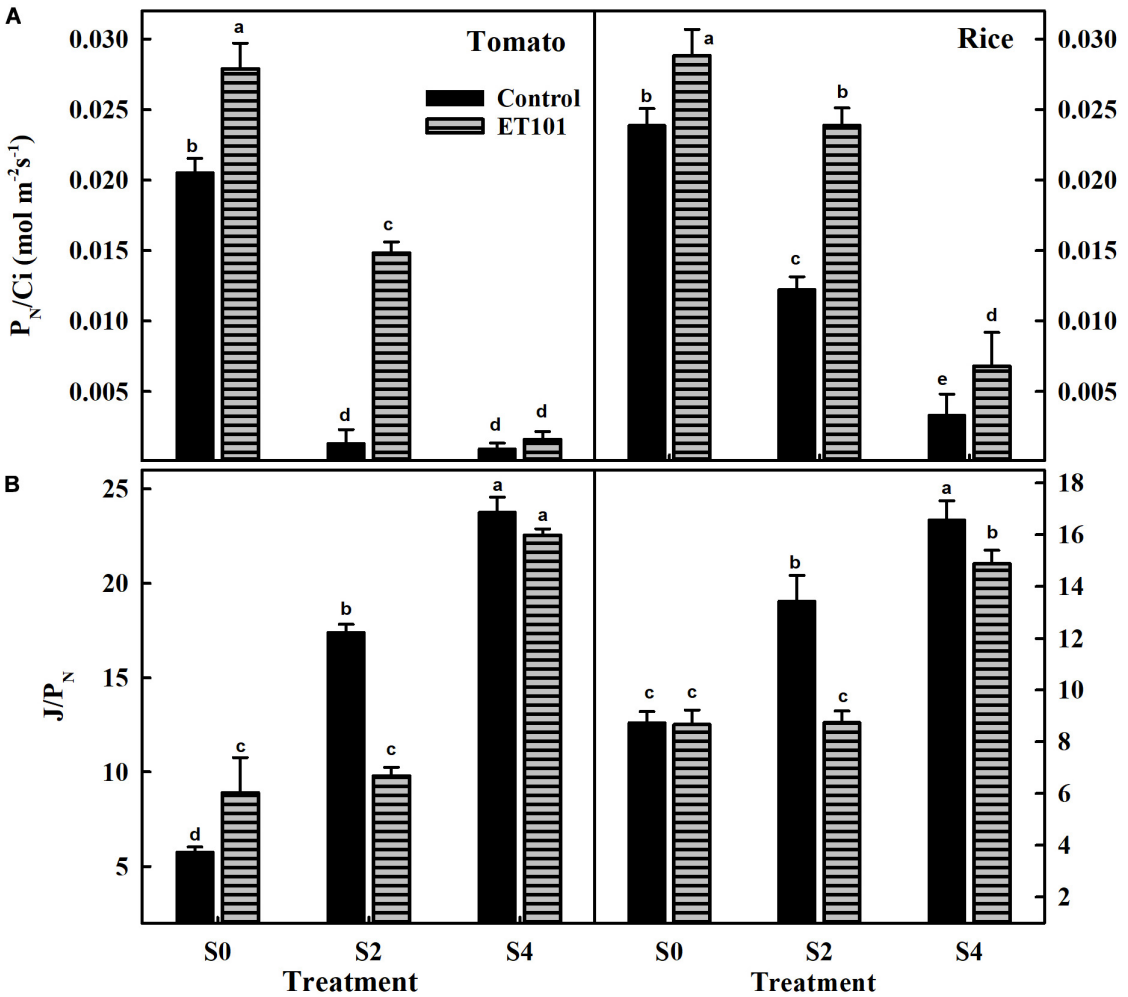

FIGURE 7 | (A) The rate of apparent RuBisCO efficiency (P_N/Ci) and **(B)** the ratio of electron transport and net photosynthesis (J/P_N) of uninoculated and ET101-inoculated tomato and rice plants subjected to different salt stress treatments (S0: 0 mM NaCl, S2: 200 mM NaCl, S4: 400 mM NaCl). Bars represent mean ± SE of triplicates. Different letters indicate significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

of ET101-inoculated plants subjected to E4 stress, the P_R rates were 97.82% in tomato and 71.63% in rice plants (**Figure 9B**).

Adenylate Levels in Uninoculated and Inoculated Tomato and Rice Plants Exposed to Salinity Stress Conditions

Under salinity stress conditions, the production of ATP and ADP in C2 and C4 plants decreased in both the tomato and rice plants (**Figures 10A,B**). However, in inoculated tomato and rice plants at E2 and E4 treatments, the ATP levels are either maintained or increased than unstressed uninoculated plants (**Figures 10A,B**). The drastic changes in levels of adenylates (ATP, ADP, and ATP/ADP) act as proof for disruption of total cellular respiration in plants by the salinity stress. The increase in ATP/ADP levels was observed in leaves of ET101-inoculated tomato and rice plants under control and salt stress conditions (**Figure 10C**).

ROS Production in Leaves of Uninoculated and Inoculated Tomato and Rice Plants Exposed to Salinity Stress

The salinity stress in plants leads to the accumulation of ROS, which can cause cellular damage leading to cell death. The ROS produced in leaves of the plants subjected to salinity stress can be detected by histochemical staining. The accumulation of H_2O_2 and superoxide ions in the leaf tissues of inoculated and uninoculated tomato and rice plants has been visualized by DAB and NBT staining. The uninoculated plants grown under salinity stress conditions showed an increase in H_2O_2 and superoxide levels in leaves. However, leaves of plants inoculated with ET101 isolate showed considerable decrease in both H_2O_2 and superoxide levels under E2 and E4 salinity stress conditions (**Figures 11A–D**). The substantial decrease in ROS accumulation in ET101-treated plants under salinity stress emphasizes the importance of the *S. sciuri* in ameliorating the ROS levels inside

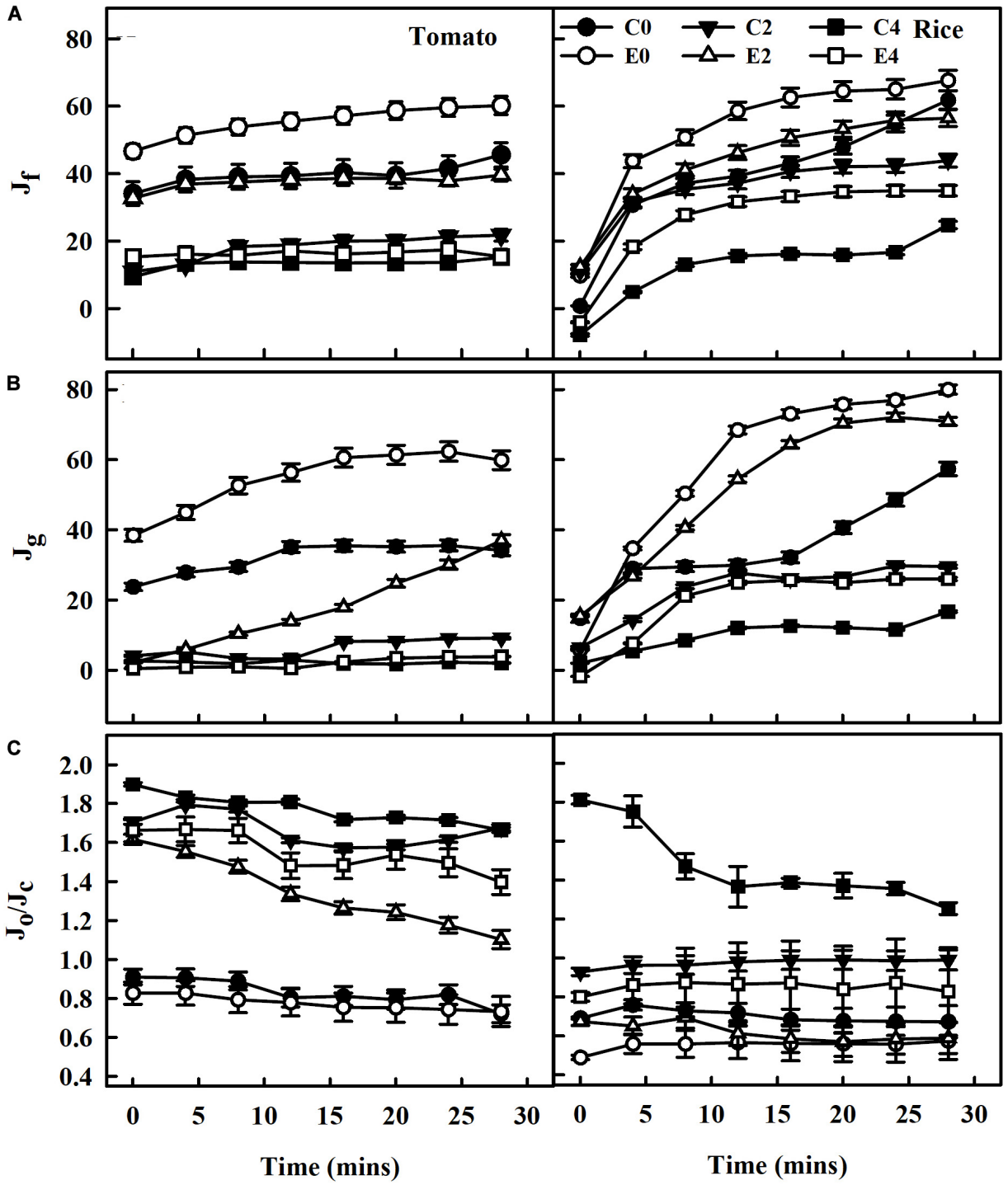

FIGURE 8 | The rate of electron transport derived from gas exchange and chlorophyll fluorescence measurements during photosynthetic induction following dark-adapted state. **(A)** Electron transport rate (J_f) calculated based on measurements of PSII quantum yields, assuming the equal distribution of absorbed light between PSI and PSII in leaves of tomato and rice plants subjected to salinity stress at 1,500 μmol photons m^{-2} s^{-1} PPFD up to saturation of 30 min. **(B)** Rate of electron transport consumed by carboxylation plus oxygenation of RuBP (J_g), calculated by the data from gas exchange measurements. **(C)** Ratio of electron transport consumed by photorespiration (J_o) to the total PSII electron transport (J_c) in leaves of tomato and rice plants (J_o/J_c). The average values ± standard errors from triplicates of sample size $n = 6$ are presented. The open symbols denote ET101-inoculated plants, whereas the closed symbols represent uninoculated tomato and rice plants (C0: control + 0 mM NaCl, C2: control + 200 mM NaCl, C4: control + 400 mM NaCl; E0: ET101 + 0 mM NaCl, E2: ET101 + 200 mM NaCl, E4: ET101 + 400 mM NaCl).

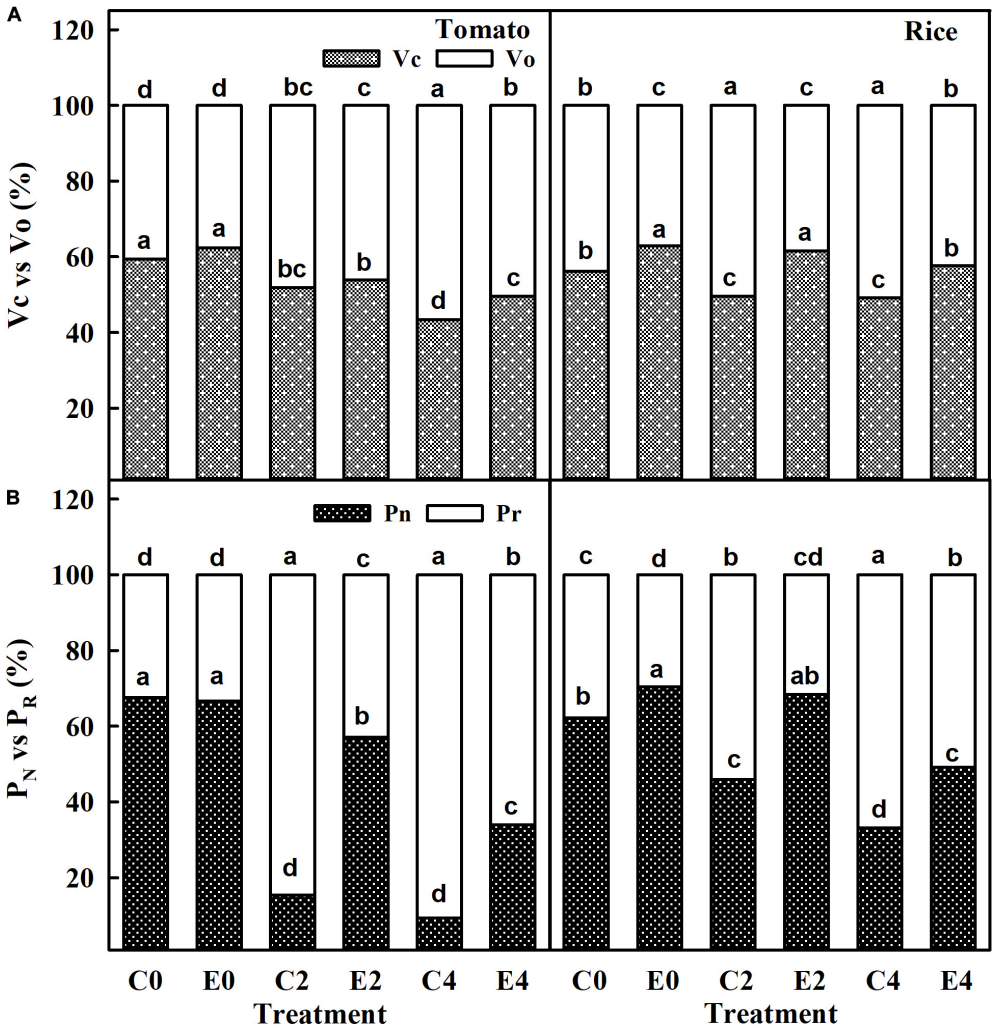

FIGURE 9 | (A) Carboxylation (V_C) and oxygenation (V_o) catalyzing efficiency of RuBisCO enzyme in uninoculated and ET101-inoculated tomato and rice plants subjected to salinity stress conditions. **(B)** Rate of net photosynthesis (P_N) and rate of photorespiration (P_R) calculated by the data from simultaneous gas exchange and chlorophyll fluorescence measurements in uninoculated and ET101-inoculated tomato and rice plants subjected to salinity stress conditions (C0: control + 0 mM NaCl, C2: control + 200 mM NaCl, C4: control + 400 mM NaCl; E0: ET101 + 0 mM NaCl, E2: ET101 + 200 mM NaCl, E4: ET101 + 400 mM NaCl). Bars represent mean ± SE of triplicates. Different letters on the bars indicate significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

the plant cells. Trypan blue is a vital stain typically used for visualization of dead cells in tissues. While intense blue staining is observed in leaves of uninoculated tomato plants subjected to S4 salinity stress, the blue spots were marginal in leaves of ET101-inoculated tomato plants subjected to S4 salinity stress indicating the protection afforded by ET101 isolate against salinity stress induced cell death (**Figures 11E,F**).

DISCUSSION

S. sciuri Is a Halophilic PGPR

In the current study, we have isolated a halotolerant bacterium, *S. sciuri* ET101, which has the potential to improve plant growth under salinity stress conditions. Rhizospheric halotolerant bacteria with plant growth promotion ability are known to be the prime candidates to test their capability to alleviate salt stress symptoms in crop species (Akram et al., 2016; Bharti et al., 2016; Ilangumaran and Smith, 2017; Shahid et al., 2019; Bakka and Challabathula, 2020; Kumar et al., 2020). The isolation and employment of potent rhizospheric halotolerant bacteria were shown to have significant beneficial effect on plant growth and development under varied abiotic stress conditions (Palacio-Rodríguez et al., 2017; Sapre et al., 2018; Vives-Peris et al., 2018; Prittesh et al., 2020). Although many species of *Staphylococcus* genus are commensal pathogens of animals and plants (Prithiviraj et al., 2005; Nemeghaire et al., 2014), the species of *Staphylococcus* possessing various plant growth-promoting traits have also been reported (Khan et al., 2015). Salt-stressed maize plants inoculated with halophilic *S. sciuri*

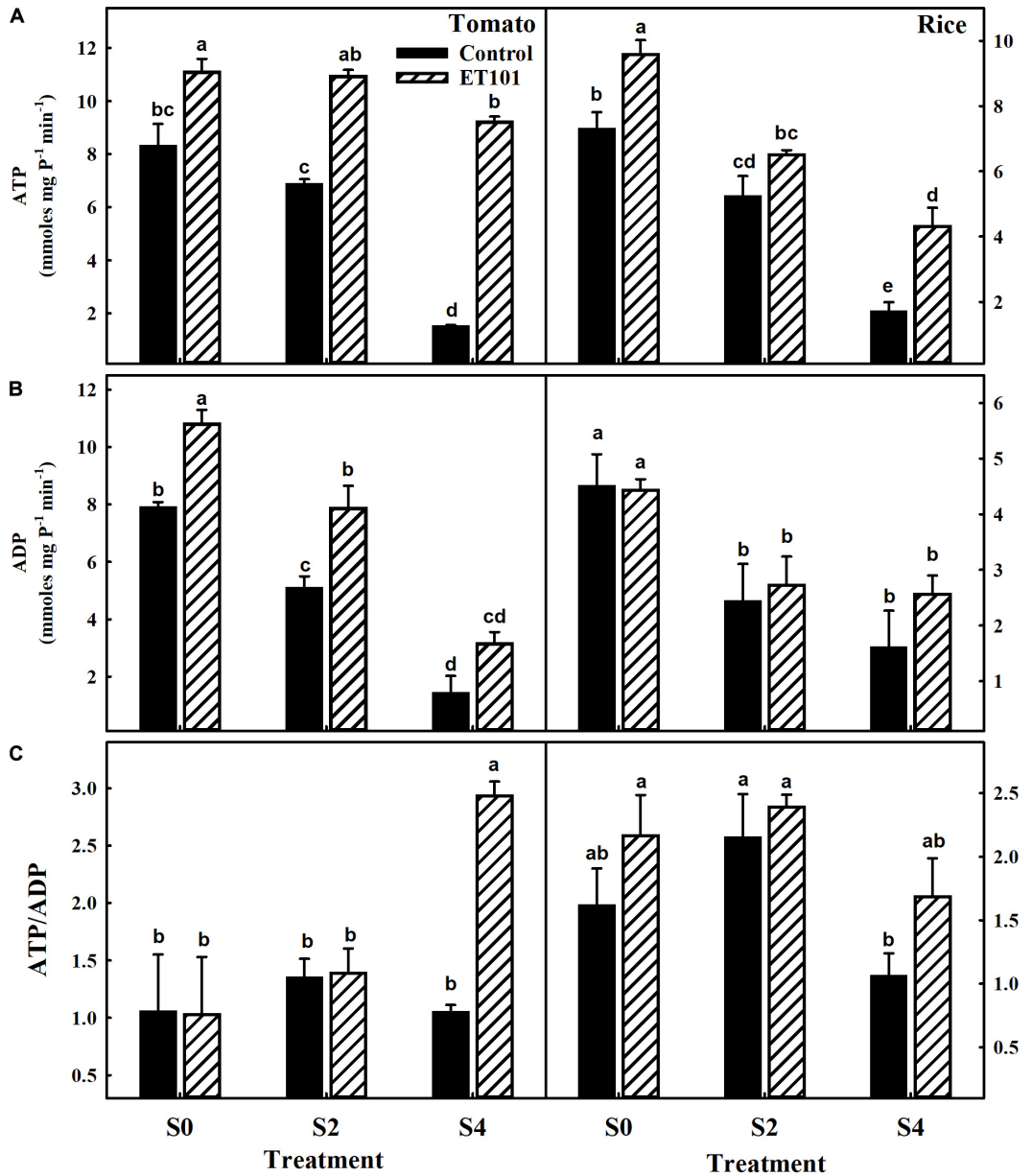

FIGURE 10 | (A) Estimation of ATP levels, **(B)** estimation of ADP levels, and **(C)** ratio of ATP to ADP levels in leaves of uninoculated and inoculated tomato and rice plants subjected to different salt stress treatments (C0: control + 0 mM NaCl, C2: control + 200 mM NaCl, C4: control + 400 mM NaCl; E0: ET101 + 0 mM NaCl, E2: ET101 + 200 mM NaCl, E4: ET101 + 400 mM NaCl). Bars represent mean ± SE of triplicates. Different letters indicate significant difference between the salt treatments and bacterial inoculation (ANOVA; $P < 0.001$). Other details are mentioned in Section "Materials and Methods."

SAT-17 isolate showed plant growth-promoting capability along with increased salinity tolerance and enhanced antioxidative defense (Akram et al., 2016). Maize plants inoculated with the bacterial strains (STN-1 and STN-5) taxonomically classified as *Staphylococcus* spp. showed enhanced growth along with increased antioxidant enzyme activity and decreased ROS under salt stress conditions (Shahid et al., 2019). Production of plant hormones and plant beneficial compounds are key factors for considering a bacterium as PGPR. As IAA acts as a major contributor for enhancement of plant growth by root elongation (Liu et al., 2018), the bacterial production of IAA by tryptophan-independent and tryptophan-dependent means and the production of GA confirm the bacteria to be a PGPR. Additionally, the production of ACC-deaminase activity in the presence of NaCl in the culture medium is evident by the production of α-ketobutyrate. Possessing the ACC deaminase activity by bacteria is an important trait for rendering stress tolerance to the plants as it reduces the production of ACC by cleaving it into α-ketobutyrate and ammonia (Glick, 2014). Although the isolate is a non-nitrogen fixer, it has the ability

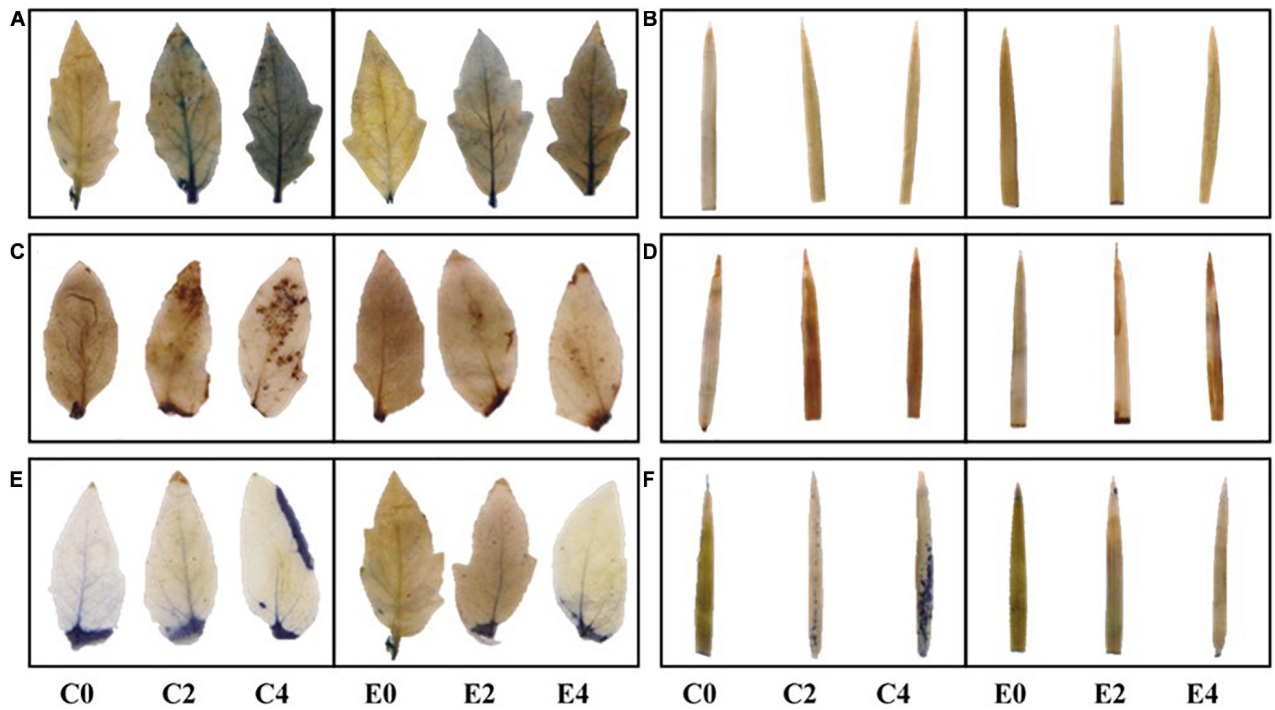

FIGURE 11 | Changes in ROS levels and cell death assay in leaves of uninoculated and inoculated tomato and rice plants. **(A,B)** *In vivo* superoxide ion O_2^- localization in uninoculated and inoculated leaves of tomato and rice plants subjected to different salt stress conditions using NBT (nitroblue tetrazolium) staining. **(C,D)** *In vivo* H_2O_2 localization in uninoculated and inoculated leaves of tomato and rice plants treated with different salt stress conditions using DAB (diaminobenzidine) staining. **(E,F)** *In vivo* localization of dead cells in uninoculated and inoculated leaves of tomato and rice plants subjected to different salt stress conditions using trypan blue staining. Other details are mentioned in Section "Materials and Methods."

to produce ammonia from nitrogen-containing organic matter. Similar to our results, the inoculation of exopolysaccharide and ACC-deaminase producing *Bacillus* spp. showed higher germination rate and increased salinity tolerance in wheat seedlings under *in vitro* conditions (Amna et al., 2019). Results suggest that the traits possessed by ET101 isolate convincingly confirm it as halophilic PGPR.

S. sciuri Protected Tomato and Rice Plants From Salinity Injury

Plants experiencing salinity stress downregulate and shut down photosynthesis mainly due to stomatal closure (Chaves et al., 2009; Igamberdiev, 2015). The leaf RWC serves as a principal factor for monitoring the water status in plants. Several studies have reported less decrease in RWC exhibited by tolerant genotypes than the sensitive plants due to salinity stress (Mekawy et al., 2015; Hossain and Dietz, 2016; Negrão et al., 2017). Salinity stress induces the osmotic stress in plants causing the depletion of water content in leaves with devastating effect on the cellular membranes and organelles. Compared to uninoculated plants, the lesser EL observed in the ET101-inoculated plants could possibly be due to the production of osmolytes and compatible solutes required for cellular protection. The proline and glycine betaine are the most important and efficient compatible solutes accumulated in many of the living organisms during stress conditions (Slama et al., 2015). Proline acts as a molecular chaperone to protect the biological macromolecules during abiotic stress conditions, and glycine betaine is an osmoprotectant known to protect PSII during salinity. Additionally, the integrity of the membranes and the activity of the key enzymes are regulated by the accumulated glycine betaine during stress conditions (Chen and Murata, 2008; Giri, 2011). Exogenous application of proline and/or glycine betaine to salt-stressed rice plants improved plant growth and salinity tolerance suggesting the importance of osmolytes for abiotic stress tolerance (Wutipraditkul et al., 2015). The higher accumulation of proline and glycine betaine in ET101-inoculated tomato and rice plants are important for cellular protection during salt stress conditions. Our results are in congruence with reports on salinity tolerance in various plants by the inoculation of PGPR (Singh et al., 2015; Khan et al., 2016; Cordero et al., 2018; Sapre et al., 2018; Sarkar et al., 2018; Prittesh et al., 2020).

Inoculated plants performed better than the uninoculated plants by showing higher plant growth rate, higher RWC, and maintenance of cellular integrity as indicated by EL. All these parameters serve as good indicators of salt tolerance in glycophytes (Neto et al., 2014; Kumar et al., 2020). The development of root system and architecture is important for the plants to acquire water and nutrition from the soil and thereby increase the replacement rate of water lost during transpiration. Higher proliferation of roots was observed in

ET101-inoculated plants with increased lateral root formation and growth (data not shown), thereby allowing better penetration into the soil to acquire water and nutrition. Salt stress is known to cause damage to the chloroplast structure and instability of the chlorophyll protein complexes resulting in decreased chlorophyll content of leaves (Parida and Das, 2015; Zhang et al., 2020). Although this is true for uninoculated tomato and rice plants, the inoculated plants accumulated greater amounts of photosynthetic pigments (chlorophyll and carotenoids). The ability of plants to synthesize more chlorophyll during salinity stress is a perceptible criterion to a stress-tolerant species. Tolerant genotypes of rice cultivars contained significantly higher chlorophyll content than sensitive genotypes under salinity conditions (Kanawapee et al., 2012). Because higher chlorophyll content contributes to the improvement of photosynthesis process in plants (Pavlović et al., 2014), the PGPR-inoculated plants performed better in terms of photosynthesis than the uninoculated plants. In comparison to these, ET101-inoculated plants showed higher chlorophyll and tend to gain more stress tolerance than the uninoculated plants. Many reports are published showing increased chlorophyll content in plants with PGPR inoculation (Li and Jiang, 2017; Ansari et al., 2019; Prittesh et al., 2020). The presence of high amount of photosynthetic pigments in the PGPR-inoculated plants aids in increasing the biochemical rate of CO_2 fixation, thereby maximizing the net photosynthesis and growth rate of plants during normal and salt stress conditions.

S. sciuri Modulates Photosynthetic Responses in Tomato and Rice Plants Subjected to Salinity Stress

A decline in net photosynthesis rate during salinity stress is due to lower intracellular CO_2 levels due to stomatal closure. The P_N/Ci curves showed the sensitivity of the photosynthesis to salt stress conditions and the induction in photosynthesis to increasing CO_2 levels in leaves of both uninoculated and inoculated tomato and rice plants. However, the induction is more in leaves of inoculated tomato and rice plants. Photosynthetic CO_2 fixation is regulated by both stomatal and non-stomatal limitations. The reduction in P_N in leaves under salinity stress is associated with the increase in intercellular CO_2 (Ci) content in both tomato and rice plants. Stomatal closure during salinity stress is an adaptive measure employed by the plants to minimize water loss during transpiration (Engineer et al., 2016). In our study, the decrease in P_N and g_s is observed in tomato and rice plants subjected to salt stress treatment. Higher P_N along with high g_s rates was observed in tolerant variety IR651 during salinity stress (Foad and Abdelbagi, 2007). As increased photosynthetic capacity is directly linked with the increase in yields (Ambavaram et al., 2014), the employment of bacteria for protecting the photosynthesis during salinity stress and amelioration of salt stress symptoms in plants by bacteria can be considered a feasible approach. Reduction in g_s and transpiration (E) rate are also important adaptive mechanisms of plants for salinity tolerance (Flowers and Yeo, 1981). Impairment of ATP synthesis could also be one of the reasons apart from stomatal closure for decrease in photosynthesis during salinity stress (Cruz et al., 2004). The chloroplasts have the capacity to cope up with the changes for energy demands under stress conditions (Kramer and Evans, 2010). The optimization of ATP and NADPH ratio generated during the light-dependent reactions plays a major role in the prevention of over reduction of chloroplast electron transport chain and ROS production. In our study, although ET101-inoculated plants showed stable ATP/ADP ratio during S0 and S2 stress conditions, the ratio increased significantly during S4 stress conditions due to substantial decrease in ADP levels in tomato plants (**Figure 10**). Decreased ADP levels in S4 plants can possibly be due to suppressed production of ADP or higher phosphorylation of ATP.

Increased photosynthesis during salinity stress conditions can be due to increase in stomatal conductance, possessing a large number of open stomata, or due to high photosynthetic pigment content. Salinity impacted the photosynthesis of both tomato and rice plants by reducing stomatal conductance and leading to decreased diffusion of CO_2 to the carboxylation sites. Salinity stress negatively affects the RuBisCO activity by modulating its rate of biosynthesis and degradation. The Ci/Ca ratio indicates the non-stomatal limitation, which is controlled by enzymatic CO_2 fixation of RuBisCO and production of carbohydrates in the Calvin cycle (Ueda et al., 2013). The observed increase of Ci/Ca ratio in S2- and S4-treated uninoculated plants depicts the non-stomatal limitations in usage of CO_2 by RuBisCO enzyme. The Stevia plants inoculated with the plant growth-promoting *Streptomyces* spp. promoted the accumulation of the RuBisCO protein along with the maintenance of its stability under salinity stress conditions (Tolba et al., 2019). The ET101-inoculated plants sustained the level of Ci/Ca ratio indicating the maintenance of balance between carboxylation and oxygenation of RuBisCO enzyme by enhancing the availability of CO_2. The plants inoculated with ET101 isolate showed an increase in photosynthesis along with stomatal conductance, suggesting bacterial-mediated increase in photosynthesis in plants.

Chlorophyll fluorescence analysis serves as an essential and quick tool for evaluation of plant survival and performance in response to salinity stress (Baker and Rosenqvist, 2004). Salinity stress downregulates photosynthesis and diminishes the quantum yield and efficiency of PSII. In our study, uninoculated plants exposed to S2 and S4 salinity treatments exhibited strong inhibition of photosynthetic capacity, along with diminished PSII activity. As the light-dependent reactions are disrupted during salinity stress, the excess reducing equivalents should be dissipated via non-photochemical processes such as heat or chlorophyll fluorescence to avoid damage to the leaf tissues (Genty et al., 1989; Maxwell and Johnson, 2000). Decreased F_v/F_m indicates that PSII reaction center is damaged subsequent to photoinhibition (Murata et al., 2007). In this study, F_v/F_m was affected when plants were subjected to salt stress indicating damage to the PSII reaction center. However, ET101-inoculated tomato and rice plants showed marginal decrease in F_v/F_m indicating that quantum efficiency of PSII is well maintained by the bacterial inoculation. The actual quantum yield of PSII photochemistry (ΦPSII) was also enhanced by ET101 inoculation. The increase in the NPQ is the major

process carried out by plants to prevent photo damage induced by salinity stress (Maxwell and Johnson, 2000; Stepien and Johnson, 2009). Apparently, the increase in NPQ in ET101-inoculated plants was effective in preventing the decline of F_v/F_m. The recovery of chlorophyll fluorescence parameters upon inoculation of plants with ET101 confirms the tolerance of the plants to salt stress conditions. The dissipation of excitation energy by photochemical utilization contributes to downregulation of PSII to avoid overreduction of primary electron acceptor Q_A (Zribi et al., 2009). The study of Wu et al. (2018) stated that the reduction in ΦPSII and increase in NPQ are the adaptive responses of salt stress in cucumber plants. In our study, ΦPSII and *qP* were significantly declined by salt stress, whereas NPQ is increased in both uninoculated and inoculated plants during salt stress. The shutdown of active photosystem II reflects in higher NPQ due to reduction in the quantity of quantum light absorbed by the reaction centers of photosystem II (Wu et al., 2018). Although the E4 plants exhibited higher NPQ rates, the increase in ΦPSII confirms the active photosystem II activity and importance of bacteria for rendering the protection to photosystem II to tomato and rice plants experiencing salinity stress. Although both uninoculated and inoculated plants showed damage to PSII during salinity stress, our results showed a dramatic decrease in the reduction of photosynthesis in inoculated plants suggesting the positive effect of bacterial inoculation in mitigating salinity stress. The capability of the photosynthetic apparatus to retain its activity ensuring the maintenance of plant productivity is associated with the activation of photo protective mechanisms during bacterial inoculation.

Salinity stress causes imbalance between PSII photochemistry and electrons required for efficient photosynthesis leading to photo inhibition culminating in a stronger decrease in, apparent RuBisCO efficiency (P_N/Ci) (Pérez-López et al., 2013; Wang et al., 2017). The partitioning of reductive power between photosynthesis and alternative sinks, which consume electrons, could be estimated by J/P_N ratio. The higher J/P_N ratio exhibited in tomato and rice plants during S4 stress treatment indicates the downregulation CO_2 assimilation mechanism by salinity stress and the divergence of the flow of electron transport to other metabolic processes. Conversely, in ET101-inoculated plants, the J/P_N ratio was lowered compared to uninoculated plants, indicating involvement of bacterial-derived processes, which drive electron flow to carbon metabolism, reflecting enhanced photosynthesis rate.

Decreased ROS in *S. sciuri* Inoculated Tomato and Rice Plants Is Important for Cellular Homeostasis

The reduction in carboxylation activity of RuBisCO is correlated with the decrease in P_N/Ci ratio and excessive generation of ROS. The oxidative damage occurs due to the imbalance between ROS production and quenching. Strong NBT and DAB staining was observed in C4 plants in both tomato and rice plants, indicating that they have higher stress levels than E2 and E4 plants. The NBT and DAB staining of leaves from tomato and rice plants indicated that H_2O_2 and O_2^- anions accumulated in the cells, suggesting that leaf cells under salinity stress are under oxidative stress. Furthermore, the intense staining of trypan blue in C2 and C4 depicts the damages of leaf tissues in leaves of uninoculated plants. H_2O_2 and O_2^- are primarily generated by the electron transport chain of mitochondria and the membrane-bound PSI electron acceptor found in chloroplast thylakoids (Gill and Tuteja, 2010). The ROS levels were lower in the leaves of inoculated plants than the uninoculated tomato and rice plants, suggesting the likely role of ET101 isolate in activating the plant antioxidant defense mechanism to scavenge the ROS during salinity stress conditions, thereby maintaining cellular redox homeostasis.

S. sciuri Mediated Interplay Between Carboxylation and Oxygenation for Salt Stress Mitigation

The drastic reduction in apparent efficiency of RuBisCO in the uninoculated plants exposed to salinity stress (C2 and C4) suggests the degradation or deactivation of RuBisCO. This difference depicts the imbalance between carboxylase/oxygenase activity, as confirmed by the significant decrease in V_C and increase in V_o. The reduction in the synthesis of ATP and NADPH in the uninoculated plants during salinity stress is related to the unavailability of sources needed for CO_2 fixation in Calvin-Benson cycle (Farquhar and Sharkey, 1982). Photorespiration plays a major role in protecting the plant cells against various abiotic stresses by maintaining the electron flow and activity of Calvin cycle enzymes, thereby preventing the accumulation of enzyme inhibiting metabolites and photoinhibition (Wingler et al., 2000; Voss et al., 2013). Although photorespiration diminishes the potential photosynthetic activity of the plants through catalyzing the oxygenase reaction by RuBisCO, it prevents the damage to the photosynthetic apparatus caused by excessive salinity stress (Salazar-Parra et al., 2015). Adopting combined measurement of gas exchange and Chl fluorescence to calculate J_o and photorespiratory rate, we observed that while the J_o of the uninoculated plants exposed to salinity stress increased (i.e., the photorespiration was activated by the stimulation from salinity stress), the J_o of the inoculated plants decreased significantly. Decrease in the intracellular CO_2 concentration due to decrease in g_s along with increase in oxygenase activity of RuBisCO enzyme could be the responsible factors for the increase in photorespiratory activity during salinity stress (Farquhar and Sharkey, 1982; Hartman and Harpel, 1994). Correspondingly, *S. sciuri* ET101-inoculated plants sustained relatively high F_v/F_m and ΦPSII levels and higher P_N rates along with lower photorespiratory rates. The photorespiratory rates of uninoculated plants increased markedly during salinity stress, and so did F_v/F_m and ΦPSII to a relatively low degree. The ET101-inoculated plants during salinity stress maintained a lower photorespiration, thereby providing photoprotection to the stressed plants. The sustainability of relatively high F_v/F_m and ΦPSII by ET101-inoculated plants could possibly provide higher photosynthetic capacity. The involvement of photoprotective

mechanism as an important contributor for salt tolerance in *Ricinus communis* was shown by Neto et al. (2014).

CONCLUSION AND FUTURE PERSPECTIVES

The results from the current study indicated that the isolated halotolerant bacteria *S. sciuri* ET101 plays a crucial role in protecting the tomato and rice plants against damaging effects of salt stress. The inoculation of bacteria led to a biomass enhancement, increased photosynthetic performance, alterations in leaf gas exchange, and photosynthetic pigment contents under salt stress. The alteration in the apparent RuBisCO activity by the inoculation of *S. sciuri* ET101 in tomato and rice plants can provide novel insights in the plant–microbe interaction mechanisms during salinity stress. The current study paves a way for exploration of photorespiration process in bacterial-mediated alleviation of the salt stress responses in plants. Additionally, the changes in photosynthesis and photorespiration in plants inoculated with halotolerant bacteria need to be explored at molecular level. Furthermore, it is essential to isolate more potent bacteria with PGP traits and gain deeper insights into the bacterial-mediated salinity stress mitigating mechanisms. This will be important for obtaining increased crop growth and higher productivity under salinity stress conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ZT and DC designed the research and wrote the manuscript. ZT performed the experiments and interpreted the data. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.547750/full#supplementary-material

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Restructuring the Cellular Responses: Connecting Microbial Intervention With Ecological Fitness and Adaptiveness to the Maize (*Zea mays* L.) Grown in Saline–Sodic Soil

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Salt stress hampers plant growth and development. It is now becoming one of the most important threats to agricultural productivity. Rhizosphere microorganisms play key roles in modulating cellular responses and enable plant tolerant to salt stress, but the detailed mechanisms of how this occurs need in-depth investigation. The present study elucidated that the microbe-mediated restructuring of the cellular responses leads to ecological fitness and adaptiveness to the maize (*Zea mays* L.) grown in saline–sodic soil. In the present study, effects of seed biopriming with *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 singly and in consortium on different growth parameters were recorded. Soil biochemical and enzymatic analyses were performed. The activity and gene expression of High-Affinity K^+ Transporter (*ZmHKT-1*), Sodium/Hydrogen exchanger 1 (*zmNHX1*), and antioxidant enzymes (*ZmAPX1.2*, *ZmBADH-1*, *ZmCAT*, *ZmMPK5*, *ZmMPK7*, and *ZmCPK11*) were studied. The expression of genes related to lateral root development (*ZmHO-1*, *ZmGSL-1*, and *ZmGSL-3*) and root architecture were also carried out. Seeds bioprimed with consortium of all three strains have been shown to confer increased seed germination (23.34–26.31%) and vigor indices (vigor index I: 38.71–53.68% and vigor index II: 74.11–82.43%) as compared to untreated control plant grown in saline–sodic soil at 30 days of sowing. Results indicated that plants treated with consortium of three strains induced early production of adventitious roots (tips: 4889.29, forks: 7951.57, and crossings: 2296.45) in maize compared to plants primed with single strains and untreated control (tips: 2019.25, forks: 3021.45, and crossings: 388.36), which was further confirmed by assessing the transcript level of *ZmHO-1* (7.20 folds), *ZmGSL-1* (4.50 folds), and *ZmGSL-3* (12.00 folds) genes using the qPCR approach. The uptake and translocation of Na^+, K^+, and Ca^{2+} significantly varied in the plants treated with bioagents alone or in consortium. qRT-PCR analysis also revealed that the *ZmHKT-1* and *zmNHX1* expression levels varied significantly in the maize root upon

inoculation and showed a 6- to 11-fold increase in the plants bioprimed with all the three strains in combination. Further, the activity and gene expression levels of antioxidant enzymes were significantly higher in the leaves of maize subjected seed biopriming with bioagents individually or in combination (3.50- to 12.00-fold). Our research indicated that *ZmHKT-1* and *zmNHX1* expression could effectively enhance salt tolerance by maintaining an optimal Na^+/K^+ balance and increasing the antioxidant activity that keeps reactive oxygen species at a low accumulation level. Interestingly, up-regulation of *ZmHKT-1*, *NHX1*, *ZmHO-1*, *ZmGSL-1*, and *ZmGSL-3* and genes encoding antioxidants regulates the cellular responses that could effectively enhance the adaptiveness and ultimately leads to better plant growth and grain production in the maize crop grown in saline–sodic soil.

Keywords: seed biopriming, rhizosphere microorganisms, Maize (*Zea mays* L), saline–sodic soil, antioxidant enzymes, salt tolerance, High-Affinity K+ Transporter, *Sodium/hydrogen exchanger*

INTRODUCTION

Soil salinity and sodicity are major abiotic stresses, causing considerable yield loss in a wide range of crops worldwide. An estimate indicated that 7% of the world's total arable land (955 million ha) is affected by salt, and in the next 25 years, this may reach up to 30% of the total cultivable land (Flowers et al., 1997; Singh S. et al., 2019). Further, it is estimated that up to 50% of total cultivable land will be lost due to salinity and sodicity by 2050 (Shahbaz and Ashraf, 2013). In India, 7 million ha of land is affected by salt stress, while the Indo-Gangatic Plain (IGP) region alone has approximately 2.7 million ha of area being affected by soil salinity and sodicity (Singh S. et al., 2019). These soils are usually unfit for crop production due to high pH, low organic matter content, and high concentrations of soluble salts such as Na_2CO_3, $NaHCO_3$, NaCl, etc., and together with sufficient exchangeable sodium, they cause poor soil physio-biochemical characteristics (Das, 2009). Excessive salt/Na^+ in the soil solution affects absorption of water and mineral nutrient and, therefore, causes seedling mortality by pulling water from the root system (exo-osmosis) and disrupting cellular function (Das, 2009; Shrivastava and Kumar, 2015; Singh et al., 2016a; Singh S. et al., 2019).

Maize (*Zea mays* L.) is considered as one of the most important cereal crops after rice and wheat all over the world (Nuss and Tanumihardjo, 2010). It is grown under a wide spectrum of soil and climatic conditions. In addition, maize is moderately sensitive to salt stress and is responsible for significant loss of crop yield worldwide (Anonymous, 2018; Singh S. et al., 2019). Soil salinity and sodicity reduce seed germination, early crop growth by suppressing leaf initiation and expansion, as well as internode growth and poor crop establishment. Further, salt stress leads to membrane damage, reduction in leaf relative water content, denaturation of proteins, accumulation of oxidizing substances, inactivation of enzymes, and decline of photosynthesis rate, which ultimately leads to stunted growth and low yield in maize (Apse et al., 1999; Shrivastava and Kumar, 2015; Singh S. et al., 2019). Therefore, salinity and sodicity proved to be two of the most serious threats to maize production (Singh S. et al., 2019).

To withstand salt stress, plants have evolved sophisticated and complex signaling mechanisms/response pathways resulting in adaptive responses through morphological and physio-biochemical changes (Gholami et al., 2009; Jiang et al., 2016, 2017; Komis et al., 2018). Further, roots are the first and foremost part of the plants exposed to any stress. The development of root architecture is in general regulated by genetic make-up of the plants including inbred lines or cultivars and environmental factors including abiotic and biotic stresses (Zimmermann et al., 2010; Zhang et al., 2018). Several studies reported that a number of genes have been found to be involved in lateral root development in maize (Zimmermann et al., 2010; Ding et al., 2013; Zhang et al., 2018). In general, several research findings reported the role of microbial inoculants on the root growth and biomass accumulation (Singh et al., 2016a,b, 2019). However, none of the studies showed the involvement of rhizospheric microbes on the activation of genes involved in lateral root development in maize under salt-stressed conditions.

To exploit the salt-stressed soil, new and more efficient ways to increase crop yield are important for sustainable crop production and food security, particularly in saline–sodic soil of India (Das, 2009; Shrivastava and Kumar, 2015). Plant breeders have tried to develop salt-tolerant cultivars in many crops including maize. However, none of the maize cultivars are commercially available so far, which possess high degree of salt tolerance (Farooq et al., 2015). Therefore, we still need such donor parents having quantitative trait loci (QTLs) or gene(s) with salt tolerance to prevent losses to our maize production (Singh S. et al., 2019). Several alternatives have been utilized to improve salt tolerance, and among them, exploitation of salt-tolerant compatible microbial inoculants is an emerging approach to ameliorate the toxic effects of salt in crop plants (Ashraf and Foolad, 2007; Agbodjato et al., 2016; Latef and Tran, 2016). In the recent past, attention has been given to identify and utilize the consortia of compatible salt-tolerant rhizospheric microorganisms (STRM) that can mediate induced systemic tolerance (IST) to sustain and improve plant growth under such stressful conditions (Gaiero et al., 2013; Egamberdieva et al., 2019; Irfan et al., 2019). The salt

tolerance responses are triggered by STRM or STRM-derived elicitors/signaling molecules and eventually lead to extensive transcriptional reprogramming (Munns and Tester, 2008; Pitann et al., 2013; Kifle and Laing, 2016a,b). Numerous salt tolerance-related genes are up-regulated in the plant under salt stress and primed with STRM (Sandhya et al., 2010; Shi et al., 2010; Wang et al., 2010; Szoboszlay et al., 2015; Sun et al., 2015; Soares et al., 2018). Considerable efforts have been made to utilize plant growth-promoting (PGP) rhizosphere microorganisms for enhanced salt tolerance in many crops (Sandhya et al., 2010; Singh et al., 2016a; Tiwari et al., 2018; Singh S. et al., 2019; Vaishnav et al., 2020). More importantly, these studies have shown the rhizosphere microbe-mediated reprogramming in the cellular physiological networks, genetic base of salt tolerance, regulation of antioxidant defense systems, and photosynthesis, and finally alleviate the detrimental effects of salt in many plant species in a fragmented way (Yamaguchi and Blumwald, 2005; Bais et al., 2006; Liu et al., 2006; Young et al., 2013; Mahmood et al., 2016; Bokhari et al., 2019; Wang et al., 2020). Though the rhizosphere microbiome constitutes a rich gene pool, selecting rhizospheric partners from the same plant species would offer a competitive advantage for the microbe to succeed (Mahoney et al., 2017; Singh S. et al., 2019; Molina-Montenegro et al., 2020). Moreover, information regarding the effects of native rhizobacteria on physiological and antioxidant machinery of salt-stressed maize plants is limited and needs to be further explored. The objective of the present study was to elucidate the microbe-mediated physio-biochemical and molecular mechanisms that provide salt tolerance, ecological fitness, and adaptiveness to maize plants grown in saline–sodic soil. This systematic investigation provided novel insights to better understand the mechanisms of the microbe-induced plant to salt tolerance and enhancement in the maize production under saline–sodic soil.

MATERIALS AND METHODS

Isolation and Characterization of Bacterial Isolates

Twenty-five samples of maize rhizosphere soil were collected from different parts of Uttar Pradesh and Madhya Pradesh (India) during 2015–2016 and brought to the laboratory (**Supplementary Table 1**). Soil samples were sieved (2 mm pore size), air dried under shade to remove the excess moisture, and used for isolation. Two hundred fifty bacterial isolates were isolated from rhizosphere soil by plating serial decimal dilution on HiChrome Bacillus Agar (HiMedia, India), and all the isolates were propagated and kept on Nutrient Agar (NA) medium at 4°C until further use.

These isolates were evaluated for salt tolerance capability on NA supplemented with different concentrations of NaCl (1–10%) as per methods described by Singh et al. (2015). Further, the isolates tolerating above 5% of NaCl were screened for P (Nautiyal, 1999), K (Rajawat et al., 2016), and Zn (Sharma et al., 2014) solubilization using the standard protocols. Based on salt tolerance, and P, K, and Zn solubilization, 154 potential isolates were selected for identification at the molecular level. The selected isolates were further screened for their PGP traits, *viz.*, indole acetic acid (Brick et al., 1991), siderophore (Schwyn and Neilands, 1987), and ammonia (Dey et al., 2004) production. The production of HCN, H_2O_2, urease, catalase, and starch hydrolysis test was performed as per the methods described in Whitman et al. (2012). However, protease estimation was carried out as per the methods described by Boller and Mauch (1988).

16S rRNA Gene Sequencing and Phylogenetic Analysis

Molecular identification of the shortlisted isolates was conducted by DNA extraction and 16S rRNA gene amplification using universal primers pair 27F and 1492R 42 (Edwards et al., 1989). The 16S rRNA gene amplicons were further subjected to restriction endonuclease digestion by using endonucleases *Hae-III*, *Alu-1*, and *Msp-1*. The digested amplicons were separated in 2.5% agarose gel added with ethidium bromide, and electrophoresis was done at 45 V for 1.5 to 2 h in 1 × TAE buffer. Grouping was done using NTSYSpc, version 2.02 software and at least one representative isolate was selected for sequencing of 16S rRNA gene amplicon. RFLP pattern revealed that all 154 isolates fall into 23 different clusters. From each group, best-performing isolates were selected and ended with a total of 50 isolates. In this way, 16S rRNA gene of 50 isolates was sequenced through Eurofin Pvt. Ltd. (India) and the sequence similarity was matched using the EzBiocloud database for identification. Phylogenetic analysis was carried out using the Molecular Evolutionary Genetics Analysis (MEGA-X) tools and 16S rRNA gene sequences were submitted to the NCBI GenBank.

In planta Assay

Experimental Setup

The experiments consisted of five different treatments: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15 (1:1:1), and T_5—control (untreated). Each treatment consisted of 10 replications under nethouse conditions, whereas 5 replications were maintained under field conditions in a randomized block design (RBD). The nethouse experiments and field experiment were conducted during *Summer* (March to May) and *Kharif* season (June to August) in 2017–2018.

Soil Collection, Preparation, and Analysis

To lay out the pot experiments, soil was collected from Research Farm, ICAR-National Bureau of Agriculturally Important Microorganisms (ICAR-NBAIM), Kushmaur, air dried in shade, sieved (2 mm), and brought to the laboratory. The soil belongs to texture class Dystric Eutrudepts (Inceptisols). Further, soil was mixed with nitrogen, phosphorus, and potassium at 150, 80, and 40 kg ha^{-1}, respectively. Soil was filled in HiDispo polythene bags (HiMedia, India) and autoclaved twice for 30 min (121°C at 15 PSI) at 24-h intervals. The autoclaved soil was kept as such for 3–5 days under shade to maintain the natural ionic equilibrium

inside the soil. The initial soil properties were analyzed using standard protocols and presented in **Supplementary Table 2**.

Development of Liquid-Based Bioformulation

The new medium (liquid base) was designed and developed by US and his team and was taken for preparation of bioformulations (composition was not shown due to IP protection). Broadly, liquid-based formulation of *Bacillus* spp. was developed using nutrient broth constituent as a base (peptone, 5 *g*; beef extract, 3 *g*; NaCl, 5 *g*; water, 1000 ml; and pH at 25°C: 7.3 ± 0.2). After 96 h of growth, 15% sterile glycerol along with 0.01% PVP was added to the formulation. The growth kinetics of selected strains was tested and found to be almost the same. For preparation of bioformulations, all the three strains were grown separately in the liquid medium till CFU count reached 2.5×10^8 and were taken as primary inoculums/mother culture. Further, 1 ml of primary inoculums was added in the 100-ml liquid medium for preparation of working formulation. During consortium development, primary inoculums were mixed in a 1:1:1 ratio to develop bioformulation. The selected strains were inoculated singly and in combination of the three in the medium, and incubated for 5 days in the incubator shaker at 150 RPM at 28°C. After 5 days, 1 ml of culture suspension was taken and colony-forming units were measured by plating serial decimal dilution on NA medium. The CFU count of the final product was 2.24×10^8 at the time of application.

Planting Material and Growth Conditions

Maize seeds (*cv*. Sachin 777) used in this study were procured from an open market. Surface sterilization was done with sodium hypochlorite (NaOCl, 1%) for 2 min, followed by three washing cycles with sterile distilled water under aseptic conditions. Maize seeds were bioprimed with liquid bioformulation (10 ml kg^{-1} seed suspended in 40 ml of water containing 0.01% gum acasia and 0.01% trehalose) and incubated overnight under the shade (Singh et al., 2020). Seeds treated with sterile liquid bioformulation containing 15% sterile glycerol along with 0.01% PVP served as control. The bioprimed and untreated control seeds were sowed in the plastic post (20 × 20 cm) containing sterilized experimental soil (5 kg). Two plants were maintained in each pot and a control set (untreated) was maintained in each experiment. The pots were watered manually on alternate days to maintain the moisture content (60%).

Field experiments were conducted at Research Farm, ICAR-NBAIM, Kushmaur in *Summer* and *Kharif* maize. The experiments consisted of five different treatments in five replicates. The size of the individual plot was 5 × 4 m^2 with a border space of 1 m. The bioprimed seeds were sown in the field manually in the evening hours with a spacing of 45 × 30 cm (row-to-row and plant-to-plant spacing). The average mean temperature and relative humidity during the experimentation in the *Summer* maize were 27.36°C and 56–61%, respectively, whereas it was 26.25°C and 76–81%, respectively, in the *Kharif* maize.

Effect of Seed Biopriming on Seed Germination and Vigor Indices

Effects of biopriming on seed germination (%) and vigor indices were studied in sterile sand and soil mixture (1:3) under nethouse conditions as per methods described by Singh et al. (2016b). In brief, for seed germination (%) test, 300 seeds were sterilized with sodium hypochlorite (1%) and washed thrice in sterile distilled water. Thereafter, sterilized seeds were bioprimed with selected strains *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually and in combination of three as per treatments and sown in tray (45 × 30 × 10 cm) containing sterile sand and soil mixture. After 15 days of sowing, percent germination was calculated. To measure the vigor indices, sterile seeds (3) were planted in each pot (20 cm diameter) containing sterile sand and soil mixture (3 kg). After sowing, the pots were irrigated on alternate days with sterile water throughout the experimental period. After 30 days of sowing, the vigor indices were calculated as per methods described by Singh et al. (2016b). The growth conditions in the nethouse were as follows: 14/10 h day/night photoperiod, 38/29°C day/night temperature, and 50/60% day/night humidity in *Summer* maize. Growth conditions were 13/11 h day/night photoperiod, 34/25°C day/night temperature, and 70/85% day/night humidity in *Kharif* maize.

Effect of Seed Biopriming on Root Architecture and Root Development

To analyze the effect of seed biopriming on root architecture, bioprimed seed (2 No.) was planted in the pots containing sterile sand soil mixture as discussed in the section *Effect of seed biopriming on seed germination and vigor indices*. After seed germination, a single seedling was maintained in each pot throughout the experiment. Thereafter, plants were allowed to grow for the next 30 days. On the 30th day, plants were uprooted gently and brought to the laboratory. The roots were washed carefully under running tap water and clean roots were scanned (Regent Instrument, Canada). The scanned images were analyzed using image analysis software "WinRhizo Pro 2017" (Client# IN1803202) to study the different parameters of root architecture. To see the root colonization potential of selected strain(s), plants were uprooted gently and washed in running tap water, the root samples were fixed with the help of glutaraldehyde (2.5%) and formaldehyde (37%; 1:1), and microscopy was done using a scanning electron microscope (Hitachi S-3400N, United States) as per methods described by Singh U. B. et al. (2019).

A quantitative real time-PCR (qPCR) analysis was performed to investigate the expression of 11 genes conferring secondary root development, plant adaptiveness, and salinity tolerance in maize. For qPCR analyses, root samples were collected after 30 days of sowing. The root samples were quick-frozen in liquid nitrogen and ground, and total RNA was isolated using RNA isolation kit (Agilent, India) following the manufacturer's protocol. One microgram of RNA was used to synthesize cDNA with oligo-dT using cDNA Synthesis Kit (BioRAD,

India) according to the manufacturer's instructions/protocols. The concentration of cDNA was determined using Nanodrop 2000c (Thermo Scientific, United States). The housekeeping gene actin was used as an endogenous standard to normalize the quantitative expression data of *ZmHO-1* (*Zea mays Haem Oxygenase-1*), *ZmGSL-1* (*Zea mays Gibberellic Acid Stimulated-Like 1*), and *ZmGSL-3* (*Zea mays Gibberellic Acid Stimulated-Like 3*). The gene expression was analyzed using gene-specific primers (**Table 1**). The qRT-PCR was performed using the SYBR Green master mix (Thermo Scientific) on the BioRAD Real-Time PCR System (MJ MiniOpticon System, BioRAD). The specificity of the amplification was verified by melting-curve analysis. Three replications were performed for each sample. The relative transcription levels were calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001).

Effect of Seed Biopriming and Microbial Intervention on Physio-Biochemical Parameters and Antioxidant Enzymes

The quantitative estimation was done to see the effects of seed biopriming on physio-biochemical properties and antioxidant enzymes in the leaves of plants bioprimed with selected strain(s) at 30 days of sowing. Total chlorophyll, carotenoids, total soluble sugar, and total protein were estimated quantitatively according to Sadasivam and Manickam (1996). Plants tend to overproduce proline, phenolics, flavonoids, and antioxidant enzymes under stress conditions. The synthesis and accumulation of total proline, total phenolics, and total flavonoids in the maize leaves were assayed as per methods described by Thimmaiah (2012). The activity of catalase and peroxidase was assayed according to Sadasivam and Manickam (1996). However, the activity of superoxide dismutase (SOD) was assayed spectrophotometrically as per methods described by Singh S. et al. (2019).

To investigate whether seed biopriming with selected strain(s) up-/down-regulate the antioxidant gene expression such as *ZmAPX1.2* (*Ascorbate peroxidase 1.2*), *ZmBADH-1* (*betaine aldehyde dehydrogenase-1*), *ZmCAT* (*Catalase*), *ZmMPK5*, *ZmMPK7*, *ZmCPK11*, *zmHNX1* (*Zea mays Sodium/hydrogen exchanger 1*), and *zmHKT1* (*Zea mays high-affinity K^+ transporter 1*) was studied in the maize plants under salt-stressed conditions. Total mRNA was extracted from maize leaves at 15 days of sowing; cDNA synthesis and quantification of cDNA were done as per methods described in the section *Effect of seed biopriming on root architecture and root development*. The qRT-PCR was performed using the SYBR Green master mix (Thermo Scientific) and primer pairs (**Table 1**) on the BioRAD Real-Time PCR System (MJ MiniOpticon system, BioRAD). The specificity of the amplification was verified by melting-curve analysis. The relative transcription levels were calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001) and housekeeping gene actin was used as an endogenous standard to normalize the quantitative expression data. Three replications were performed for each sample.

Effect of Seed Biopriming and Microbial Intervention on Plant Growth Attributes, Physiological Traits, and Yield

To determine the effects of seed biopriming on plant growth attributes, physiological traits, and yield, and pot and field experiments were conducted on maize grown in saline–sodic soil. From nethouse experiments, five plants were selected randomly and uprooted from each treatment to observe average shoot and root length, average number of leaves per plant, and average fresh and dry biomass of shoot and root in the *Summer* and *Kharif* maize at 45 DAS. However, five plants were selected from the field experiments to measure the average plant height, average number of leaves, and dry biomass of shoot and root in the in the *Summer* and *Kharif* maize at 45 DAS. To see the effects of seed biopriming and microbial intervention on physiological traits, five plants were sampled randomly and we measured the leaf area index, mean crop growth rate, mean relative growth rate, and mean assimilation rate in the *Summer* and *Kharif* maize grown under field conditions at 45 DAS as per methods described by Rajput et al. (2017). Further, the effects of microbial intervention on yield-attributing traits such as average number of cobs/plant, average number of grain/cob, test weight (weight of 1000 grains), and yield/plot were measured in the *Summer* and *Kharif* maize grown under field conditions at harvest.

Effect of Seed Biopriming and Microbial Intervention on Soil Enzymes, Microbial Biomass Carbon, and Soil Respiration

Rhizosphere soil samples were collected from *Kharif* maize grown under nethouse experiments to measure the activity of soil enzymes such as dehydrogenase, alkaline phosphatase, acid phosphatase, protease, cellulase, invertase, microbial biomass carbon (MBC), and soil respiration at 45 DAS. The quantitative estimation of dehydrogenase activity (μg TPF/g of dry soil/24 h) in the rhizospheric soil samples was carried out using spectrophotometric method as given by Casida et al. (1964). The alkaline phosphatase activity (μg PNP/g of dry soil/2 h) was assayed as per methods described by Tabatabai and Bremner (1969). Briefly, soil samples (1 *g*) were taken in a 50-ml Erlenmeyer flask containing 0.2 ml of toluene, 4 ml of modified universal buffer, and 1 ml of *p*-nitrophenol phosphate solution and incubated at 37°C for 1 h. Further, calcium chloride (1 ml) and sodium hydroxide (4 ml) were added in the flasks. The intensity of yellow color (*p*-nitrophenol) developed in the filtrate was measured colorimetrically at 440 nm. Further, to estimate acid phosphatase activities in the soils, samples were incubated with Na *p*-nitrophenyl phosphate at 37°C in universal buffer (pH 6.5) for 1 h by adding 0.25 ml of toluene to control microbial growth (Tabatabai and Bremner, 1969). The protease activity in the soil was determined by following the protocols described by Rejšek et al. (2008). Briefly, 0.05 M tris–HCl buffer (2 ml) and 1% casein (2 ml) were added in the soil samples (1 *g*) and incubated at 49°C for 2 h. Trichloroacetic acid (1 ml) was added to stop the reaction. Further, 1 ml of supernatant was added to the Na_2CO_3 and $CuSO_4$ solution and mixed properly. Thereafter,

TABLE 1 | Primers used in qRT-PCR analyses.

S. No.	Gene name	Primer sequences	References
1.	**Housekeeping gene**		
	ZmActin-RT-F	CTGAGGTTCTATTCCAGCCATCC	Jiang et al., 2018
	ZmActin-RT-R	CCACCACTGAGGACAACATTACC	Jiang et al., 2018
2.	**High-Affinity K^+ Transporter**		
	ZmHKT1-RT-F	TCGGCTCTGGACCTACTCTT	Zhang et al., 2017
	ZmHKT1-RT-R	ACGACGACGACTCTGCTCTA	Zhang et al., 2017
3.	**Sodium/hydrogen exchanger 1**		
	ZmNHX1-RT-F	ATGCAGGGTTCCAAGTGAAG	Jiang et al., 2016
	ZmNHX1-RT-R	AATATTGCCCCAAGTGCAAG	Jiang et al., 2016
4.	**Genes related to lateral root development**		
	ZmHO-1-RT-F	ACACTGTTGGCTGATCCAGT	Zhang et al., 2018
	ZmHO-1-RT-R	AAACGTATCTGGGGGAGGGA	Zhang et al., 2018
	ZmGSL-1-RT-F	CTAATTTGCTGCGCGGCAATG	Zhang et al., 2018
	ZmGSL-1-RT-R	CACTTGCGGCAGAAGAAGAG	Zhang et al., 2018
	ZmGSL-3-RT-F	GCTCTGCGGCAGCAGGTGAAG	Zimmermann et al., 2010
	ZmGSL-3-RT-R	GATGTTCCTCATCAATCCGGGG	Zimmermann et al., 2010
5.	**Antioxidant enzymes**		
	ZmAPX-1.2-RT-F	GGCAAGCAGATGGGTTTGA	Phillips et al., 2018
	ZmAPX-1.2-RT-R	CTCCACAAGAGGGCGGAAGA	Phillips et al., 2018
	ZmBADH-1-RT-F	ATTGGGGTTGTTGGACTGATCACTC	Phillips et al., 2018
	ZmBADH-1-RT-R	TGGGAATGTGAGGATAATGGAGCAC	Phillips et al., 2018
	ZmCAT-RT-F	TCAAGCCGAATCCAAAGACCA	Phillips et al., 2018
	ZmCAT-RT-R	TCGAGCAAGCATTTCACACCA	Phillips et al., 2018
	ZmMPK-5-RT-F	GATTATCAGTAGCCAAAGTTCAA	Jiang et al., 2016
	ZmMPK-5-RT-R	ACACCGTCACCAGCTTTTAATC	Jiang et al., 2016
	ZmMPK-7-RT-F	CCAGTAGCCAAAGTTCGGTTC	Jiang et al., 2016
	ZmMPK-7-RT-R	TACAGACAACACCGAGAAGTACTTA	Jiang et al., 2016
	ZmCPK-11-RT-F	AGAACGAAATCCAGGCTCTAATG	Jiang et al., 2016
	ZmCPK-11-RT-R	ATTCGCGACATGCTTGTGAC	Jiang et al., 2016

Folin–Ciocalteau reagent (1 ml) was added and the concentration of aromatic amino acids was determined spectrophotometrically at 648 nm. L-Tyrosine was taken as standard and activity was denoted as μg tyrosine g^{-1} of dry soil h^{-1}.

To estimate the cellulose activity in the soil (μg glucose g^{-1} of dry soil h^{-1}), the soil samples (5 *g*) were taken into an Erlenmeyer flask (50 ml) containing 0.5 ml of toluene, 10 ml of acetate buffer, and 10 ml carboxymethyl cellulose solution (Pancholy and Rice, 1973). The flasks were incubated at 30°C for 24 h. The reducing sugar content in the samples was determined by Nelson–Somogyi's method by using D-glucose as standard. The invertase activity (mg glucose g^{-1} of dry soil h^{-1}) in the maize rhizosphere soil was determined as per methods described by Gianfreda et al. (1995) with slight modifications. Briefly, the soil samples (1 *g*) were taken into an Erlenmeyer flask (50 ml) containing saccharose (5 ml) and sodium acetate buffer, incubated at 30°C for 1 h, and centrifuged at 3,500 *g* for 10 min. The concentration of sugar produced from saccharose was determined by Nelson–Somogyi's reagent. D-glucose was taken as standard.

To estimate the MBC, soil samples (10 *g*) were added into the beakers containing 25 ml of chloroform and fumigated in airtight desiccators for 5 min. Thereafter, 0.5 M K_2SO_4 (40 ml) was added to the beaker and shaken for 30 min. Further, 8 ml of the filtrate was taken into conical flask (500 ml) containing 2 ml of $K_2Cr_2O_7$ (0.2 N), 10 ml of concentrated sulfuric acid, and 5 ml of orthophosphoric acid, and the content was refluxed at 100°C for 30 min. After cooling the sample, ferroin indicator was added and titrated against standard ferrous ammonium sulfate to obtain a brick-red end point. The organic carbon was determined in non-fumigated soil by following a similar method for determining MBC in terms of mg kg^{-1} of dry soil. However, soil respiration was measured as per methods described by Bekku et al. (1995). Briefly, for each replication, 50 *g* of moist soil was incubated in a 500-ml jar (along with two blanks) with an alkali trap containing 20 ml of 1 *N* NaOH to capture CO_2. To measure soil respiration, alkali traps after 10 days of incubation were drawn out of the jars. Amount of CO_2 trapped was determined by back titration of the 1 *N* NaOH with 0.5 *N* HCl at pH 8.3 in the presence of saturated $BaCl_2$ using phenolphthalein indicator. The following equation was used to assess CO_2 evolved:

$$CO2\ C\,evolved\ =\ (AB)\ \times\ N\times\ 6\ \ldots \qquad (1)$$

where A and B are the volumes (ml) of HCl consumed for titrating 10 ml 1 *N* NaOH of flask without soil and flask with soil, respectively; *N* is the normality of HCl, and six is the equivalent weight of C. The value was expressed in mg CO_2-C $(100\ g)^{-1}$ $(10\ day)^{-1}$ on dry weight basis.

Effects of Seed Biopriming and Microbial Intervention on Na^+, K^+, and Ca^{2+} Uptake

Five plants were sampled to measure the uptake of Na^+, K^+, and Ca^{2+} from the *Kharif* maize grown under field experiments at harvest. The quantitative measurements of Na^+, K^+, and Ca^{2+} content in the root and shoot were performed as per methods described by Zhang et al. (2008) using the inductively coupled plasma spectrograph (Optima 2100DV, Perkin Elmer).

Statistical Analysis

Nethouse and field experiments were carried out in complete RBD in 10 and 5 replications, respectively. Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) at $p \leq 0.05$ using SAS 9.2 version. Data were compared with Duncan's multiple range test at $p \leq 0.05$. Graphs were prepared using statistical software Origin 9.0.

RESULTS

Microbial Strains

During the course of exploration and isolation of microbial strains for alleviation of salt stress, 250 strains were isolated from different soil samples collected. These strains were screened for salt tolerance (NaCl 1–10%). Based on the salt tolerance (above 5% NaCl), P, K, and Zn solubilization, and PGP traits, 154 potential isolates were selected for identification at the molecular level. Molecular characterization of 154 selected strains was carried out based on 16S rRNA gene amplification and its RFLP pattern with a set of three endonuclease restriction enzymes *Hae-III*, *Alu-1*, and *Msp-1*. A total of 50 strains were selected as representatives based on RFLP clustering at 70% similarity level and the PGP traits they have shown (**Supplementary Table 3**). These 50 strains were sequenced by Sanger's di-deoxy nucleotide sequencing method, and identification was done based on percentage similarity (EzBiocloud, a public database of type strains) by BLAST homology (**Supplementary Table 4**). These sequences were submitted to NCBI GenBank and accession numbers were obtained. Based on BLAST homology results, 23 different species including 20 species of *Bacillus* and one each of *Pseudomonas aeruginosa*, *P. geniculata*, and *Enterobacter cloacae* subsp. *dissolvens* were reported. It was further observed that *Bacillus altitudinis* was the most dominant species with nine strains followed by *B. safensis* with seven strains and *B. aryabhattai* with six strains (**Figure 1**). Therefore, on the basis of salt tolerance (>8% NaCl), P, K, and Zn solubilization, and other PGP traits tested *in vitro*, the most promising strains *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 were selected for further *in planta* experimentation on maize grown in saline–sodic soil.

Effect of Microbial Intervention on Seed Germination and Seedling Vigor

Effects of seed biopriming and microbial intervention on seed germination (%), vigor index I, and vigor index II were studied under nethouse conditions after 30 DAS. All the three strains, i.e., *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually or in consortium, were found to increase percent seed germination in *Summer* and *Kharif* maize. Results showed that maximum germination was recorded in the seeds treated with *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 in combination compared to individual inoculation in both *Summer* (94.36%) and *Kharif* (96.25%) maize. However, no significant difference was recorded for the percent seed germination in case of individual inoculation. The least seed germination (%) was recorded in untreated control (**Table 2**). Similarly, the highest vigor index I and vigor index II were recorded in the plants inoculated with consortium of all three strains in *Summer* (4550.33 and 405.22, respectively) and *Kharif* (4675.29 and 444.50, respectively) maize. However, significant differences in the vigor index I and vigor index II were recorded in the plants treated with either bioinoculant individually in *Summer* and *Kharif* maize at 30 DAS. The lowest vigor index I and vigor index II were recorded in the untreated control plants in *Summer* (3280.43 and 222.75, respectively) and *Kharif* (3042.25 and 255.75, respectively) maize (**Table 2**). It was further observed that *Kharif* maize showed higher vigor indices compared to *Summer* maize. None of the microbial strains tested individually or in combination had a phytotoxic as well as an adverse effect on maize plants (visual observations, data not shown) grown in *Summer* and *Kharif* season. It was concluded that consortium of all three strains gave significantly higher percentage of seed germination and vigor indices as compared to treatments and untreated control.

Effects of Seed Biopriming and Microbial Inventerization on Root Architecture

To see the effects of seed biopriming and microbial inventerization on root architecture, growth, and development, experiments were conducted in *Kharif* season under nethouse conditions. Scanning electron microphotographs clearly indicated that selected strains have the potential to colonize maize root even under saline–sodic soil at 30 DAS (**Figure 2A**). Upon inoculation and proper colonization of bioinoculants in the rhizosphere, an increased level of IAA was estimated (data not shown). Further, root scanning results clearly indicated that microbial inventerization has positive effects on root architecture and different parameters of root development. A significant ($p \leq 0.05$) increase in the surface area (449.73 cm^2), projected area (143.15 cm^2), root length (2151.01 cm), length per volume (2151.01 cm/m^3), total root volume (7.48 cm^3), average diameter (0.75 mm), number of tips (4889.29), number of forks (7951.57), and number of crossings (2296.45) was recorded in the plant inoculated with all three strains, *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 in combination compared to those inoculated with *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually and untreated control under salt-stressed conditions. These values were as high as 2.5- to 3.5-fold over the untreated control plants (**Table 3**). However, significant differences in different parameters of the

FIGURE 1 | Neighbor joining tree derived by CLUSTAL W and MEGA X using analysis of 16S rRNA sequences of bacterial strains isolated from maize rhizosphere at different growth stages. The numbers at nodes indicate bootstrap support values, as calculated by MEGA X.

TABLE 2 | Effects of seed biopriming and microbial intervention on germination (%) and vigor indices in maize grown in *Summer* and *Kharif* season under nethouse experiments at 30 days of sowing.

Treatments	Germination (%)	Vigor index I	Vigor index II
***Summer* maize**			
T_1—*B. safensis* MF-01	86.25^{b}	3987.57^{d}	280.75^{c}
T_2—*B. altitudinis* MF-15	85.33^{b}	4094.25^{b}	294.10^{b}
T_3—*B. velezensis* MF-08	84.36^{b}	4051.25^{c}	300.25^{b}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	94.36^{a}	4550.33^{a}	405.92^{a}
T_5—Control (untreated)	76.50^{c}	3280.43^{e}	222.75^{d}
***Kharif* maize**			
T_1—*B. safensis* MF-01	86.33^{b}	3806.15^{d}	344.50^{d}
T_2—*B. altitudinis* MF-15	85.75^{b}	3890.25^{c}	351.85^{c}
T_3—*B. velezensis* MF-08	86.11^{b}	3975.21^{b}	360.01^{b}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	96.25^{a}	4675.29^{a}	444.50^{a}
T_5—Control (untreated)	76.20^{c}	3042.25^{e}	255.75^{e}

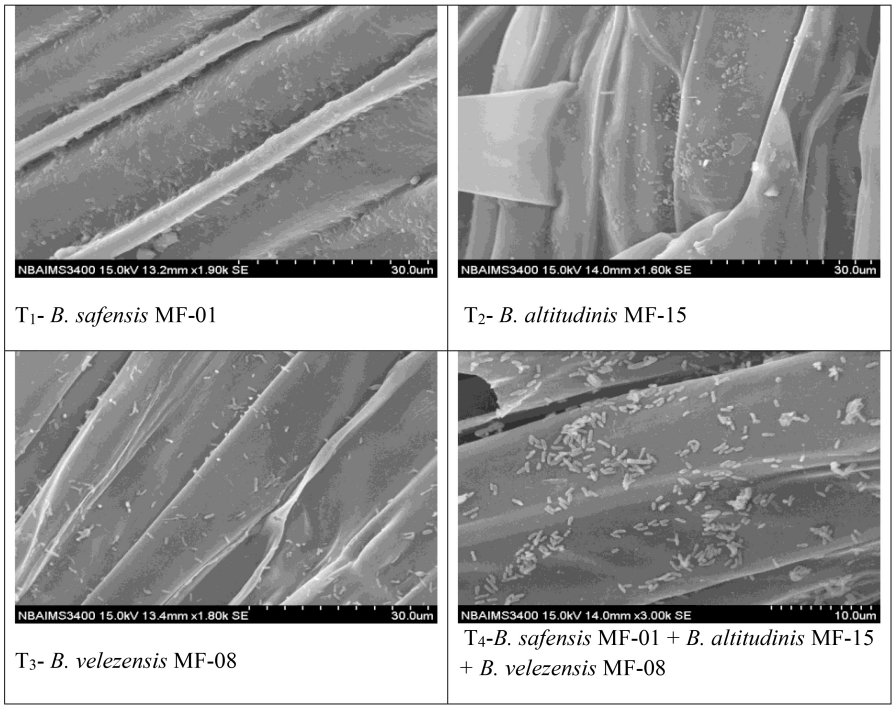
Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

root growth and development were recorded in the plants inoculated with *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually at 30 DAS (**Table 3**). The effects of seed biopriming with *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually or in combination on enhancement of root growth and development were also evident from **Figure 2B**.

Effect of Seed Biopriming and Microbial Inventerization on Expression of Genes Related to Root Development

After analyzing the root architecture data obtained from root scanning experiments, results prompted us to further investigate the up/down regulation of some of the important genes responsible for lateral root development in maize. Results

A



B

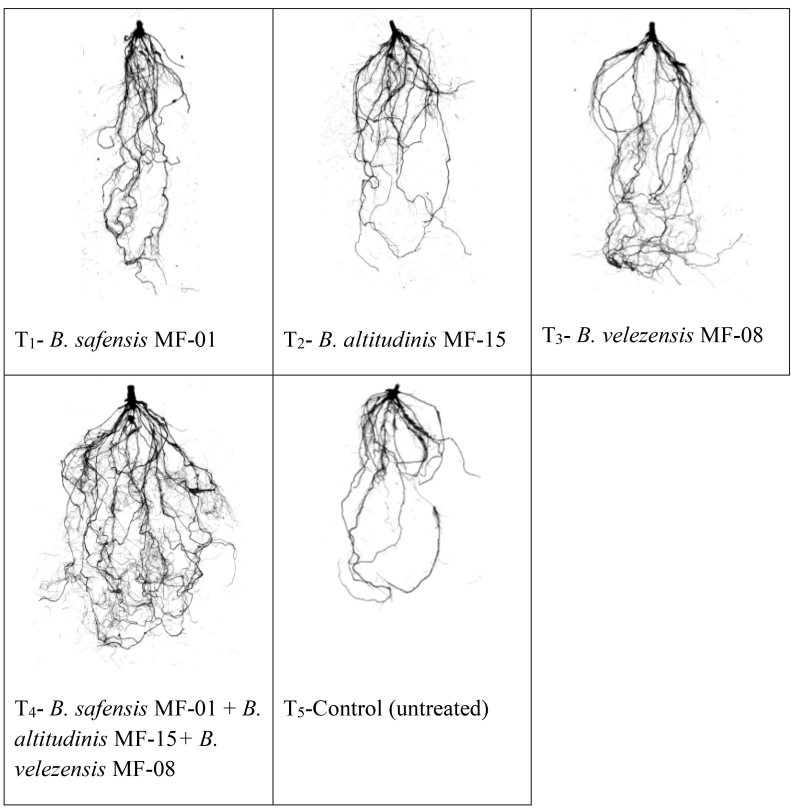


FIGURE 2 | (A) Scanning electron microphotographs showing root colonization by selected strains of *Bacillus* spp. at 30 days of sowing. **(B)** Effects of seed bio-inoculation on root growth and development in the maize grown in saline sodic soil at 30 days of sowing. Treatments were as follows: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15, and T_5—control.

TABLE 3 | Effects of seed biopriming and microbial intervention on root development and attributes in maize grown in *Kharif* season under nethouse experiments at 30 days of sowing.

Treatments	Surface area (cm^2)	Projected area (cm^2)	Root length (cm)	Length per volume (cm/m^3)	Root volume (cm^3)	Average diameter (mm)	Number of tips	Number of forks	Number of crossings
T_1 — *B. safensis* MF-01	174.46^{d}	60.54^{d}	1033.25^{d}	1005.66^{d}	2.04^{b}	0.50^{c}	2605.62^{c}	4154.36^{d}	595.25^{d}
T_2 — *B. altitudinis* MF-15	190.21^{c}	74.77^{c}	1265.75^{b}	1033.25^{c}	2.12^{b}	0.51^{c}	2500.01^{d}	4295.40^{c}	805.26^{c}
T_3 — *B. velezensis* MF-08	221.84^{b}	78.57^{b}	1138.28^{c}	1138.82^{b}	2.78^{b}	0.54^{b}	2727.50^{b}	4410.25^{b}	859.45^{b}
T_4 — *B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	449.73^{a}	143.15^{a}	2151.01^{a}	2151.01^{a}	7.48^{a}	0.75^{a}	4889.29^{a}	7951.57^{a}	2296.45^{a}
T_5 — Control (untreated)	131.54^{e}	41.87^{e}	756.77^{e}	756.77^{e}	1.82^{b}	0.48^{d}	2019.25^{e}	3021.45^{e}	388.36^{e}

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

indicated that *zmHO-1*, *zmGSL-1*, and *zmGSL-3* genes were up-regulated upon inoculation of *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually or in combination in the maize roots even under saline–sodic soil at 30 DAS. It was observed that twofold increase in the expression of *zmHO-1* gene was recorded in the maize roots inoculated with the consortium of *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 as compared to individual inoculation, whereas this value was as high as sevenfold over the untreated control plants (**Figure 3A**). Similarly, 2.75- to 3-fold increase in the expression of *zmGSL-1* (**Figure 3B**) and *zmGSL-3* (**Figure 3C**) was recorded in the maize roots inoculated with the *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 in combination as compared to individually inoculated plants grown under saline–sodic soil. However, least expression of these genes was recorded in the untreated control plants. Results clearly indicated that these bioinoculants have the potential to modulate gene expression related to primary and secondary rooting in maize, which was also evidenced from root scanning results (**Table 3**).

Effect of Seed Biopriming and Microbial Inventerization on Plant Growth Under Nethouse Conditions

Because of the significant differences observed in root architecture and growth parameters in response to seed biopriming in maize, nethouse experiments were conducted to evaluate the impact of selected strains on plant growth attributes under salt-stressed conditions. Results revealed that shoot length, root length, number of leaves, and fresh and dry weight of shoot and root were significantly increased by 2- to 2.5-fold in the plants bioprimed with consortium of all three strains *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 as compared to untreated control plants in *Summer* maize grown in saline–sodic soil at 45 DAS (**Table 4**). In *Kharif* maize, shoot length, root length, number of leaves, and fresh weight of shoot were increased by ∼2-fold; however, fresh weight of root and dry weight of shoot and root were increased by ∼3-fold at 45 DAS under nethouse conditions. It was observed that individual application of either bioinoculant significantly enhanced the plant growth attributes tested by 1.25- to 1.5-fold as compared to untreated control plants in *Summer* and *Kharif* maize at 45 DAS under nethouse conditions (**Table 4**).

Effect of Seed Biopriming and Microbial Inventerization on Accumulation of Stress-Responsive Biomolecules and Antioxidant Enzymes

Biosynthesis and accumulation of total chlorophyll, stress-responsive biomolecules, and antioxidant enzymes were significantly affected by seed biopriming and microbial inventerization in maize plants grown in saline–sodic soil. Results indicated that increased accumulation of total chlorophyll was recorded in the leaves of *Kharif* maize plants bioprimed with either bioagent (1.25- to 1.5-fold) or in combination of all three strains, i.e., *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 (∼2-fold) as compared to untreated

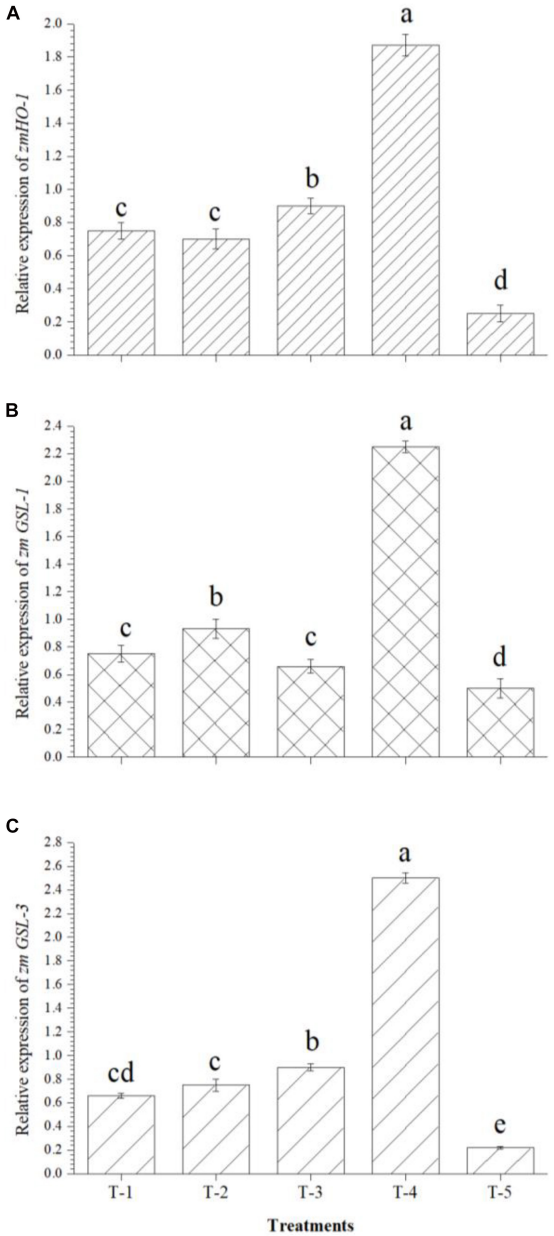


FIGURE 3 | Effects of seed bio-inoculation on expression profile of genes (fold change) related to primary and secondary rooting in the maize roots grown in saline sodic soil at 30 days of sowing. **(A)** Relative expression of *zmHO-1*, **(B)** relative expression of *zmGSL-1*, and **(C)** relative expression of *zmGSL-3*. Treatments were as follows: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15, and T_5—control (untreated). Data are mean (n = 10) and vertical bar represents standard deviation. Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

control plants (4.25 mg g^{-1} fresh wt.) at 45 DAS under nethouse conditions (**Figure 4A**). Similarly, total carotenoid content was increased by threefold in the leaves of *Kharif* maize plants bioprimed with consortium of all three strains (**Figure 4B**). Seed biopriming and microbial inventerization resulted in a significant increase in the total soluble sugar (**Figure 4C**) and total protein (**Figure 4D**) content in the leaves of *Kharif* maize plants treated with either of bioagent or in combination of all three relative to untreated control plants at 45 DAS under nethouse conditions.

It was also observed that maximum accumulation of total proline (3.85 mg g^{-1} dry wt.), total phenolics (9.05 μmol g^{-1} fresh wt.), and total flavonoids (1.55 μmol g^{-1} fresh wt.) was recorded in the leaves of *Kharif* maize plants bioprimed with consortium of all three strains, i.e., *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 as compared to untreated control plants at 45 DAS under nethouse conditions. However, accumulation of total proline, total phenolics, and total flavonoids did not significantly differ in the leaves of maize plants bioprimed with either bioinoculant (**Figures 5A–C**). The data presented herein showed significant differences in the activity of catalase, peroxidase, and SOD recorded in the leaves of *Kharif* maize plants bioprimed with either strain and the consortium of all three strains. Significantly higher activity of catalase (1580.40 units g^{-1} fresh wt.), peroxidase (1885.45 units g^{-1} fresh wt.), and SOD (622.50 units g^{-1} fresh wt.) was recorded in the leaves of plants bioprimed with consortium of all three strains, i.e., *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 followed by the plants bioprimed with *B. velezensis* MF-08, *B. altitudinis* MF-15, and *B. safensis* MF-01 individually at 45 DAS under nethouse conditions (**Figures 5D–F**). However, the lowest activity of catalase (452.25 units g^{-1} fresh wt.), peroxidase (890.25 units g^{-1} fresh wt.), and SOD (205.77 units g^{-1} fresh wt.) was recorded in the leaves of untreated control plants grown in saline–sodic soil under nethouse conditions at 45 DAS (**Figures 5D–F**).

Effects on Expression of Stress-Responsive Genes and High-Affinity K^+ Transporter

Quantitative real time-PCR analysis was carried out to study the effects of seed biopriming and microbial inventerization on the regulation of genes related to antioxidants and MAP kinase in the leaves of *Kharif* maize grown in saline–sodic soil under nethouse conditions at 45 DAS. Interestingly, seed biopriming with either of the strains or a consortium of all three strains, i.e., *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 under salt stress significantly induced the expression levels of *zmAPx-1.2*, *zmBADH-1*, *zmCAT*, *zmMPK-5*, *zmMPK-7*, and *zmCPK-11* genes in leaf tissues as compared to the untreated control plants (**Figures 6A–F**). The results indicated that an increased expression was recorded in *zmAPx-1.2* by 12-fold, *zmBADH-1* by 5-fold, *zmCAT* by 3.5-fold, *zmMPK-5* by 4-fold, *zmMPK-7* by 8-fold, and *zmCPK-11* by 5-fold genes in the leaves of plants bioprimed with consortium of all three strains compared to untreated control. Additionally, differential expression of these genes was also observed in the plants bioprimed with either of the bioinoculants individually, while untreated control plants showed the lowest expression of all these genes at 45 DAS under nethouse conditions (**Figures 6A–F**).

TABLE 4 | Effects of seed biopriming and microbial intervention on plant growth parameters in maize grown in *Summer* and *Kharif* season under nethouse experiments at 45 days of sowing.

Treatments	Shoot length (cm)	Root length (cm)	Number of leaves plant^{-1}	Fresh wt. of shoot (*g*)	Fresh wt. of root (g)	Dry wt. of shoot (g)	Dry wt. of root (g)
***Summer* maize**							
T_1—*B. safensis* MF-01	27.33^c	24.16^b	4.06^c	9.39^b	3.66^c	1.41^d	1.25^c
T_2—*B. altitudinis* MF-15	27.66^c	20.73^c	4.33^c	8.06^c	4.09^c	1.85^c	1.33^c
T_3—*B. velezensis* MF-08	34.03^b	25.06^b	5.08^b	10.66^b	5.50^b	2.05^b	1.45^b
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	45.35^a	40.36^a	7.48^a	16.44^a	8.33^a	3.56^a	2.50^a
T_5—Control (untreated)	24.26^d	16.23^d	3.15^d	8.60^c	3.05^d	1.05^e	0.95^d
***Kharif* maize**							
T_1—*B. safensis* MF-01	32.50^d	28.46^b	6.45^c	10.25^b	5.05^b	1.62^c	1.33^c
T_2—*B. altitudinis* MF-15	34.75^c	25.55^c	6.55^c	10.45^b	5.50^b	1.95^b	1.46^b
T_3—*B. velezensis* MF-08	36.46^b	29.05^b	7.25^b	9.37^b	5.33^b	2.05^b	1.55^b
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	48.25^a	42.33^a	9.05^a	15.44^a	10.50^a	4.75^a	3.03^a
T_5—Control (untreated)	26.25^e	20.23^d	4.15^d	7.05^c	3.06^c	1.25^d	1.05^d

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

Further, the possible role of *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 in modulating the expression of gene(s) involved in the abiotic stress tolerance especially salt was further investigated by analyzing the expression profile of *zmHKT1* and *zmNHX1* in maize roots under salt-stressed conditions. In addition, at least *zmHKT1* and *zmNHX1* genes were identified as being up-regulated under salt-stressed conditions. qRT-PCR results revealed that 2- to 2.25-fold increase in the transcript level of *zmHKT1* and *zmNHX1* was recorded in the maize roots inoculated with the *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 in combination as compared to the maize plants inoculated with individual strain. However, the lowest expression of *zmHKT1* and *zmNHX1* was recorded in the roots of uninoculated control plants (0.45 and 0.66, respectively), emphasizing the role of bioinoculants in modulating gene expression and strengthening the physio-biochemical homeostasis that leads to salt tolerance in maize grown in saline–sodic soil at 45 DAS (**Figure 7**).

Effect of Seed Biopriming and Microbial Inventerization on Soil Enzymes, MBC, and Soil Respiration

Seed biopriming and microbial inventerization led to differential activation of soil enzymes in the rhizosphere of *Kharif* maize grown in saline–sodic soil under nethouse conditions. Maximum dehydrogenase activity was recorded in the rhizosphere of plants bioprimed with consortium of all three strains (28.56 μg TPF/g of dry soil/24 h) as compared to untreated control (18.93 μg TPF/g of dry soil/24 h). However, dehydrogenase activity did not differ significantly in the rhizosphere of *Kharif* maize bioprimed with either of the bioinoculants (**Table 5**). The alkaline and acid phosphatase activity was found to be significantly higher in rhizosphere soil of *Kharif* maize bioprimed with consortium of all three strains (124.50 and 115.85 μg PNP/g of dry soil/2 h, respectively). A more or less similar pattern was recorded for protease, cellulose, and invertase activity in the rhizosphere of maize bioprimed with consortium of all three microbial strains. On the other hand, the increment in the activity of soil enzymes was also significantly higher in the rhizosphere of maize plants bioprimed with individual microbial strains as compared to untreated control at 45 DAS (**Table 5**).

Effect of Seed Biopriming and Microbial Inventerization on Under Field Conditions

Looking at the significant impact of biopriming and microbial inventerization on seed germination, vigor indices, root architectural development, plant growth, activity of soil enzymes, and physio-biochemical modulation in maize grown in saline–sodic soil under nethouse experiments, field experiments are conducted to evaluate the impact of these strains on plant growth attributes, physiological traits, yield, and yield-attributing parameters and uptake of Na^+, K^+, and Ca^{2+} in maize plants grown in saline–sodic soil.

Effects on Plant Growth and Physiological Traits

Plants grown under saline–sodic soil and seed biopriming with *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 individually or in combination of all three strains significantly enhance the plant height in *Summer* and *Kharif* maize grown under field conditions. The maximum plant height was recorded in the *Summer* and *Kharif* maize bioprimed with consortium of all three strains (175.66 and 206.75 cm, respectively) followed

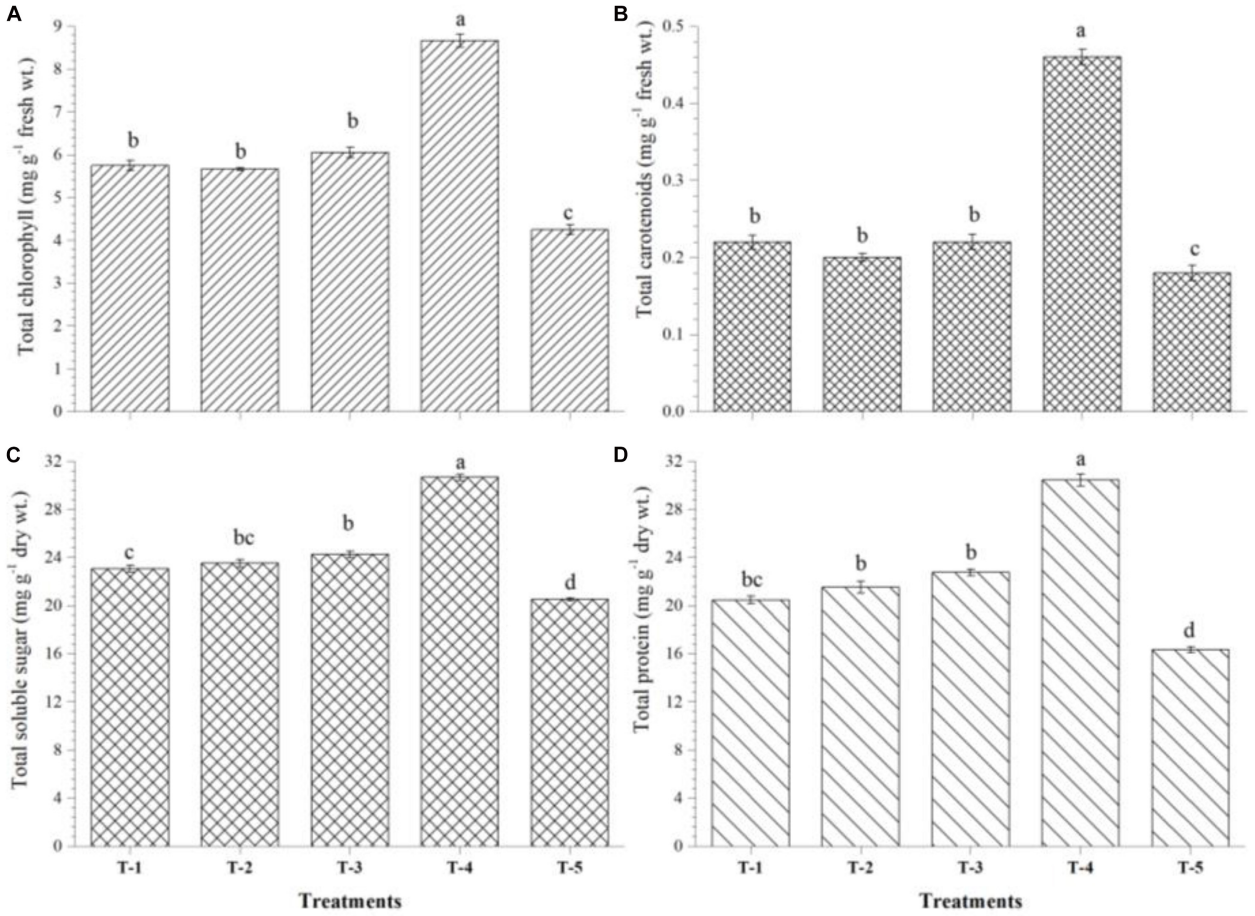


FIGURE 4 | Effects of seed bio-inoculation on activities and accumulation of **(A)** total chlorophyll, **(B)** total carotenoids, **(C)** total soluble sugar, and **(D)** total protein in the maize leaves grown in saline sodic soil at 45 days of sowing. Treatments were as follows: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15, and T_5—control (untreated). Data are mean (n = 10) and vertical bar represents standard deviation. Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

by *B. velezensis* MF-08 (140.25 and 148.96 cm, respectively) and *B. altitudinis* MF-15 (132.33 and 142.33 cm, respectively) individually inoculated plants (**Table 6**). The minimum plant height was recorded in the untreated control plants during the *Summer* and *Kharif* season (120.23 and 120.25 cm, respectively). Similarly, maximum number of leaves and dry weight of shoot and root were recorded in the *Summer* (17.25, 42.33 *g*, and 22.66 *g*, respectively) and *Kharif* (10.23, 62.46 *g*, and 36.40 *g*, respectively) maize plants bioprimed with consortium of all three strains. However, a slight difference in the number of leaves and dry weight of shoot and root was recorded in the *Summer* and *Kharif* maize plants bioprimed with either strain but significantly higher than untreated control plants at 45 DAS. Further in *Kharif* maize, all growth parameters were significantly higher when compared with the *Summer* maize under salt-stressed conditions (**Table 6**).

It was clearly indicated that seed biopriming and microbial inventerization have a positive impact on plant growth and physiological traits of *Summer* and *Kharif* maize grown in saline–sodic soil. According to data obtained and analyzed, maximum leaf area index, mean crop growth rate, mean relative growth rate, and mean net assimilation rate were recorded in the plants bioprimed with consortium as compared to individual inoculation and untreated control plants in both *Summer* (2.66, 15.10, 15.75, and 5.86, respectively) and *Kharif* (3.50, 20.45, 21.76, and 8.02, respectively) maize at 45 DAS (**Table 7**). However, slight deviation in the leaf area index, mean crop growth rate, mean relative growth rate, and mean net assimilation rate was recorded in the *Summer* and *Kharif* maize plants bioprimed with either strain, but these values were significantly higher than those of the untreated control plant at 45 DAS (**Table 7**). Therefore, these strains were able to promote plant growth and physiological traits of *Summer* and *Kharif* maize grown under salt-stressed conditions. It was concluded that the highest growth attributes were recorded in the plants treated with consortium. In general, among the individual inoculation, the highest growth attributes were recorded with *B. velezensis* MF-08.

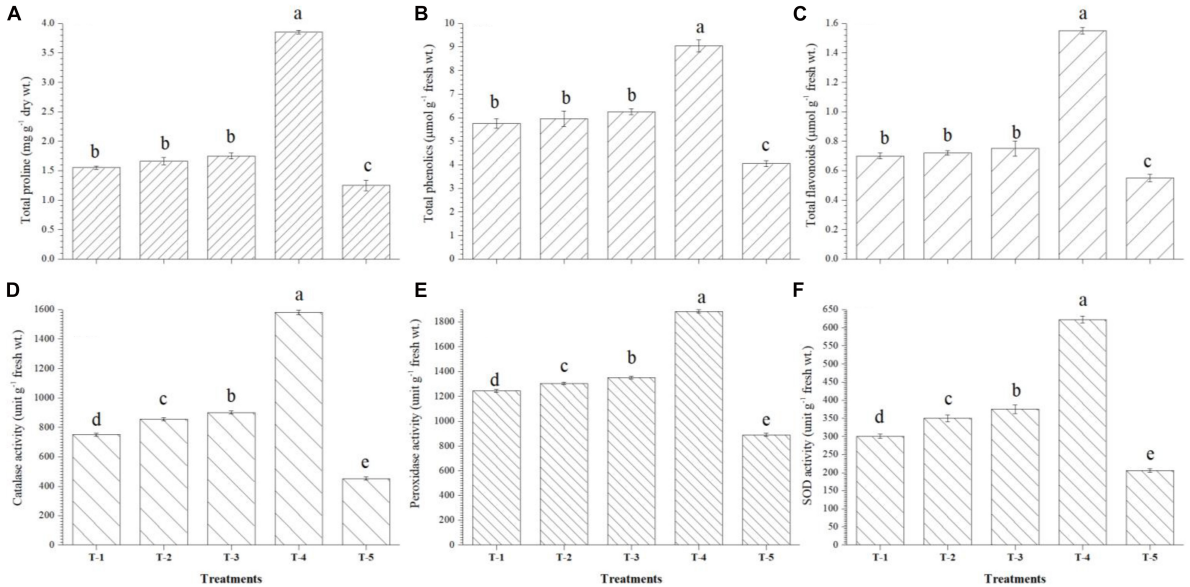


FIGURE 5 | Effects of seed bio-inoculation on activities and accumulation of **(A)** total proline, **(B)** total phenolics, **(C)** total flavonoids, **(D)** catalase, **(E)** peroxidase, and **(F)** SOD in the maize leaves grown in saline sodic soil at 45 days of sowing. Treatments were as follows: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15, and T_5—control (untreated). Data are mean (n = 10) and vertical bar represents standard deviation. Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

Effects on Yield and Yield-Attributing Traits

Seed biopriming and microbial inventerization significantly affect the yield-attributing traits (number of cobs/plant, cob size, number of grain/cob, and test weight) and grain yield of *Summer* and *Kharif* maize grown in saline–sodic soil. The maximum number of cobs/plant, cob size, number of grain/cob, test weight, and grain yield was recorded in the *Summer* (3.50, 18.46 cm, 314.25, 344.75 *g*, and 24.56 kg, respectively) and *Kharif* (3.15, 29.45 cm, 385.44, 365.33 *g*, and 33.46 kg, respectively) maize plants bioprimed with consortium of all three strains (**Table 8**). In individually inoculated plants, a slight deviation was recorded in the number of cobs/plant, cob size, number of grain/cob, test weight, and grain yield in the maize grown in salt-stressed conditions, but these values were significantly higher as compared to un-inoculated control (**Table 8**). However, minimum grain yield was reported in the untreated control plants in both seasons (15.33 and 24.81 kg, respectively). The data presented herein showed significant differences in the yield-attributing traits and grain yield between *Summer* and *Kharif* maize across the treatments under salt-stressed conditions. In conclusion, the highest grain yield was recorded in *Kharif* maize as compared to the *Summer* maize bioprimed with consortium. Among the individual inoculation strains, MF-08 bioprimed plants gave maximum yield in both seasons.

Effects on Uptake of Na^+, K^+, and Ca^{2+}

To see the effects of seed biopriming and microbial inventerization on the uptake and translocation of Na^+, K^+, and Ca^{2+} in maize, an analysis was done to estimate the uptake and translocation of these ions in the *Kharif* maize grown in saline–sodic soil under field experiments. Under salt stress conditions, Na^+ content in the maize roots was significantly higher in the untreated control plants (26.75) as compared to the roots of other treated plants. The least Na^+ was recorded in the roots of maize plants bioprimed with consortium of all three strains, i.e., *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 (7.25) followed by *B. velezensis* MF-08 (18.01), *B. altitudinis* MF-15, (19.25), and *B. safensis* MF-01 (20.47) inoculated plants. In contrast, maximum amounts of K^+ (45.37) and Ca^{2+} (20.33) were recorded in the roots of *Kharif* maize plants inoculated with consortium of all three strains. However, plants inoculated with either strain had a smaller deviation (in general, non-significant) in K^+ and Ca^{2+} content in the roots of maize plants (**Table 9**). Taken together, there was a ∼13-fold increment in Na^+/K^+ ratio for roots of untreated plants (2.18) and those inoculated with the consortium of all three strains (0.16).

Further, results showed that shoots of untreated plants had a Na^+ content nearly threefold higher (15.75) than plants bioprimed with consortium of all three strains (5.25), whereas plants bioprimed with either microbial strain had comparatively less Na^+ content in the shoot than the untreated control plants. Similar to the root K^+ and Ca^{2+}, maximum level of K^+ and Ca^{2+} was reported from the shoot of maize plants bioprimed with consortium of microbial strains tested (24.75 and 16.05, respectively). No significant differences were recorded in the K^+ and Ca^{2+} content in the shoot of maize plants bioprimed with either of the microbial strains at harvest (**Table 9**).

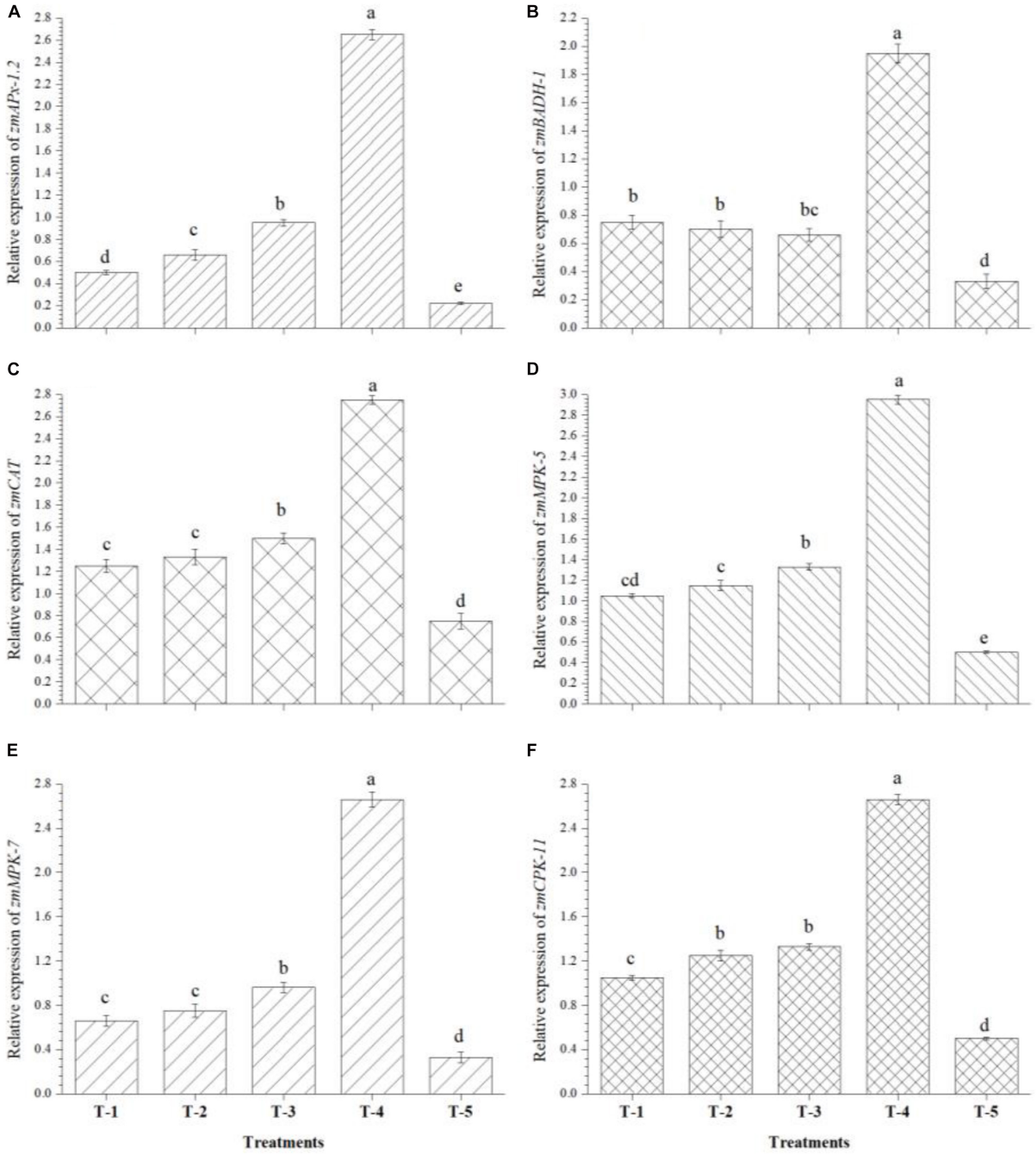


FIGURE 6 | Effects of seed bio-inoculation on expression profile of genes (fold change) related to antioxidants and abiotic stress tolerance in the maize leaves grown in saline sodic soil at 45 days of sowing. **(A)** Relative expression of *zmAPx-1.2*, **(B)** relative expression of *zmBADH-1*, **(C)** relative expression of *zmCAT*, **(D)** relative expression of *zmMPK-5*, **(E)** relative expression of *zmMPK-7*, and **(F)** relative expression of *zmCPK-11*. Treatments were as follows: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15, and T_5—control (untreated). Data are mean (n = 10) and vertical bar represents standard deviation. Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

However, the least amount of K^+ and Ca^{2+} was reported from the shoot of untreated maize plants grown in the field under salt-stressed conditions (5.67 and 7.50, respectively). The results implied that strains *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 reduced the uptake of Na^+ and increased the uptake of K^+ and Ca^{2+} and thus help in maintaining the ion balance and, thus, increase salt tolerance in bioprimed maize.

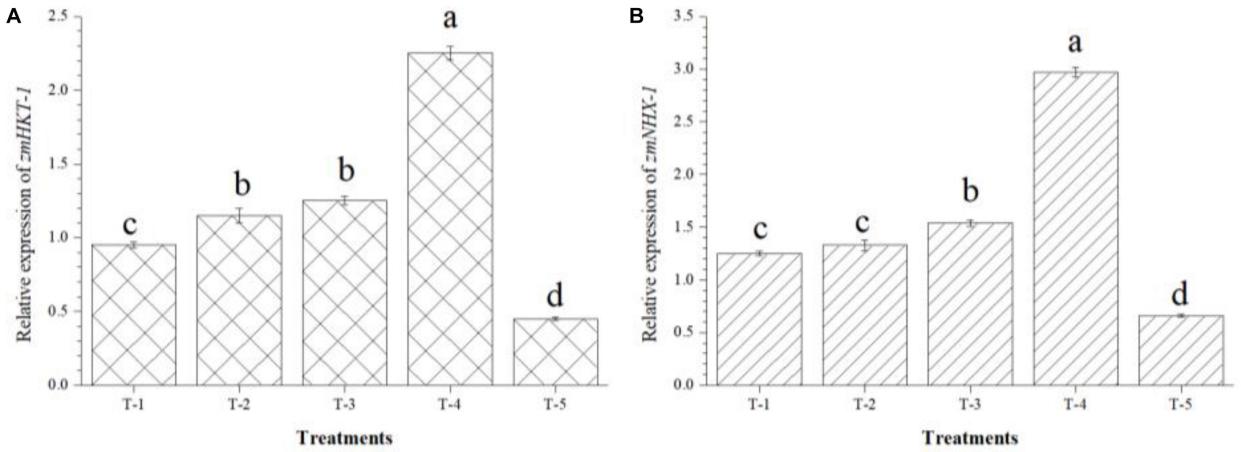


FIGURE 7 | Effects of seed bio-inoculation on expression profile of **(A)** *zmHKT-1* and **(B)** *zmNHX1* genes (fold change) in the maize roots grown in saline sodic soil at 45 days of sowing. Treatments were as follows: T_1—*B. safensis* MF-01, T_2—*B. altitudinis* MF-15, T_3—*B. velezensis* MF-08, T_4—*B. safensis* MF-01 + *B. velezensis* MF-08 + *B. altitudinis* MF-15, and T_5—control (untreated). Data are mean (n = 5) and vertical bar represents standard deviation. Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

DISCUSSION

Salt stress is one of the important abiotic stresses affecting crop establishment, survival, and productivity of maize, leading to severe economic losses year after year across the globe (Singh S. et al., 2019). Plant breeders routinely explore the wild relative, which may have prominent gene(s)/QTLs for salt stress tolerance and conduct multi-location trials over years to evaluate the performance of test entries against salt stress (Niu et al., 2012; Farooq et al., 2015; Singh S. et al., 2019). Alternatively, plant-/rhizo-microbiome plays an important role and their interactions in the rhizosphere influence plant fitness and improved functioning of ecosystems under salt-stressed conditions (Bais et al., 2006; Hameeda et al., 2008; Irfan et al., 2019). This may be accomplished through several direct and indirect mechanisms including hormone-induced growth stimulation (Zong et al., 2009), signaling (Zhu, 2002), chemotaxis, and modulation of physio-biochemical pathways in the plant system (Zhu, 2003; Zahra et al., 2018). The aim of the present study was to elucidate the microbe-mediated physio-biochemical and molecular mechanisms that provide salt tolerance, ecological fitness, and adaptiveness to maize plants grown in saline–sodic soil. In the current study, we have focused on the cooperative interactions of native *Bacillus* spp. with host defense responses underlying activation of stress tolerance cascades in maize plants grown in saline–sodic soil. This study provides some of the unique findings with a mechanistic overview of microbe-mediated stress tolerance in maize. Results of this investigation clearly indicated that maize rhizosphere harbors rich diversity of *Bacillus* spp. Results show that 20 different species of *Bacillus* were isolated and characterized from maize rhizosphere. These isolates have the potential to tolerate high concentrations of NaCl and have good PGP traits. They solubilize phosphorus, potassium, and zinc, and make them available to crop plants and promote plant growth directly and/or indirectly under salt-stressed conditions. Moreover, several reports indicated that rhizosphere microorganisms play an important role in alleviating salt stress in many crop plants in a different manner (Saleem et al., 2018; Rouphael et al., 2020; Wang et al., 2020). *Bacillus* spp. are a root-associated mutualistic plant symbiont with a capability to colonize roots, nourish the host, and protect plants from biotic and abiotic stresses via developing biofilm on the host root system (Ahmad et al., 2008; Hameeda et al., 2008; Singh et al., 2016a). Results clearly indicated that test strains, *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 profusely colonized the maize root inoculated individually or in consortium and formed a thin biofilm on the root system (**Figure 2A**). Host species, plant secretome, and microenvironment of a niche are the key deciding factors in the recruitment and shaping of a rhizosphere microbiome (Bais et al., 2006; Micallef et al., 2009; Mahoney et al., 2017). These microorganisms formed biofilm on the root and established a close relation with their host system and thereby stimulate plant vigor under biotic and abiotic stresses (Sahu et al., 2019; Singh S. et al., 2019; Singh U. B. et al., 2019; Singh et al., 2020). However, interactions between host and rhizosphere microorganisms may change due to the increasing global temperature, salt, and environmental factors (Liu et al., 2006; Souza et al., 2015; Szoboszlay et al., 2015). In the changing climatic scenario and constant increase in the area under salinity and/or alkalinity, these microbial inoculants play a key role in the establishment of young seedlings under salt-stressed conditions. This phenomenon is called IST (Bais et al., 2006; Singh et al., 2016a; Singh U. B. et al., 2019).

It is necessary to explore and characterize microbial strain(s) and to develop effcient, stable, and eco-friendly bioformulations that can be utilized to protect plants from toxic effects of salt stress. In the present study, liquid-based bioformulations have been developed using a unique medium that supports the bacterial population (log colony-forming units) for a longer time (data not shown). For a long time, biological preparations

TABLE 5 | Effects of seed biopriming and microbial intervention on activity of soil enzymes in maize rhizosphere grown in *Kharif* season under nethouse experiments at 45 days of sowing.

Treatments	Dehydrogenase activity (μg TPF/g of dry soil/24 h)	Alk. phosphatase activity (μg PNP/g of dry soil/2 h)	Acid phosphatase activity (μg PNP/g of dry soil/2 h)	Protease activity (μg tyrosine/g of dry soil/h)	Cellulase activity (μg glucose/g of dry soil/h)	Invertase activity (mg glucose/g of dry soil/h)	Microbial biomass carbon (mg/kg of dry soil)	Soil respiration (mg CO_2-C/100 g of dry soil/10 day)
T_1—*B. safensis* MF-01	20.33^{b}	101.50^{b}	94.33^{bc}	105.50^{c}	70.50^{d}	5.25^{c}	114.56^{c}	7.85^{c}
T_2—*B. altitudinis* MF-15	20.80^{b}	98.05^{c}	95.85^{b}	110.50^{b}	73.57^{c}	6.05^{b}	112.75^{d}	8.01^{c}
T_3—*B. velezensis* MF-08	20.50^{b}	101.95^{b}	96.33^{b}	109.75^{b}	75.50^{b}	6.45^{b}	116.25^{b}	9.33^{b}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	28.5^{a}	124.50^{a}	115.85^{a}	119.50^{a}	88.85^{a}	10.01^{a}	143.60^{a}	14.59^{a}
T_5—Control (untreated)	18.93^{c}	88.45^{d}	83.25^{d}	98.25^{d}	60.33^{e}	4.35^{d}	111.25^{de}	6.50^{d}

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

from spore-forming *Bacillus* spp. were preferred and used as efficient microbial inoculants successfully for the management of salt stress in many crops (Liu et al., 2006; Singh et al., 2016a). Because of their wider adoptability, positive rhizosphere effects, and long-term viability (shelf life), they facilitate the development of successful commercial products (Singh et al., 2016a; Bokhari et al., 2019; Sahu et al., 2019). They have the potential to colonize and spread in the root, rhizosphere soil, and foliar environments. Simultaneously, they are capable of suppressing growth of harmful biotic entities and/or ill effects of abiotic stresses effectively (Liu et al., 2006; Singh et al., 2016a; Sahu et al., 2019). Results indicated that seed biopriming and microbial inventerization have positive effects on root architecture and root development. Many fold increases in the surface area, projected area, root length, length per volume, total root volume, average diameter, number of tips, number of forks, and number of crossings were reported in the plants inoculated with either of bioagents or in combination compared to untreated control under salt-stressed conditions. Indeed, lateral roots are critical to plant anchorage, nutrient acquisition, and water uptake. Development of secondary and tertiary roots (lateral roots) of a plant is greatly influenced under salt stress conditions. It was shown that seed biopriming and microbial inventerization modulate the expression of several genes (*ZmHO-1*, *ZmGSL-1*, and *ZmGSL-3*) involved in lateral root development. Further, auxin (IAA) synthesized by the microbial inoculants in the rhizosphere soil might be involved in the modulation of cascades responsible for root architecture development (Berberich et al., 1999; Du and Scheres, 2018). Moreover, lateral root formation is a highly complex process that involves in general three major stages such as lateral root initiation, formation of the lateral root primordia, and finally lateral root emergence (Lalle et al., 2005; Kashyap et al., 2018; He and Meng, 2020). Several pathways/cascades were investigated in model plants including *Arabidopsis thaliana* in which plant growth regulators, auxins, play an important role at multiple stages of the lateral root development (Lalle et al., 2005; Lynch and Brown, 2012; Santos Teixeira and Ten Tusscher, 2019). The auxin gradient and signaling determine cell fate during lateral root primordial development while, during emergence, auxin regulates cell separation in the roots (Tian et al., 2014). Further, a small amount of auxin activates the MKK4/MKK5–MPK3/MPK6 cascade via TMK1/TMK4, which regulates the cell division pattern during lateral root morphogenesis (Zhu et al., 2019; Xu et al., 2020). The increased expression of *ZmHO-1*, *ZmGSL-1*, and *ZmGSL-3* genes and secondary and tertiary roots in the bioprimed plants provides a proof of concept that bioinoculant(s) might modulate the physio-biochemical pathways at the molecular level, which could potentially lead to lateral root development and substantially increase the establishment of maize plants even under salt-stressed conditions (Zimmermann et al., 2010; Zhang et al., 2018). However, the exact role of microbial inoculants in the activation of MAPK signaling during lateral root formation is not yet fully elucidated and needs further in-depth investigation.

Moreover, high salt concentration in the soil solutes causes ionic imbalance and osmotic stress in the root system. Later on, excess Na^{+} inhibits/alters cellular metabolism, photosynthesis,

TABLE 6 | Effects of seed biopriming and microbial intervention on plant growth parameters in maize grown in *Summer* and *Kharif* season under field conditions at 45 days of sowing.

Treatments	Plant height (cm)	Number of leaf	Dry wt. of shoot (g)	Dry wt. of root (g)
***Summer* maize**				
T_1—*B. safensis* MF-01	125.51^{d}	12.66^{b}	29.66^{b}	14.35^{c}
T_2—*B. altitudinis* MF-15	132.33^{c}	13.02^{b}	28.25^{b}	15.03^{c}
T_3—*B. velezensis* MF-08	140.25^{b}	13.66^{b}	30.50^{b}	16.46^{b}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	175.66^{a}	17.25^{a}	42.33^{a}	22.66^{a}
T_5—Control (untreated)	120.23^{e}	9.75^{c}	25.67^{c}	11.42^{d}
***Kharif* maize**				
T_1—*B. safensis* MF-01	137.45^{d}	6.96^{b}	31.96^{c}	18.33^{c}
T_2—*B. altitudinis* MF-15	142.33^{c}	7.25^{b}	32.33^{c}	20.65^{b}
T_3—*B. velezensis* MF-08	148.96^{b}	7.06^{b}	36.44^{b}	20.25^{b}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	206.75^{a}	10.23^{a}	62.46^{a}	36.40^{a}
T_5—Control (untreated)	120.25^{e}	6.06^{c}	26.45^{d}	15.25^{d}

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

TABLE 7 | Effects of seed biopriming and microbial intervention on physiological traits in maize grown in *Summer* and *Kharif* season under field conditions at 45 days of sowing.

Treatments	Leaf area index	Mean crop growth rate (g/m^2/day)	Mean relative growth rate (mg/g/day)	Mean net assimilation rate (g/m^2 leaf area/day)
***Summer* maize**				
T_1—*B. safensis* MF-01	2.05^{b}	11.27^{c}	13.50^{b}	4.50^{b}
T_2—*B. altitudinis* MF-15	2.15^{b}	12.50^{b}	13.75^{b}	4.45^{b}
T_3—*B. velezensis* MF-08	2.25^{b}	12.66^{b}	13.05^{c}	4.25^{bc}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	2.66^{a}	15.10^{a}	15.75^{a}	5.86^{a}
T_5—Control (untreated)	1.86^{bc}	10.05^{d}	12.47^{d}	3.85^{d}
***Kharif* maize**				
T_1—*B. safensis* MF-01	2.75^{c}	14.35^{c}	17.25^{b}	5.79^{c}
T_2—*B. altitudinis* MF-15	2.81^{b}	15.06^{b}	18.46^{c}	5.85^{c}
T_3—*B. velezensis* MF-08	2.89^{b}	15.33^{b}	17.75^{b}	6.05^{b}
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	3.50^{a}	20.45^{a}	21.76^{a}	8.02^{a}
T_5—Control (untreated)	2.55^{d}	12.46^{d}	15.67^{d}	5.25^{d}

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

and protein and lipid biosynthesis, thereby limiting the root growth and ultimately leading to early seedling mortality (Cicek and Çakirlar, 2002; Chaves et al., 2009; Niu et al., 2012; Delavar et al., 2018; Cao et al., 2020). Additionally, excess Na^+ in the soil solute had negative effects on plant growth, soil microbial activity, and uptake and translocation of essential nutrients in the plants (Singh S. et al., 2019). In the present study, a dramatic decline was reported in plant growth parameters such as plant height, shoot and root length, number and size of leaf, and fresh and dry weight in untreated *Summer* and *Kharif* maize grown in saline–sodic soil, possibly due to higher accumulation and toxicity of Na^+ and osmotic stress caused by impaired ion homeostasis and hampered overall growth performance (Zhu, 2003; Zhang et al., 2006, 2019). However, under salt-stressed conditions, *Summer* and *Kharif* maize bioprimed with either of the strains or in consortium of all three significantly enhanced plant growth and fresh and dry biomass accumulation compared to untreated control under nethouse and field conditions. These results were in agreement with previous work, which showed that rhizospheric as well as endophytic microbes increased shoot and root growth of several agriculturally important crops like rice (Jeong et al., 2006), maize (Singh U. B. et al., 2019), wheat (Singh et al., 2016a), and tomato (Vaishnav et al., 2020) under salt stress conditions. This might be due to the increased availability of essential nutrients, ionic balance in the soil solutes, and reduced osmotic stress in the plants. Further, several plant-associated beneficial microorganisms produce plant growth regulators, small peptides, and signaling molecules in the rhizosphere that would directly encourage/regulate plant growth or modulate certain pathways directly and/or indirectly under different circumstances (Singh et al., 2016a,b; Singh S. et al., 2019; Singh U. B. et al., 2019). In the present

TABLE 8 | Effects of seed biopriming and microbial intervention on yield and yield-attributing traits in maize grown in *Summer* and *Kharif* season under field conditions at harvest.

Treatments	Number of cobs/plant	Cob size (cm)	Number of grain/cob	Test weight (*g*)	Yield/plot (kg)
***Summer* maize**					
T_1—*B. safensis* MF-01	1.80^b	11.85^b	240.57^d	288.47^b	18.66^b
T_2—*B. altitudinis* MF-15	2.05^b	12.75^b	250.47^c	290.37^b	19.01^b
T_3—*B. velezensis* MF-08	2.15^b	12.45^b	260.45^b	285.49^c	19.55^b
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	3.05^a	18.46^a	314.25^a	344.57^a	24.56^a
T_5—Control (untreated)	2.00^b	10.15^c	225.45^e	275.75^d	15.23^c
***Kharif* maize**					
T_1—*B. safensis* MF-01	2.25^b	14.75^b	309.25^b	298.23^c	28.10^b
T_2—*B. altitudinis* MF-15	2.30^b	14.96^b	290.75^d	300.97^c	28.50^b
T_3—*B. velezensis* MF-08	2.50^b	15.25^b	300.25^c	315.46^b	28.01^b
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	3.15^a	29.45^a	385.44^a	365.33^a	33.46^a
T_5—Control (untreated)	1.95^b	12.47^c	254.33^e	285.25^d	24.81^c

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

TABLE 9 | Effects of seed biopriming and microbial intervention on Na^+, K^+, and Ca^{2+} uptake in maize grown in *Kharif* season under field conditions at harvest.

Treatments	Na^+	K^+	Ca^{2+}	Na^+/K^+
Content in root				
T_1—*B. safensis* MF-01	20.47^b	20.33^b	12.66^b	1.01^b
T_2—*B. altitudinis* MF-15	19.25^b	20.25^b	12.75^b	0.95^b
T_3—*B. velezensis* MF-08	18.01^b	22.35^b	12.50^b	0.81^b
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	7.25^c	45.37^a	20.33^a	0.16^c
T_5—Control (untreated)	26.75^a	12.25^c	11.50^c	2.18^a
Content in shoot				
T_1—*B. safensis* MF-01	13.06^b	8.25^{bc}	9.25^b	1.58^b
T_2—*B. altitudinis* MF-15	12.96^b	9.75^b	9.33^b	1.33^c
T_3—*B. velezensis* MF-08	12.47^b	10.50^b	9.56^b	1.19^c
T_4—*B. safensis* MF-01 + *B. altitudinis* MF-15 + *B. velezensis* MF-08	5.25^c	24.75^a	16.05^a	0.21^d
T_5—Control (untreated)	15.75^a	5.67^d	7.50^c	2.78^a

Data with different letters show significant difference in column data in randomized block design test at $p < 0.05$ under Duncan's multiple range test.

study, a drastic decline in leaf chlorophyll was recorded in the untreated control plants as compared to bioprimed plants grown in saline–sodic soil. Seed biopriming with compatible microbe(s) was found to improve chlorophyll content under salt stress conditions, which ultimately enhanced the photosynthesis and significantly increased the level of total soluble sugar and protein recorded. The drastic reduction in chlorophyll and other pigments might be due to inefficient activities of the enzymes α-aminolevulinic acid dehydratase and proto chlorophyllide reductase, which are coordinately involved in biosynthesis of chlorophyll and other pigments. Our results are in agreement with Latef and Tran (2016) who reported that Na^+ toxicity reduced chlorophyll contents in maize plants subjected to salt stress.

The inherent redox nature of salts, specifically sodium salts, accelerates toxicity by generating reactive oxygen species (ROS), such as hydroxyl radicals ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), and superoxide anions ($O_2^{\cdot-}$) in the plant system (Liu et al., 2012, 2013; Meng et al., 2016). To conquer salt-induced oxidative stress, plant cells have a well-developed inherent antioxidant capability that is composed of non-enzymatic components, such as glycine betaine, proline, trehalose, ascorbic acid, glutathione, and other organic osmolytes and enzymatic components, such as catalase, peroxidase, SOD, glutathione peroxidase, ascorbate peroxidase, dehydroascorbate reductase, glutathione *S*-transferase, glutathione reductase, monodehydroascorbate reductase, etc. (Pan et al., 2012; Jiang et al., 2016; Phillips et al., 2018; Nawaz and Wang, 2020). Moreover, high salt stress causes cytotoxicity to cellular biomolecules. More specifically, it causes brutal oxidative damage to proteins and nucleic acids either (i) directly through accelerating ROS production, or (ii) indirectly by the overproduction of advanced glycation end products, which leads to membrane disruption, exo-osmosis, and cell death (Jiang et al., 2016; Phillips et al., 2018). In this study,

significantly higher accumulation of compatible osmolyte proline (∼3.5-fold), phenolics (∼2.5-fold), and flavonoids (∼3.0-fold) has been reported in the plants bioprimed with consortium of compatible strains *B. safensis* MF-01, *B. altitudinis* MF-15, and *B. velezensis* MF-08 grown in saline–sodic soil. They are a noble indicator of salt stress tolerance (Sakamoto and Murata, 2002; Wu et al., 2011). Likewise, several-fold increment in the activity of catalase, peroxidase, and SOD was reported in the plants bioprimed with either of the strains or in combination of all three strains. The present result is supported by several researchers who reported that activity of these antioxidant enzymes in maize under salt stress has been significantly increased by the application of compatible microbial inoculants (Mittler et al., 2004; Singh U. B. et al., 2019). Further, *zmAPx-1.2*, *zmBADH-1*, *zmCAT*, *ZmMPK5*, *ZmMPK7*, and *ZmCPK11* were markedly up-regulated in the plants bioprimed with microbial inoculants individually or in combination, expected to contribute in the improvement of antioxidant defense systems under salt-stressed conditions (Zhang et al., 2006; Yamane et al., 2010; Jiang et al., 2016; Phillips et al., 2018). It was reported that *ZmMPK5* is the key gene activated by H_2O_2 and regulates the antioxidant defense systems, while *ZmCPK11* increases the activity and expression of antioxidant enzymes such as SOD and ascorbate peroxidase under stressed conditions (Zhang et al., 2010; Ding et al., 2013). Moreover, increased activation of *ZmMPK7* was reported during osmotic stress for alleviation of toxic effects of reactive oxygen species (ROS) and SA-regulated broad-spectrum resistance to biotic stresses, thereby regulating plant growth and development (Zong et al., 2009; Shi et al., 2010).

It is well established that Na^+/K^+ homeostasis and low cytosolic Na^+ concentrations are the key factors for salt tolerance in plants (Jiang et al., 2016; Singh et al., 2016a; Singh S. et al., 2019). Generally, under salt-stressed conditions, comparatively higher uptake and bioaccumulation of Na^+ inside the plant cells inhibit K^+ uptake, resulting in an increase in Na^+/K^+ ratio that adversely affects plant growth and development (Jiang et al., 2016). In the present study, a significant decrease in Na^+ content and an increase K^+ and Ca^{2+} were recorded in the root and shoot of the maize plants bioprimed with all three strains individually and in consortium. It was reported that microbial inoculants may regulate the uptake of Na^+, K^+, and Ca^{2+} and maintain ionic homeostasis/equilibrium in the plants directly and/or indirectly. These results were in agreement with the previous report where plants bioprimed with endophytic *P. geniculata* MF-84 showed significantly high K^+ and Ca^{2+} concentrations in the plants as compared to untreated control that resulted in enhancement of salt tolerance (Singh S. et al., 2019). In maize roots, significantly high expression of Na^+ and K^+ transporter genes such as *zmNHX1* and *zmHKT1* was detected in plants bioprimed with bioinoculants alone or in combination. These results were positively correlated with increased salt tolerance. In the present study, we found that the expression of *zmNHX1 and zmHKT1* was up-regulated in the roots of maize plants bioprimed with microbial inoculant(s) and exposed to salt concentrations in the soil. In untreated plants, the expression of *zmNHX1* and *zmHKT1* is usually very weak. Thus, the above studies involving microbial inoculation confirmed this point. These results suggested that Na^+ compartmentalization is a crucial factor and plays an important role in the roots during early establishment of crop plants under salt stress. The transport of Na^+ into the vacuoles of the roots was likely facilitated by tonoplast Na^+/H^+ antiporter encoded by *zmNHX1* genes under high salinity, which prevented the toxic effect of Na^+ to the plant system (Jiang et al., 2016; Zhang et al., 2017). The present study, showed novel insights into microbe-mediated mechanisms of root architecture development, expression of several genes related to lateral root development, antioxidant enzymes, Na^+/K^+ transporter, effects of microbial inoculation on soil physio-biochemical properties, and crop establishment under salt stress in detail. However, the exact mechanisms of microbe-mediated Na^+ compartmentalization and regulation of physiological and metabolic pathways in maize are still unclear, and the molecular mechanism needs to be further analyzed in the future. Further, it would be interesting to explore how the overexpression of *zmNHX1*, *zmHKT1*, and other antioxidant genes could be used to enhance salt tolerance and better crop growth in maize, similar to achievements made with *A. thaliana* and other plants.

CONCLUSION

The present study reveals that seed biopriming and microbial inventerization restructured the cellular responses that provide early establishment, ecological fitness, salt tolerance, and adaptiveness to the maize grown in saline–sodic soil. The increased number of lateral roots and vigorous growth of shoots was discovered to be phenotypic adaptation and the higher accumulation of compatible solutes, phenolics, flavonoids, and reduced ROS contributed to the enhanced salt tolerance in the maize plants bioprimed with either of strains or in combination. A reduced Na^+ content and increase in K^+ and Ca^{2+} were positively correlated with an increased transcript level of Na^+/K^+ transporter in plants. The highly expressed *ZmHO-1*, *ZmGSL-1*, *ZmGSL-3*, *zmAPx-1.2*, *zmBADH-1*, *zmCAT*, *zmMPK-5*, *zmMPK-7*, *zmCPK-11*, *zmNHX1*, and *zmHKT1* genes could play important roles in lateral root development, antioxidant properties, and ion homeostasis at the cellular level. Moreover, this study provides new insights into the molecular mechanism of microbe-mediated salt tolerance and could lay a foundation for crop management under salt stress. Furthermore, analyses of the soil enzymes and uptake and translocation of Na^+ revealed that some Na^+/K^+ transporters responded positively to microbial inoculation and salt stress, suggesting that these transporters are probably key molecular targets of microbe-mediated salt tolerance in maize. Further, this study clearly indicated that biological interactions between beneficial microorganisms and roots of maize plants could be an alternative for salt stress management in maize.

The present investigation tried to elucidate the microbe-mediated mechanisms of salt tolerance, but a key knowledge gap remains unclear, especially regarding metabolome and metatranscriptome changes during salt stress in maize. The present study is a step ahead from the current knowledge

on plant–microbe interactions under salt stress. Going beyond the simple biopriming and interaction study, a comprehensive and multi-omics based study is needed to analyze the physiological responses with proper validation and testing of hypothesis *via in vitro* and *in vivo* experiments and should be the critical next step. Herein, based on observation and recent research, we proposed a multi-disciplinary system-based approach that includes plant genetics, physiology, soil science, and plant biotechnology to better understand the plant–microbe interactions with special reference to salt/abiotic stress tolerance for improving agricultural productivity and environmental sustainability.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SS, US, PSh, MT, and AS conceived and designed the experiments. SS, US, DM, MM, and PKS performed the experiments. US, HS, and PKS analyzed the data. MR did the SEM. SS, US, HS, and MM wrote the manuscript. All authors have reviewed the manuscript and have given approval to the final version.

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SUPPLEMENTARY MATERIAL

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