

ORIGINAL ARTICLE

## A comparative study of native growth-promoting rhizobacteria and commercial biofertilizer on maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) development in a saline environment

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### Abstract

Salinity is one of the most significant constraints to crop production in dry parts of the world. This research emphasizes the beneficial effects of plant growth-promoting rhizobacterial isolates (PGPR) on the physiological responses of maize and wheat in a saline (NaCl) environment. Soil samples for the study were collected from a maize field in Baddi, Himachal Pradesh, India. Isolated bacterial strains were screened for salt (NaCl) tolerance and plant growth-promoting characters (i.e., indole acetic acid (IAA) production, siderophore production, amino cyclopropane-1-carboxylic acid (ACC) deaminase activity, hydrogen cyanide (HCN) production, and mineral phosphate solubilization). Screened bacterial isolates were further tested in pot experiments to examine their effects on wheat and maize growth. The treatments included five levels of bacterial inoculation (P0: control, P1: ACC deaminase positive + siderophore producer + NaCl tolerant bacteria, P2: mineral phosphate solubilizer + HCN producer + NaCl tolerant bacteria, P3: IAA producer + ACC deaminase positive + NaCl tolerant bacteria, P4: bacterial consortium, P5: Phosphomax commercial biofertilizer) and salt stress at 6 dS/m. Research findings found that exposure to a bacterial consortium led to the highest growth parameter in maize, including shoot length, root length, shoot and root dry weight followed by P2, P3, and P5 treatments at 6 dS/m salinity levels. However, P2 showed the best results for wheat at the same salinity levels, followed by P3, P4 and P5 treatments. P1 treatment did not show a significant result compared to control at 6dS/m salt level for both crops. The maximum proline content in maize and wheat was observed in P4 ( $23.28 \mu\text{mol} \cdot \text{g}^{-1}$ ) and P2 ( $15.52 \mu\text{mol} \cdot \text{g}^{-1}$ ) treatments, respectively, followed by P5 with Phosphomax biofertilizer. Therefore, the study proposed the application of growth-promoting bacterial isolates as efficient biofertilizers in the Baddi region of Himachal Pradesh, India.

**Keywords:** abiotic stress, maize, plant growth promotion characters, rhizobacteria, salinity tolerance, wheat

## Introduction

Plants have to manage numerous biotic and abiotic conditions in a natural environment. Abiotic constraints are categorized as inanimate elements of the ecosystem, with adverse impacts on living things (Bharti and Barnawal 2019). The major abiotic stresses influencing plant development in agriculture include: depleting minerals, increased salt concentrations, drought

conditions, temperature, and pH changes. Increased salinity levels are critical growth-reducing factors for most crops grown in arid and semi-arid regions worldwide (Goswami *et al.* 2016; Bharti and Barnawal 2019; Karnwal 2019). Many crops cannot withstand an increased salt environment, which restricts soil utilization for plantations and productivity. The Food and

Agriculture Organization (FAO) and United Nations Environment Programme (UNEP) statistics have reported that 5.2 billion hectares of agricultural land are currently ruined through erosion, salinity and deterioration of soil (Numan *et al.* 2018). Out of this, 0.4 billion hectares of land is ruined globally due to salinity (20% of agricultural and 50% of croplands) and threaten agricultural productivity (Karnwal 2020). To overcome the negative impact of the salt environment, researchers are developing various strategies to produce salt stress-resistant plant varieties. Such methods are time-consuming and expensive.

Most plants have various mechanisms for minimizing negative salinity impacts, such as controlling and separating ions, synthesizing suitable solutes, triggering antioxidative supporting enzymes, activating plant bioactive compounds, hormones, and modifications in the photosynthesis process (Akhtar 2019). Several studies have suggested that salt tolerance in plants is improved through beneficial soil microbes during cultivation and could be used as a mitigation tool in salt-sensitive fields for farming (Karnwal and Manan 2018; Emami *et al.* 2019; Bhatt and Maheshwari 2020). Microbial communities play a crucial role in the performance of plants by controlling their physiology and growth. Recently, Arora and Verma (2017), Numan *et al.* (2018), and Akhtar (2019) targeted applications of advantageous microbiomes and their formulations to increase plant growth and productivity by mitigating abiotic and biotic stress. Their research has attracted attention and is considered to be an exciting research field. The microbial diversity associated with the plant root system is immense. This multifaceted microbial community, also known as the second genome of the plant, is linked with plants and is vital to plant development. In plants, unique microbiota are found in different habitats of the plant, i.e., above the soil surface or aerial parts (phyllosphere), in the living tissue as endophytes (endosphere), and below the soil surface or with roots (rhizosphere) (Gottel *et al.* 2011; Johnston-Monje *et al.* 2016).

Rhizospheric microbes are an alternate solution, particularly beneficial bacteria, which can optimize the production potential of various plants under different abiotic stress environments and thus improve plant growth through various mechanisms (Egamberdieva *et al.* 2015). Rhizobacteria are plant root-colonizing soil bacteria, capable of multiplying and occupying all the food resources found at all phases of plant development in the roots (Akter and Oue 2018). These bacteria may also negatively interfere with plant development by competing directly for available soil nutrients (Akhtar 2019) or positively by competing with phytopathogens for survival and encouraging mutual interactions with the associated plants to allow nutrient exchange and trigger antimicrobial

secretion against the associated plants' plant pathogenic microbes (Egamberdieva *et al.* 2015).

Plant growth-promoting soil bacteria have been used to enhance plant growth and productivity for many decades. These bacteria improve plant growth and yield using different direct or indirect mechanisms, i.e., biological N<sub>2</sub> fixation, siderophore synthesis, mineral phosphorus solubilization, plant hormone production, hydrogen cyanide (HCN) production, antimicrobial activity, amino cyclopropane-1-carboxylic acid (ACC) deaminase activity, and mineral solubilization (Egamberdieva *et al.* 2015; Goswami *et al.* 2016; Akter and Oue 2018). Rhizobacteria with mineral solubilization, siderophore production, indole acetic acid (IAA) production, and ACC-deaminase potential have been extensively studied by scientists using solid and liquid mediums (Goswami *et al.* 2016; Akter and Oue 2018; Karnwal 2019). The solubilization of inorganic phosphate by soil microbes is of economic significance in crop development. Several soil bacterial genera have been reported and identified for phosphate solubilization, i.e., *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Enterobacter*, *Serratia*, *Bacillus*, *Mycobacterium*, *Erwinia*, *Agrobacterium*, and *Escherichia* (Gottel *et al.* 2011; Johnston-Monje *et al.* 2016). IAA production is among the most significant properties of many soil microorganisms used to encourage plant growth by natural methods (Karnwal 2009). IAA is a growth hormone involved in rhizome proliferation and extension, plant cell multiplication, and cell replication, which makes it crucial for plant growth (Gottel *et al.* 2011; Goswami *et al.* 2018). Of all recorded auxins IAA is the most effective growth stimulant for the plant root system. Karnwal (2019) documented the importance of rhizo-competent stress tolerant bacteria with different IAA roles expected to reduce crop salt stress. The synthesis of siderophores is another important plant growth-promoting trait. It has a dual effect on plant development by regulating the availability of required nutrients and reducing available iron for phytopathogens (Bharti and Barnawal 2019). Plant growth-promoting rhizobacterial isolates (PGPRs) in soil are capable of generating ACC deaminase. They can promote plant growth and protect plants from abiotic (water shortage, salinity, flooding conditions, inorganic and organic contaminants) and biotic stresses (bacterial and fungal pathogens) (Karnwal 2017). Such rhizobacteria help to improve plant development by enhancing the supply of minerals, phytohormones, and iron to plants and creating stress resistance traits as well as so-called plant growth-promoting rhizobacteria. This study aimed to examine the response of maize and wheat crops to salinity stress (6 dS/m NaCl) in the vicinity of the PGPR extracted from a maize agriculture field and a commercial biofertilizer.

## Materials and Methods

### Sample collection and culturing bacterial isolates

Mixed soil samples from 5 to 10 cm underneath the soil surface were taken at field sites under maize farming (30° 57' 36.5286" N, 76° 49' 13.2702" E), near Badli, Himachal Pradesh, India. A total of 10 soil samples was collected from the maize farming sites in pre-sterile lockable plastic bags and kept at room temperature ( $28 \pm 2^\circ\text{C}$ ). A 2 mm mesh sieve was used to segregate the stone and debris from collected soils (Chen *et al.* 2019). The sieved soil sample was then used immediately for bacterial isolation and cultivation. The microbes were cultivated on Nutrient Agar Medium (NAM) using the serial dilution technique. One gram of soil sample was gently mixed separately in 10 ml of sterilized distilled water for each soil sample and vortexed for 10 min.

Each soil mixture was diluted in series from  $10^{-1}$  to  $10^{-6}$ , as described by Karnwal (2009). A lawn plate procedure was performed to isolate the bacteria from soil suspension. The serially diluted soil sample (0.1 ml) was spread with a sterilized L-shaped glass spreader and cultivated at  $28 \pm 2^\circ\text{C}$  for 48 h on NAM plates. Pinpoint bacterial colonies were selected at random to check for plant growth-promoting attributes.

### Screening for salt stress and plant growth-promoting traits

Thirty morphologically distinct individual bacterial colonies were selected for salt stress and plant growth-promoting (PGP) assay. The colonies were tested for salinity tolerance using NAM plates amended with various NaCl concentrations (5%, 7% and 10%) at  $28 \pm 2^\circ\text{C}$  for 48 h. Isolates were graded from the most sensitive to the most resistant isolate under different salt concentrations based on colony consistency and diameter. Consequently, the moderate and most tolerant isolates were chosen to evaluate plant growth-promoting characters (Karnwal 2019). A total of 09 salt-tolerant isolates was selected and examined qualitatively and quantitatively for siderophore production (Schwyn and Neilands 1987; Arora and Verma 2017), IAA production (Karnwal 2009), phosphate solubilization (Pikovskaya 1948; Kaushal *et al.* 2013), ACC-deaminase activity (Dworkin and Foster 1958), and HCN production (Karnwal 2019) ability. Finally, three isolates with the following characters were selected for pot experiments:

1) RB1: siderophore producer + ACC-deaminase positive + NaCl tolerant bacteria,

- 2) RB7: mineral phosphate solubilizer + HCN producer + NaCl tolerant bacteria, and
- 3) RB18: IAA producer + ACC-deaminase positive + NaCl tolerant bacteria.

### Development of bacterial consortium

For the development of the microbial consortium, all three isolates (RB1, RB7 and RB18) were examined for their compatibility (Bhatt and Maheshwari 2020). Bacterial cultures were inoculated and grown independently in Luria Bertani (LB) broth. The RB1 (0.1 ml) strain was inoculated on respective LB plates and placed for 15 min at room temperature, followed by spot inoculation of RB7 and incubated for 24–48 h at  $28 \pm 2^\circ\text{C}$ . A similar methodology was used to examine the mutualistic behavior of RB1+RB18, RB7+RB18, and RB1+RB7+RB18. Isolates without an inhibition zone were found as mutually amicable and deemed suitable for consortium formulation.

### Pot experiments

*Zea mays* var. PSCL-4642 maize and *Triticum aestivum* L. var. HD 2687 wheat genotypes were selected for the pot experiment. Seeds for the present study were procured from the Indian Agricultural Research Institute (IARI), Pusa, Delhi, India. Seeds were rinsed thoroughly three times with sterile distilled water and then surface sterilized by dipping in 3% sodium hypochlorite (NaClO) solution for 5–6 min at room temperature  $28 \pm 2^\circ\text{C}$ . The remaining NaClO from the seed surface was removed by rewashing with sterile distilled water five times (Karnwal and Mannan 2018). Subsequently, the surface-sterilized seeds were immersed and kept for 20 min with bacterial suspension in a conical flask containing 48 h old bacterial growth ( $10^9$  CFU · ml<sup>-1</sup>). Control seeds were managed with sterile distilled water only.

In the pot experiment, one commercial biofertilizer (Phosphomax) was used to study the efficacy of rhizobacterial isolates and for enhanced development and productivity of maize and wheat. Twenty rhizobacterial inoculated maize and wheat seeds were incubated for 24 h at  $28 \pm 2^\circ\text{C}$  and then transferred to Petri dishes with sterile filter paper (Whatman No. 42) and planted in pots after germination. The applied treatments included bacterial inoculation at five levels with salt (NaCl) stress at 6 dS/m (P0: control, P1: ACC deaminase positive + siderophore producer + NaCl tolerant bacteria, P2: mineral phosphate solubilizer + HCN producer + NaCl tolerant bacteria, P3: IAA producer + ACC deaminase positive + NaCl tolerant bacteria, P4: bacterial consortium, P5: Phosphomax commercial biofertilizer).

Salt stress was imposed on pots after 4 months of visible germination in pots using NaCl solution. Initially, 6 dS/m salt solution was prepared, and the corresponding pots were irrigated with saline water eight times during growth (Emami *et al.* 2019). Over the cultivation period, 70–80% of the moisture in the pots was retained to prevent drought stress. About 5 months after germination, the mature plants were harvested. At harvest, traits, including shoot length, root length, shoot and root dry weights, total proline, and SPAD (Soil Plant Analysis Development) values, were measured.

### Statistical analysis

During the study, all trials were conducted using a randomized block design. All values are the standard means of triplicates unless otherwise specified. Plant development data were analysed by analysis of variance (ANOVA). Fisher's significant difference (LSD) test at  $p$  values of 0.05 was used to compare the means of the treatments.

## Results and Discussion

### Isolation and screening of rhizobacteria for salt tolerance and PGP traits

Plant growth-promoting rhizobacteria (PGPR) grow and survive under the influence of plant roots and actively participate in plant growth and mitigation of abiotic stress. Such microbes improve soil-water-plant association, manage plant hormone production and signalling, and stimulate several other pathways that act in an integrative manner to enhance plant responses towards salt and drought stress (Bhatt and Maheshwari 2020). In the present study, a total of 30 rhizobacteria was successfully checked for sali-

nity tolerance at 5, 7 and 10% NaCl enriched nutrient agar media and labelled as RB1 to RB30. A total of 09 isolates was selected for further study based on moderate to strong tolerance against saline conditions and further evaluated for various PGP activities. Table 1 displays colony features of 09 salt-tolerant isolates. All 09 isolates were found to have irregular to circular colonies. Bacterial colonies of RB1, RB11, RB17 and RB22 were elevated; RB31 and RB38 curved outward, and RB7, RB18 and RB25 were flat. The results of colony margin, colour, and Gram staining differed considerably for all bacterial isolates, as seen in Table 1.

The diversity and nature of microbes in the soil depended on the native plant variety in the cultivated region. Many studies have reported the diverse range of rhizospheric soil bacteria which vary in numbers and type from  $10^6$  to  $10^9$  CFU · g<sup>-1</sup> of soil (Kaushal *et al.* 2013; Goswami *et al.* 2016; Karnwal 2019). In recent years, various research reports have described the broad number of rhizobacteria-associated pathways used to enhance plant growth under different abiotic stresses (Vejan *et al.* 2016; Kalam *et al.* 2017; Zheng *et al.* 2018; Emami *et al.* 2019). These include phytohormone synthesis, ACC deaminase production, HCN synthesis, solubilization of various minerals in bioavailable form, and antagonistic activity. In the present study, 09 salt tolerating isolates were further evaluated for the PGP trait, i.e., siderophore production, IAA production, phosphate solubilization, ACC-deaminase activity, and HCN production (Table 2).

Phosphorus is an essential macronutrient for all forms of life. Although plants need this individual macronutrient much less than animals, a significantly low supply might lead to deficiency and adversely affect plant development and yield (Pikovskaya 1948; Karnwal 2019). Phosphorus requirements for plants vary from 25  $\mu\text{mol} \cdot \text{l}^{-1}$  to 30  $\mu\text{mol} \cdot \text{l}^{-1}$  for optimum productivity, but in most soil types, the total quantity of bioavailable phosphorus ranges from 1  $\mu\text{mol} \cdot \text{l}^{-1}$  to

**Table 1.** Micro- and macroscopic characteristics of bacterial isolates

Bacterial isolates	Shape	Colour	Margin	Elevation	Gram stain
RB1	cocci	whitish	entire	elevated	+
RB7	cocci	whitish	entire	flat	+
RB11	rod	pale yellow	irregular	elevated	-
RB17	cocci	whitish	irregular	elevated	-
RB18	rod	creamy	irregular	flat	+
RB22	rod	creamy	entire	elevated	-
RB25	cocci	yellow	entire	flat	-
RB31	rod	yellow	entire	curved outward	-
RB38	rod	pale yellow	regular	curved outward	+

**Table 2.** Plant growth-promoting traits testing results for native salt-tolerant isolates screened from a maize growing field

Rhizobacterial isolates	Qualitative analysis of plant growth-promoting characters				
	siderophore production	IAA production	ACC-deaminase activity	phosphate solubilization	HCN production
RB1	+++	-	+++	-	-
RB7	-	-	-	+++	++
RB11	-	-	+++	-	-
RB17	-	-	-	+++	+
RB18	-	+++	++	-	+
RB22	-	-	+	-	-
RB25	++	+	-	-	-
RB31	-	-	-	-	+
RB38	-	-	-	++	+++

“-”no activity; “+” minimum activity; “++” moderate activity; “+++” maximum activity; all responses relative to the maximum activity category  
 IAA – indole acetic acid; ACC – amino cyclopropane-1-carboxylic acid; HCN – hydrogen cyanide

1.7  $\mu\text{mol} \cdot \text{l}^{-1}$  (dos Reis *et al.* 2017). The maximum amount of phosphorus in soil is primarily available in solid or powder form which plants cannot utilize. Different researchers (Johnston-Monje *et al.* 2016; Kalam *et al.* 2017; Arora and Verma 2017) reported the ability of soil bacteria to mobilize mineral phosphates into a usable plant form by its liquefaction. Quantitative phosphate liquefaction results from the current research exhibited that only three isolates have a translucent area around bacterial growth. Qualitative assessment of phosphate solubilization for the three isolates in 0.5% tricalcium phosphate supplied by Pikovskaya’s broth indicate that all three isolates effectively liquefy mineral phosphate in the Pikovskaya’s broth. Phosphate solubilizing bacteria (PSB) RB7, RB17, and RB38 were estimated with 217.00  $\mu\text{g} \cdot \text{ml}^{-1}$ , 163.00  $\mu\text{g} \cdot \text{ml}^{-1}$ , and 80.00  $\mu\text{g} \cdot \text{ml}^{-1}$  soluble phosphate in the broth after

48 h incubation, respectively. Phosphate solubilizing efficiency results are reported in Table 3.

In the present study, all 09 isolates were analyzed for IAA production in the availability of L-tryptophan. A total of 02 bacterial isolates (RB18 and RB25) showed positive attributes from minimum to maximum IAA production ranging from 3.6  $\mu\text{g} \cdot \text{ml}^{-1}$  to 25.2  $\mu\text{g} \cdot \text{ml}^{-1}$ , as shown in Table 3.

In the present study, a total of 05 isolates exhibited a positive response for HCN synthesis, 02 siderophore production, and 04 ACC deaminase activity, as shown in Table 3. These bioactive compounds have an immediate influence on seedling, shoot and root development of the respective crops. Based on all PGP attribute results, the three top salinity stress tolerant isolates (RB1, RB7 and RB18) were chosen for further research.

**Table 3.** Quantitative estimation results of selected salt-tolerant isolates for multifarious plant growth-promoting traits

Rhizobacterial isolates	Plant growth-promoting traits		
	phosphate solubilization [ $\mu\text{g} \cdot \text{ml}^{-1}$ ]	IAA production [ $\mu\text{g} \cdot \text{ml}^{-1}$ ]	siderophore amount [% siderophore unit, psu]
RB1	0	0	28.44 ± 4.76
RB7	217 ± 8.32	0	0
RB11	0	0	0
RB17	163 ± 5.85	0	0
RB18	0	25.2 ± 1.66	0
RB22	0	0	0
RB25	0	3.6 ± 0.48	13.87 ± 1.48
RB31	0	0	0
RB38	80 ± 2.54	0	0

Values are mean ± SE; IAA – indole acetic acid

## Pot experiments

Several studies with different plants, like eggplant, chili, pepper, and wheat, documented the beneficial application of PGPRs to minimize salt anxiety in plants (Abbas *et al.* 2010; Sharifi 2017; Karnwal 2019). In earlier research, the inoculation effect of PGPRs at early growth stages was documented and led to improved plant growth, development and grain yield through direct influences on root and shoot development (Johnston-Monje *et al.* 2016; Kalam *et al.* 2017; Arora and Verma 2017). In the present study, a bacterial consortium study showed the antagonistic behavior of RB7 to RB1 and RB18 isolates, and no growth of RB1 and RB18 was observed during the study. However, RB1 and RB18 isolates showed enhanced growth under their mutual association and were selected as a bacterial consortium for the pot experiment. Results of the

pot study showed that two isolates (RB7 and RB18) significantly ( $p \leq 0.05$ ) increased the growth of maize and wheat compared to the non-inoculated and RB1 inoculated treatment (Tables 4 and 5).

Our findings are supported by other research (Egamberdieva and Kucharova 2009; Gottel *et al.* 2011; Johnston-Monje *et al.* 2016; Dixit *et al.* 2018), as the shoot and root lengths were the maxima with bacterial inoculated treatments compared to non-inoculated treatment in maize and wheat plants. A similar observation was recorded with commercial biofertilizer which showed healthier growth than non-inoculated treatment for experimental crops. Almaghrabi *et al.* (2013) found that the use of salt-resistant isolates *P. putida* and *P. fluorescens* enhanced plant growth parameters like shoot length, root length and product yield.

Salt stress in agriculture has a detrimental effect on plant growth and reduces the yield of the product.

**Table 4.** Effect of different microbial and commercial biofertilizer treatments on maize growth at 6 dS/m salinity level

Treatment	Maize growth parameters					
	shoot length [cm]	root length [cm]	shoot dry weight [g]	root dry weight [g]	proline	SPAD value
P0 (control)	69.04 ± 2.43	63.72 ± 1.66	150 ± 6.3	20 ± 2.5	12.38	32.0
P1 (RB1)	71.18 ± 3.77	68.88 ± 3.7*	157 ± 7.8	22 ± 2.8	14.56	36.0
P2 (RB7)	80.13 ± 4.15*	70.34 ± 6.12*	168 ± 5.2*	25 ± 3.5*	16.44*	37.8*
P3 (RB18)	78.11 ± 3.12*	70.88 ± 3.66	170 ± 8.2*	24.33 ± 1.5*	17.36*	43.0*
P4 (RB1+RB18)	82.47 ± 6.72*	81.11 ± 1.76*	174 ± 6.8*	30 ± 4.2*	23.28*	40.0*
P5 (Phosphomax)	74.23 ± 2.61	70.22 ± 2.56*	166 ± 5.1*	23.5 ± 2.6	16.87*	34.75

Values are mean ± SE, values with \* superscript symbol in a column are significantly different ( $p < 0.05$ ) and show the difference between control and inoculated treatments

P0: control non-inoculated, P1: ACC deaminase positive + siderophore producer + NaCl tolerant bacteria, P2: mineral phosphate solubilizer + HCN producer + NaCl tolerant bacteria, P3: IAA producer + ACC deaminase positive + NaCl tolerant bacteria, P4: bacterial consortium (RB1+RB18), P5: Phosphomax commercial biofertilizer

SPAD – Soil Plant Analysis Development

**Table 5.** Effect of different microbial and commercial biofertilizer treatments on wheat growth at 6 dS/m salinity level

Treatment	Wheat growth parameters					
	shoot length [cm]	root length [cm]	shoot dry weight [g]	root dry weight [g]	proline	SPAD value
P0 (control)	66.67 ± 1.76	55.23 ± 4.56	93.44 ± 2.76	18.62 ± 1.28	7.83	42.40
P1 (RB1)	68.5 ± 2.44	67.1 ± 4.99*	94.6 ± 5.98	21.75 ± 2.54	10.51	43.00
P2 (RB7)	92.34 ± 4.2*	73 ± 7.34*	127.34 ± 7.29*	28 ± 2.87*	15.52*	53.27*
P3 (RB18)	86.4 ± 3.21*	72.55 ± 4.28*	117.44 ± 4.87*	26 ± 3.2*	14.28*	47.32*
P4 (RB1+RB18)	83.32 ± 1.98*	71.87 ± 4.40*	114 ± 2.87*	23.55 ± 3.2*	13.22*	45.33*
P5 (Phosphomax)	74.39 ± 3.6*	68.99 ± 7.23*	106.33 ± 8.34*	22.33 ± 1.74	12.66*	44.18*

Values are mean ± SE, values with \* superscript symbol in a column are significantly different ( $p < 0.05$ ) and show the difference between control and inoculated treatments

P0: control non-inoculated, P1: ACC deaminase positive + siderophore producer + NaCl tolerant bacteria, P2: mineral phosphate solubilizer + HCN producer + NaCl tolerant bacteria, P3: IAA producer + ACC deaminase positive + NaCl tolerant bacteria, P4: bacterial consortium (RB1 + RB18), P5: Phosphomax commercial biofertilizer

SPAD – Soil Plant Analysis Development

Salt-resistant and root-colonizing microbes have the potential to withstand abiotic stress and develop adaptively in extreme circumstances, which positively supports the plant in managing and tolerating salt stress (Karnwal 2019). Egamberdieva and Kucharova (2009) documented, in an earlier study, the overall impact of salt-tolerant *P. extremorientalis* on root colonization and plant growth. Dixit *et al.* (2018) reported that salt-tolerant bacteria were the most effective in tomato productivity in non-saline and saline soil.

In the current analysis, of the bacterial treatments tested, a significant increase in plant growth for maize was observed for the bacterial consortium treatment, followed by P3, P2 and P5 at 6 dS/m salinity levels. There was no significant difference in maize growth recorded with P1 treatment compared to the control. Bacterial consortium treatment showed maximum shoot length (82.47 cm), root length (81.11 cm), shoot dry weight (174 g), root dry weight (30 g), and proline content (23.28), as shown in Table 4.

However, the SPAD value for the P5 treatment was recorded with a lower value than the P3 treatment. During the study, it was observed that the P1 treatment was less effective than other treatments and showed no significant value different than the P0 treatment (Table 4). Commercial biofertilizer treatment (P5) was reported with better growth parameters than the P1 treatment; however, the P2 and the P3 treatments showed better results than the P5 treatment. Results of the present study reported the beneficial effects of multifarious PGP inoculants on plant development compared to non-inoculated and commercial biofertilizer treatments. A previous study (Akter and Oue 2018) showed that microbial inoculants improved root growth which encouraged plant growth and nutrient access to plant roots, i.e., nitrogen (N), phosphorus (P), and potassium (K). In an earlier study, Karnwal (2020) reported the beneficial effects of salt-tolerant PGP bacterial isolates BoGl17 on the growth of *Triticum aestivum* L. var. HD 2687 and *Zea mays* var. PSCL-4642 cultivar seedlings at different NaCl levels. BoGl17 inoculation increased the radicle length of wheat and corn germinated seeds by 23.1 mm and 7 mm, respectively, at salinity stress of 50 mM NaCl. Similar results were reported with plumule growth with BoGl17 isolate with wheat at 50, 100, 150 and 200 mM salinity stress. In the present study, similar results were obtained with wheat growth, where P2 treatment gave the highest shoot length, root length, shoot and root dry weights, proline content, and SPAD value, followed by P3 and P4 treatment at 6 dS/m salinity level. In wheat, the bacterial consortium treatment was less effective than P2 and P3 treatments. Inoculation of commercial biofertilizer also showed better growth parameter results than P1 and the non-inoculated control. The results of the present study showed that phosphate solu-

bilizing CN producing bacterial isolate increased the nutrient availability for the wheat plant under salinity stress and enhanced plant growth. Whereas, siderophore + ACC deaminase producing salt-tolerant bacterial isolate was less effective than other plant growth promoting inoculants (Table 5).

Orhan (2016) reported the effect of 18 halotolerant and halophilic bacteria with ammonia production, indole-3-acetic acid synthesis, 1-aminocyclopropane-1-carboxylate-deaminase production, phosphate solubilization, and nitrogen fixation abilities on *T. aestivum* growth in hydroponic culture. Orhan (2016) observed significantly increased in the plant's root and shoot length and total fresh weight in a salt stress (200 mM NaCl) environment with plant growth rates ranging from 62.2% to 78.1%.

In the present study, IAA producing isolate with ACC deaminase activity increased the wheat growth and showed better results than the P1 treatment. Inoculation of phytohormones synthesizing bacteria in soil is a beneficial method for enhancing plant growth through plant-microbe interactions. Bacterial inoculants triggered the development of lateral roots; additionally, the non-auxin strains did not show this effect in plant roots, indicating the role of IAA in the bacterial mediated plant growth (Bharti and Barnawal 2019). Our results are in agreement with these findings. Emami *et al.* (2019) reported that phosphate solubilizer + NaCl tolerant bacteria resulted in the highest maize growth at various NaCl concentrations (0, 4, 8 dS/m) and no significant changes with ACC-deaminase producer salt-tolerant bacteria and two commercial biofertilizers (Barvar-2 and Biofarm-2).

Results of the present study reported that the indigenous bacteria and agricultural biofertilizers ( $p < 0.05$ ) were greatly influenced by the proline and SPAD values of maize and wheat plants, as shown in Tables 4 and 5. The results revealed that soil salinity substantially increased the proline quantity of maize and wheat at 6 dS/m salinity levels. The maximum proline level in maize and wheat was found with the bacterial consortium at 6 dS/m salinity level by 23.88 and P2 treatment at 6 dS/m salinity level by 15.52, respectively, followed by P3 treatment for both crops by 17.36 and 14.28. At the same salinity level, there was no significant difference observed between P2 and P5 treatments for maize and between P4 and P5 treatments for wheat. Scientists (Ansary *et al.* 2012; Agami *et al.* 2016; Emami *et al.* 2019) found an increase in proline content due to salinity stress in rice. Emami *et al.* (2019) recorded maximum proline in wheat (17.48) and barley (23.42) with salt-tolerant siderophore producing bacteria at 6 dS/m, followed by salt-tolerant phosphate solubilizing bacteria by 16.53 and 19.78, respectively. These results show PGP bacteria's effectiveness against abiotic stress, which

helps plants bear this stressed environmental condition. Scientists (Ansary *et al.* 2012; Agami *et al.* 2016; Emami *et al.* 2019) demonstrated that a rise in the plant proline volume is the physiological feedback of plants towards a reduced availability of water in the root system. Under such circumstances, the root cells' osmotic stress was lowered by proline and increased water and nutrient absorption from the soil. The production of phytohormones by inoculated bacteria activates root exudation and thus provides the substratum for bacterial growth. However, the interactions of genetically distinct rhizobacteria with plants could be unique.

## Conclusions

The study's findings suggest that native rhizobacteria from fields of Baddi, Himachal Pradesh, India, enhanced maize and wheat physiological development in saline environments, and they may well compete with commercial biological fertilizer Phosphomax. These findings suggest that native bacteria are more compatible with soil and climatic conditions in the researched area than commercial biofertilizers. Therefore, isolated growth-promoting bacteria can be recommended as an effective biofertilizer in the Himachal region.

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