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Effect of halotolerant plant growth-promoting rhizobacteria from *Bougainvillea glabra* on wheat and maize seedlings under NaCl stress

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Abstract

Wheat and maize are the main staple food crops that contribute to human food security. Their growth, however, is reduced under stresses such as salinity. The plant microbiome is associated with each plant tissue and develops a holobiont in association with the plant. Plants actively manage the configuration of their related bacterial population and its function. These microorganisms provide a broad range of benefits and advantages to the plants. The present study aimed to examine the growth improvement of wheat var. HD 2687 and maize var. PSCL-4642 under salinity at the seedling stage following inoculation of salt-tolerant plant growth-promoting rhizobacteria (PGPR) BoGl120 purified from *Bougainvillea glabra.* The seed germination potential with/without bacterial inoculation was examined at 50, 100, 150, and 200 mM NaCl concentrations for both crops. Compared to controls, at 50 mM NaCl concentration, the BoGl120 isolate provided the maximum radicle length in maize (32 mm) and in wheat (24.8 mm). At 100 mM NaCl concentration, however, the radicle length of wheat and maize seedlings was decreased. Inoculation of plants with the BoGl120 isolate enhanced the plumule length of seedlings at different NaCl concentrations as compared to controls. In comparison with controls, BoGl120 improved the plumule length of wheat to 32.6, 14.0, and 8 mm at 50, 100, and 150 mM NaCl concentrations, respectively. The results of the present study support the concept that PGPRs could help to increase the tolerance against saline stress in wheat and maize at the seedling stage.

Key words: abiotic stress, salinity stress, seedling growth, rhizobacteria, staple food

Introduction

Bacteria that can promote plant growth, that is, plant growth-promoting bacteria (PGPB), include free-living bacteria, bacterial species that form specific symbiotic relationships with plants (e.g., *Rhizobia* spp. and *Frankia* spp.), bacterial endophytes that can colonize some or a portion of a plant's interior tissues, and cyanobacteria (formerly called blue-green algae). The most commonly found genera of PGPB are *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholdaria, Enterobacter, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavobacterium,* etc. (Etesami and Beattie, 2018; Etesami and Maheshwari, 2018; Saleem et al., 2018; Sen et al., 2018). Plant growth-promoting rhizobacteria (PGPR) (a class of PGPBs) are among the most closely studied plant-associated bacteria that occur both on root surfaces and in deep soil interfaces (Glick et al., 1998). Regardless of the diversity of the environment, free-living rhizobacteria and mutualistic microorganisms use numerous similar strategies to improve crop growth and manage plant diseases.

Stress tolerance traits are relevant parameters for the formulation of microbial inoculants. Such microorganisms are ideal for decreasing salt stress in crops and restoring land affected with salinity for farming purposes (Zhou et al., 2017). These microbial inoculants may support host plants directly by increasing the number of nutrients available for plants and by modulating phytohormones linked to growth and stress responses (Kumar et al., 2012). Indirectly, PGPRs can improve the

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plant health by targeting pests and pathogens with antibiotics, hydrolytic enzymes, and nutrient limitation and by priming plant defences (Chauhan et al., 2016). PGPRs also exert a beneficial role in plant growth, including the decomposition of organic materials for smooth absorption, detoxification, and reduction of the effect of phytopathogenic microorganisms (Yadav et al., 2011; Kumar et al., 2012; Karnwal, 2017).

All biological life present on the Earth relies on crops for their nutritional needs. More than 90% of global food comes from 12 varieties of crops and 14 animal species (Macauley, 2015; Miller, 2016). For over 50% of the world's population, the key nutrition crops are wheat, rice, and maize (Macauley, 2015). The major challenges in agriculture include abiotic stresses caused by climate change or salinity of soils, which have been reported to affect the fertility of soil. This has a serious negative impact on plant growth and consequently food availability (Maheshwari, 2012; Goyal and Manoharachary, 2014). An increase in the atmospheric temperature causes loss in soil humidity (Singh et al., 2015) and subsequently increases the content of NaCl in soil, thereby causing fertile lands to become arid lands and leading to poor crop development, product yield, flowering, and pollination. Generally, salinity affects plant growth in three ways: osmotic stress, ionic stress or ion imbalance, and oxidative stress (Dimkpa et al., 2009). Changes in osmotic pressure may lead to a reduced cell turgor pressure resulting from the changes in water balance both within and outside the cells. This slows down the rate of cell elongation and cell division (Kunin and Rudy, 1991). To mitigate salt toxicity during natural evolution, crop communities respond and grow successfully through various strategies such as nutrient absorption and ionic equilibrium, plant physiological reaction, protective antioxidant reaction, and proline accumulation (Cipriano et al., 2015).

Crops share a significant number of resources and nutrients with microorganisms in their natural surroundings. These highly nutritionally rich ecosystems enable beneficial reciprocal relationships among crops and microorganisms (Ashraf et al., 2019). Through various biochemical pathways and products, microorganisms help the plants to cope with abiotic stresses. Many of the identified PGPRs (e.g. *Microbacterium*, *Pantoea*, *Achromobacter*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Enterobacter*, *Burkholderia*, *Methylobacterium*, *Azospirillum*, *Variovorax*) are directly associated with several plant species and may probably be found in several environments (Fatima and Ahmed, 2018; Ashraf et al., 2019). The inoculation of crop plants with beneficial microorganisms is gaining agronomic importance as these microorganisms facilitate cultivation under saline-prone conditions by improving salt tolerance and consequently restoring the yield (Lugtenberg et al., 2013). Rhizospheric bacteria isolated from extreme environments have been shown to induce salt tolerance in crop plants. For example, a *Pseudomonas fluorescens* strain isolated from date palm rhizosphere in the Saharan region promoted root growth in maize (*Zea mays*) seedlings under salt stress (Zerrouk et al., 2016). Wheat plants (*Triticum aestivum*) inoculated with *Serratia* sp. Sl-12, a halophilic bacterium isolated from a salt lake, showed improved salt tolerance and increased shoot biomass (Singh et al., 2015). The genera *Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, and *Ochromobacter* have been reported to improve the productivity of peanut, wheat and rice under saline conditions (Sharma et al., 2016; Singh and Jha, 2016; Sarkar et al., 2018). The diazotrophic salt-tolerant bacterial strains of *Klebsiella*, *Agrobacterium*, *Pseudomonas*, and *Ochrobactrum* isolated from the roots of a halophytic plant *Arthrocnemum indicum* showed salinity tolerance ranging from 4 to 8% NaCl and improved the productivity of peanuts in saline and in control conditions (Sharma et al., 2016). *Planococcus rifietoensis*, an alkaliphilic bacterium, was reported to enhance the growth and yield of wheat crops under salinity stress (Rajput et al., 2013). The diversity of salt-tolerant bacteria isolated from paddy rhizosphere in Taoyuan, China, was reported by Zhang et al. (2018). They reported that out of 305 bacterial isolates, 74 salt-tolerant strains belonged to Bacillales (72%), Actinomycetales (22%), Rhizobiales (1%), and Oceanospirillales (4%) orders. Bougainvillea is a plant that develops and grows in saline condition. It is a highly drought-tolerant, salt-tolerant, and wind-resistant plant that grows in the well-drained acidic soil with a pH between 5.5 and 6.0 (Islam et al., 2016; Abarca-Vargas and Petricevich, 2018). Earlier, the effect of endophytic bacteria isolated from *Bougainvillea glabra* was examined (Karnwal 2020), and the beneficial effect of endophytes on *Triticum aestivum* L. and *Zea mays* seedlings under saline condition was reported. However, little attention has been given to the rhizospheric microorganisms in *B. glabra* and their

plant growth promoting and salinity tolerance traits. The objective of the current research was to isolate and screen *B. glabra* rhizobacteria for salinity tolerance and plant growth-promoting activity. Further, these isolates were examined for their plant growth-promoting effect on the growth of wheat var. HD-2687 and maize var. PSCL-4642 seedlings under saline condition.

Materials and methods

Isolation of bacteria from B. glabra rhizosphere

Bacterial isolates intended for the current research were isolated and identified from *B. glabra* rhizosphere grown at the Bhojia Institute of Life Sciences, Budh, Baddi, Himachal Pradesh, India (located at latitude 30.952802 and longitude 76.776914) as explained in an earlier investigation of Karnwal (2017). The rhizospheric soil samples were serially diluted up to 10^{-6}, and 0.1 ml of suspension from the 10^{-6} dilution was spread onto a modified nutrient agar medium (NAM) enriched with 4% salt (NaCl) as described by Karnwal (2020). The plates were incubated for up to 72 h at 28 ± 1 °C in dark. Individual bacterial colonies that developed on 4% NaCl-enriched NAM plates were further examined for their colony characteristics and Gram staining results. Next, 200 µl of overnight grown fresh bacterial culture in Luria-Bertani (LB) (Masciarelli et al., 2014) broth was thoroughly mixed with 800 µl of 100% sterilized glycerol and stored at −20 °C until further use (Kumar et al., 2015). A total of 24 bacterial colonies were isolated in their pure form and were further analysed for salt stress tolerance.

Stress tolerance screening of bacterial isolates

Salinity tolerance of isolated bacterial species was investigated on NAM supplemented with different NaCl concentration: 5% (0.86 M); 7% (1.2 M); 8.5% (1.46 M); 10% (1.71 M); and 12% (2.054 M). Bacterial isolates that grew on a medium amended with NaCl at above 4% concentration were recorded as salt stress-resistant isolates and were examined *in vitro* for plant growth promoting (PGP) traits (Kumar et al., 2015; Zhou et al., 2017; Karnwal, 2019).

Morphological characterization of isolated bacteria

Selected salt stress-resistant isolates were morphologically and biochemically characterized as described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Gram staining, urease production, lipolysis activity, gelatine liquefaction, starch hydrolysis, citrate utilization, casein hydrolysis, catalase test, indole, production of H_2S and HCN, and oxidative-fermentative (OF) reaction were used to characterize the isolates.

Screening for PGP traits

Phosphate solubilization

The phosphate solubilization assay was conducted by applying a spot inoculation of each bacterial isolate on modified Pikovskaya's agar (HiMedia) and presented in the form of the solubilization index (SI) (Karnwal, 2017). Clear halo zones around the bacterial growth were assumed to be positive for phosphate solubilization and quantified using the following equation (Edi-Premono et al., 1996):

$$\text{Phosphate solubilization index [SI]} = = \frac{\text{(colony diameter + halo zone diameter)}}{\text{colony diameter}}$$

IAA-like auxin production

IAA production was measured on DF medium (Dworkin and Foster, 1958) enriched with 0.1% L-tryptophan using the Van Urk Salkowski reagent following Salkowski's method (Armada et al., 2016). The bacterial isolates (100 µl) were incubated in DF medium supplemented with L-tryptophan for 48 h at 28 ± 1 °C. The culture broth was centrifuged at 11963 *g*, and 1 ml of supernatant with 2 ml of Salkowski reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$) was incubated in the test tube. This mixture was left without any disturbance for 30 minutes in dark at room temperature. The optical density (OD) was recorded at 530 nm, and the amount of IAA-like auxins was estimated as µg/ml against non-inoculated control. A standard curve of various concentrations (range of 0–250 µg/ml) of pure IAA (Merck, Frankfurt, Germany) was prepared by plotting the IAA concentration based on the optical density (Almaghrabi et al., 2013).

1-Aminocyclopropane-1-carboxylate (ACC) deaminase assay

The ACC deaminase assay for salt-tolerant isolates was performed on DF salt minimal medium (Dworkin and Foster, 1958) supplemented with ACC as the sole nitrogen source. DF agar plates inoculated with bacteria were incubated for 48 h at 28 ± 1 °C. The appearance of

a bacterial colony after incubation was considered to be positive.

Siderophore production

A chrome-azurol S (CAS) medium was used to confirm the siderophore production potential of bacterial isolates (Schwyn and Neilands, 1987). After 24-h incubation, the bacterial culture was inoculated on CAS agar and incubated for 48–72 h at 28 ± 1 °C. The change in colour from blue to orange or yellow around the bacterial growth on the CAS agar medium confirmed the production of siderophore.

HCN determination

A modified Bakker and Schippers method (1987) was used to verify the ability of bacterial isolates to produce HCN. NAM plates enriched with 4.4 g/l glycine and a Whatman grade 1 filter paper (Sigma-Aldrich, India) soaked in 0.5% picric acid in 1% Na_2CO_3 in the upper lids of Petri plates along with an uninoculated control were used to detect HCN production. Parafilm-sealed Petri plates were incubated at 28 ± 1 °C for 48 h. A change in the colour of the filter paper from yellow to light brown, brown, or reddish-brown was recorded as negative (–), weak (+), moderate (++), or strong (+++) reaction, respectively .

The isolate that showed the most prominent PGP traits and the highest salt tolerance activity was selected for further studies: colonization, seed inoculation, seed inoculation under salinity conditions, and bacterial identification.

Root colonization ability study

Root colonization of a chosen bacterial strain was tested with wheat and maize seeds as described by Silva et al. (2003). The seeds of selected crop varieties were obtained from the Indian Agricultural Research Institute (IARI), Pusa, Delhi, India and were surface sterilized (Karnwal, 2019). After drying under a laminar air flow, the surface-sterilized seeds were transferred onto half-strength tryptic soy agar (TSA) plates for 24 h at 28 °C to check for any form of contamination. The surface-sterilized seeds were immersed and left for 12 h in the bacterial culture (two-day-old bacterial growth) with 9 log 10 CFU ml^{-1} and then placed in culture tubes containing 0.05% Phytagel (Sigma-Aldrich) for germination. The bacterial isolate was tested in three replicates (a replicate was considered as one seed in a tube). A change in the opacity of the Phytagel near roots was considered as bacterial root colonization.

Seed inoculation study

The bacterial suspension was adjusted to the cell density of 8 log 10 CFU ml^{-1} as read at a wavelength of 600 nm. The surface-sterilized seeds were immersed in a bacterial suspension and rotated at 150 rpm for 2 h at 28 °C. The seeds were then air-dried in a laminar airflow hood for 60 min (Karnwal, 2020). Ten seeds per crop were placed on a sterile moistened Whatman No. 1 filter paper (Sigma-Aldrich) and wetted with 5 ml of sterile distilled water. All experiments were performed in a sterile laminar air flow. Petri dishes were taped and incubated at 27 °C for 7 days in the incubator in a dark room. The moisture during the germination process was maintained by providing sterile distilled water. All trials were conducted in triplicate (Kumar et al., 2015; Karnwal, 2019).

Seed inoculation under salinity conditions

The effect of the chosen bacterial isolate on seed germination under different salinity conditions was recorded for different NaCl concentrations (50, 100, 150, and 200 mM). Ten surface-sterilized bacterial-inoculated seeds were used for each treatment and placed in a Petri dish lined with a sterile Whatman No. 1 filter paper. All trials were conducted in triplicate (Karnwal, 2019). Sterile distilled water was used instead of the bacterial suspension to serve as a control. Five millilitres of sterile 50, 100, 150, and 200 mM NaCl solution were used for maintaining salinity and moisture conditions during the experiment. The germination percentage was calculated based on Kader's equation (Kader, 2005):

$$\text{Germination [\%]} = = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100$$

Characterization and identification of the bacterial isolate

The phylogenetic analysis and 16S ribosomal RNA sequencing were performed for the chosen salt-resistant bacterial isolate as reported in Karnwal (2019). DNA was isolated using the DNeasy-Plant Mini Kit (Qiagen, USA), and the 16S rRNA gene (rDNA) was amplified using universal bacterial primers: 534r (5′–ATTACCGC GGCTGCTGG–3′) and *U1517R* (5′–ACGGCTACCTT GTTACGACTT–3′).

Trial Design and Statistical Analysis

All trials were set up in a randomized block design and were conducted in triplicate. The numerical data generated from the seed germination and growth promotion experiments were subjected to analysis of variance (ANOVA). The Fisher's least significant difference (LSD) test at p values of 0.05 was used to compare the mean values of the treatments.

Results and discussion

Characterization of the bacterial isolates

In a natural environment, plants have to face various biotic and abiotic stresses (Balseiro-Romero etal., 2017). Abiotic stresses refer to inanimate components associated with the environment, such as nutrients, salt concentration, water availability, temperature change, and pH, which directly influence plant growth in the agricultural field (Kumar and Verma, 2018). In the present study, 24 rhizospheric bacterial isolates (designated as BoGl101 to BoGl124) were screened from the *B. glabra* rhizosphere having the salt-tolerance ability of at least 4% NaCl concentration, which was tested in a salt-amended NAM.

Screening for stress tolerance

Microorganisms have various mechanisms that enable them to survive in a stress environment (Singh et al., 2015). The application of PGPR is one of the most promising alternative approaches to improve crop production in saline soils (Choudhary et al., 2016; Chatterjee et al., 2017; Numan et al., 2018). Various salt-tolerant PGPRs, including *Azospirillum, Burkholderia, Rhizobium*, *Pseudomonas*, *Acetobacter,* and *Bacillus*, have been successfully applied or tested for plant growth promotion under salt stress (Egamberdieva et al., 2015; Chatterjee et al., 2017; Torres et al., 2019). In the present study, all 24 isolates were grown on media that contained NaCl. The salt stress results revealed that BoGl109, BoGl113, BoGl120, BoGl123, and BoGl124 isolates tolerated NaCl concentration higher than 4% (Table 1). The BoGl120 and BoGl123 isolate showed growth at 5%, 7%, 8.5%, and 10% NaCl concentrations as shown in Table 1. In recent years, bacteria belonging to different genera such as *Rhizobium, Bacillus, Pseudomonas, Burkholderia, Achromobacter, Methylobacterium*, and *Variovorax* have been shown to exhibit good tolerance against different abiotic stresses (Armada et al., 2016; Passari et al*.,* 2016; Karnwal, 2019). These microorganisms can help to reduce environmental stress in farming and are potentially useful for decreasing soil salinity. In the current study, the isolates BoGl120 and BoGl123 could tolerate the maximum concentration of 10% NaCl; however, no growth was observed above 10% NaCl concentration. On the basis of the salt stress tolerance results, five bacterial isolates (BoGl109, BoGl113, BoGl120, BoGl123, and BoGl124) were examined for their PGP traits: phosphate liquefaction, IAA production, ACC deaminase assay, siderophore production, and HCN production.

Phosphorus is an essential macronutrient required by all living organisms (Saleem et al*.,* 2018). Plants require it in small amounts, although its critically low availability leads to deficiency, which adversely impacts their growth (Karnwal, 2017). The required amount of phosphorus for an optimal growth ranges from 25 µmol/l to 30 µmol/l, but the actual amount of phosphorus available in most soil types ranges only from 1 µmol/l to 1.7 µmol/l (Perrig et al*.,* 2007). Several researchers (Bakker and Schippers, 1987; Perrig et al*.,* 2007; Masciarelli et al., 2014; Passari et al*.,* 2016) have documented that plants use soil bacteria for liquefaction of mineral phosphates into a utilizable form. The current study found only three isolates (BoGl113, BoGl123, and BoGl124) that had bright areas surrounding bacterial growth with diameter ranging from 6 to 28 mm and the phosphate solubilisation index ranging from 3.60 to 10.33, indicating positive phosphate solubilization (Table 1).

The production of IAA is one of the most critical traits of a wide variety of soil microorganisms and is important for plant growth (Karnwal, 2009). IAA is a growth hormone associated with rhizome propagation, plant cell proliferation, and cell duplication (Karnwal and Dohroo, 2018). PGPR that produce both IAA and ACC deaminase can effectively protect plants from a wide range of stresses (Egamberdieva et al., 2015). IAA accumulation induces the transcription of ACC synthase genes, which increases ACC concentration, leading to the production of ethylene (Numan et al., 2018). PGPRs containing ACC deaminase may break down some of the excess ACC and lower plant ethylene levels during an event of environmental stress, and simultaneously allow IAA to promote plant growth (Egamberdieva et al., 2015; Choudhary et al., 2016). In the present study, five iso-

Table 1. Screening of bacterial isolates for salt tolerance and phosphate solubilisation ability

Bacterial isolate	Salt tolerance at different NaCl concentrations					Phosphate solubilization efficiency [mm]		Phosphate solubilization index
	5%	7%	8.5%	10%	12%	colony diameter	halo zone diameter	
BoGl109	+	+	+	–	–	7 ± 0.02 [a]	0 ± 0.0	1.00 ± 0.02
BoGl113	+	+	–	–	–	2 ± 0.06	6 ± 0.01	4.00 ± 0.05
BoGl120	+	+	+	+	–	4 ± 0.10	0 ± 0.0	1.00 ± 0.10
BoGl123	+	+	+	+	–	3 ± 0.04	28 ± 0.12	10.33 ± 0.08
BoGl124	+	+	–	–	–	5 ± 0.04	13 ± 0.06	3.60 ± 0.02

(–, +) – represent negative growth, positive growth, respectively; [a] – values are mean ± SE

Table 2. Screening profile of bacterial isolates for various PGP traits

Bacterial isolate	IAA-like auxin production [μg/ml] *	ACC deaminase activity [a]	HCN production [b]	Siderophore production zone [mm] *
BoGl109	0	–	++	1.0 ± 0.01
BoGl113	1.6 ± 0.02	–	+	2.8 ± 0.02
BoGl120	6.24 ± 0.02	+	–	8.12 ± 0.02
BoGl123	8.24 ± 0.06	–	+++	12.6 ± 0.04
BoGl124	0	–	++	2.2 ± 0.02

[a] (–, +) – represent no growth, positive growth, respectively; [b] (–, +, ++, +++) – represent no activity, weak activity, moderate activity, strong activity, respectively; * – values are expressed as mean ± SE

lates, namely BoGl109, BoGl113, BoGl120, BoGl123, and BoGl124, were analysed for IAA production ability (the availability of L-tryptophan), and three isolates (BoGl113, BoGl120, and BoGl123) showed positive attributes for IAA production in the range from 1.6 μg/ml to 8.24 μg/ml IAA (Table 2). The BoGl123 isolate produced the highest amount of IAA (8.24 μg/ml) followed by BoGl120 (6.24 μg/ml). On the other hand, BoGl113 produced the lowest amount of IAA (1.6 μg/ml). During the study, it was observed that only one isolate, i.e. BoGl120, was positive for ACC deaminase activity, as other isolates were unable to use 1-aminocyclopropane-1-carboxylate as the sole nitrogen source for their growth in DF salt minimal medium (Dworkin and Foster, 1958) supplemented with ACC as the sole nitrogen source.

Karnwal (2009) reported the significance of rhizocompetent stress-resistant microorganisms with diverse functions that are primarily responsible for eliminating salt stress in crops. The biosynthesis of iron scavenger siderophores is an important function of PGPRs (Shruti et al., 2013; Chauhan et al., 2016) as it promotes plant development by increasing the nutrient supply for the plant while reducing the iron supply to soil-borne phytopathogens (Schwyn and Neilands, 1987; Etesami and Beattie, 2018). In the current study, four isolates (BoGl109, BoGl113, BoGl120, and BoGl123) showed positive results for HCN production with weak to a maximum strength (detected by colour change of the filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 from deep yellow to reddish-brown), and all isolates showed positive results for siderophore production (Table 2). A range of PGPR produce HCN that can control the level of deleterious microorganisms in the rhizosphere and increase the availability of P by metal chelation and sequestration (Kumar et al., 2015; Rijavec and Lapanje, 2016). Volatile organic carbon (VOC) such as HCN and NH_3 (Agbodjato et al., 2015) produced by PGPRs stimulate plant growth that results in an increased shoot biomass and improved plant stress resistance (Ruzzi and Aroca, 2015). The production and synthesis of HCN by PGPR are independent of their genus, and their impact suggests the possibility of using them as biological ferti-

Table 3. Effect of bacterial inoculation on seed germination under various NaCl concentrations

NaCl concentration [mM]	Seed germination % for wheat		Seed germination % for maize	
	control	BoGl120	control	BoGl120
50	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
100	48 ± 0.12^{b}	91 ± 0^{a}	60 ± 0.02^{b}	83 ± 08^{a}
150	0 ± 0^{b}	53 ± 0.2^{a}	0 ± 0^{a}	0 ± 0^{a}
200	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}

Values represented with superscript alphabets in a row are significantly different ($P < 0.05$) and show a difference between the control and NaCl treatments

Table 4. Effect of rhizobacteria on the radicle length under various NaCl concentrations

NaCl concentration [mM]	Radicle length [mm] of wheat seedlings		Radicle length [mm] maize seedlings	
	control	BoGl120	control	BoGl120
50	4.3 ± 0.10^{b}	28 ± 0.04^{a}	3.8 ± 0.01^{b}	22.4 ± 0.06^{a}
100	3.7 ± 0.02^{b}	18.2 ± 0.04^{a}	3.0 ± 0.02^{b}	13.0 ± 0.12^{a}
150	0 ± 0^{a}	1.8 ± 0.01^{a}	0 ± 0^{a}	0 ± 0^{a}
200	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}

Values represented with superscript alphabets in a row are significantly different ($P < 0.05$) and show a difference between the control and NaCl treatments

lizers or biocontrol agents to enhance crop production (Agbodjato et al., 2015; Rijavec and Lapanje, 2016). Many authors reported HCN-producing PGPBs and their use as biofertilizers in growth promotion and yield enhancement under drought and saline stress conditions (Rijavec and Lapanje, 2016; Kumar et al., 2015).

Of all the screened isolates, BoGl120 showed the highest salt tolerance with most prominent PGP traits. Therefore, only the BoGl120 strain was selected for further studies: colonization study, seed inoculation study, seed inoculation under salinity conditions, and bacterial identification.

Root colonization

It was found that the BoGl120 isolate could colonize the roots of wheat and maize seedlings as the bacteria formed a dense opaque zone around the radicle and adjacent to the roots. This indicated root colonization.

Seed inoculation and germination under salinity conditions

Table 3 shows the results of the influence of salt stress on the germination of wheat and maize seeds. A positive effect of the inoculated bacteria on the seed germination percentage under salinity was noted. Wheat seeds showed higher germination efficiency than maize seeds under salinity, when they were submerged in a suspension of BoGl120. Inoculation with BoGl120 showed 100, 91, and 53% seed germination at 50, 100, and 150 mM salt concentration in wheat, respectively, while in maize, 100 and 83% seed germination was observed at 50 and 100 mM NaCl concentration, respectively. These results confirmed the positive effect of bacterial inoculation on seeds (Table 3).

BoGl120 inoculation increased the radicle length of wheat seedlings significantly by 28, 18.2, and 1.8 mm at 50, 100, and 150 mM NaCl concentration, respectively (Table 4). The control treatment without bacterial inoculation showed radicle growth only up to 100 mM salt concentration with a significantly reduced radicle growth (at 50 mM NaCl: 4.3 mm and 100 mM: 3.7 mm) as shown in Table 4. Similarly, inoculation of maize seeds with a bacterial isolate increased the radicle length significantly at 50 and 100 mM NaCl concentration by 22.4 and 13.0 mm, respectively, when compared with controls (3.8 mm and 3.0 mm) at similar NaCl concentrations. However, at a higher level of salinity (150 and

Table 5. Effect of rhizobacteria on the plumule length under various NaCl concentrations

NaCl concentrations [mM]	Plumule length [mm] wheat seedlings		Plumule length [mm] maize seedlings	
	control	BoGl120	control	BoGl120
50	10.3 ± 0.04^{b}	32.2 ± 0.04^{a}	11.2 ± 0.12^{b}	24.0 ± 0.06^{a}
100	8.0 ± 0.12^{b}	14.0 ± 0.03^{a}	9.2 ± 0.06^{b}	10.8 ± 0.04^{a}
150	0 ± 0^{b}	8.0 ± 0.01^{a}	0 ± 0^{a}	0 ± 0^{a}
200	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}

Values represented with superscript alphabets in a row are significantly different ($P < 0.05$) and show the difference between the control and NaCl treatments

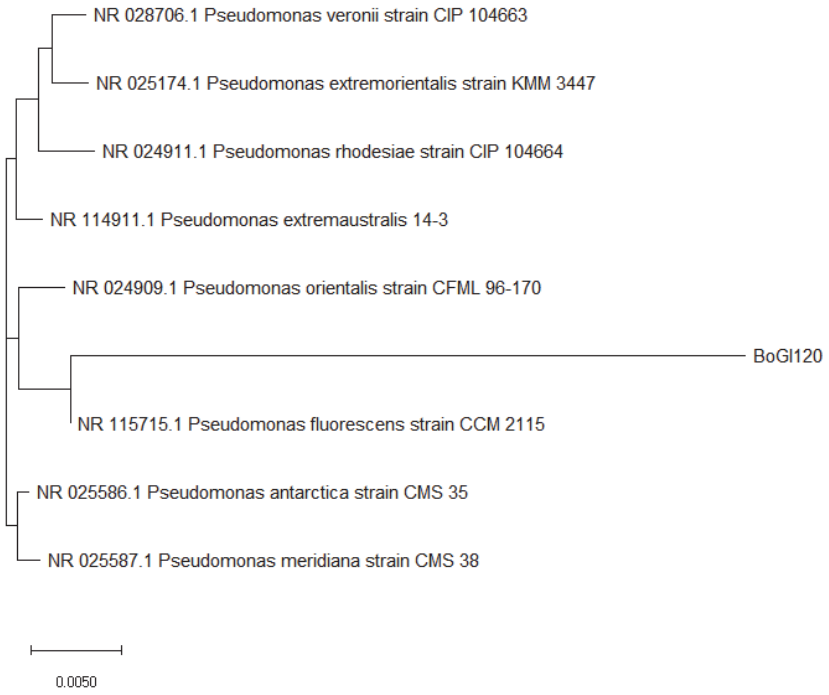

Fig. 1. A phylogenetic tree created with MEGAX software for the BoGl120 isolate

200 mM NaCl), bacterial inoculation did not stimulate any radicle growth in maize (Table 4).

Inoculation of seeds with BoGl120 at 50 mM NaCl increased the plumule length of the germinated seeds of wheat and maize by 32.2 mm for wheat and 24.0 mm for maize, respectively, when compared with the controls at a similar salt strength (Table 5). In the control treatment, wheat and maize seeds showed plumule growth at 50 mM (10.3 mm and 11.2 mm) and 100 mM (8.0 mm and 9.2 mm) NaCl level. No plumule growth was reported at 150 and 200 mM NaCl for maize in the control or bacterial treatments, whereas for wheat, a limited growth was observed at 150 mM NaCl concentration (8.0 mm) for the bacterial treatment but not for the control treatment. At 200 mM NaCl concentration, control and bacterial inoculation in wheat and maize showed no plumule growth.

Bacterial characterization and identification

Many authors (Belimov et al., 2001; Yadav et al., 2011; Passari et al., 2016; Ashraf et al., 2019) reported that major soil phyla comprised *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, and *Plantcomycetes*, although their relative abundances vary with the study site. It was reported that a significant proportion of plant growth-related bacterial communities were mainly associated with phylum *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* (mainly genera *Micro-*

bacterium, *Bacillus*, *Pantoea*, *Micrococcus*, *Burkholderia*, *Flavisolibacter*, and *Pseudomonas*) (Ashraf et al., 2019). In the present study, the microscopic, biochemical, and molecular methods were used to identify and characterize the BoGl120 bacterial isolate, and on the basis of the results, the isolate was characterized to be a rod-shaped gram-negative bacilli in nature.

Molecular characterization of the bacterial isolate was performed by 16S rDNA sequencing. The BoGl120 16S rRNA gene sequence showed 96.33% identity with *Pseudomonas fluorescens* strain CM 2115 and 95.80% identity with *Pseudomonas fluorescens* strain CFML 96-170 (according to BLAST). Nucleotide sequences were uploaded with the accession number MT704960 to GenBank (NCBI) database. The phylogenetic tree for BoGl120 was constructed using MEGA X software using the Muscle alignment method as shown in Figure 1.

Conclusions

This study confirmed an improved growth of wheat and maize in saline conditions in the presence of salinity-tolerant bacteria. Inoculation of the tested crop seeds with the chosen bacterial isolate resulted in an increase in the seed germination percentage and plumule and radicle lengths. These improvements in plant growth traits were related to phytohormone production (auxins-IAA), phosphate liquefaction, siderophore production, and HCN production, which suggested that salinity stress-tolerant bacteria were helpful and beneficial in enhancing the growth of wheat and maize. The results of the present study show the possibilities of using salt stress-tolerant rhizobacteria in wheat and maize varieties grown in Himachal region under field conditions.

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