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Isolation and characterization of plant growth promoting rhizobacteria from cacti root under drought condition

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ABSTRACT

Plant growth-promoting rhizobia (PGPR) helps plants grow and develop by protecting them from abiotic and biotic stresses, increasing the synthesis of chemicals that promote growth, and enabling the uptake of nutrients. Drought is one of the biggest problems throughout the world. The search for novel and efficient drought-resistant microorganisms that reduce the adverse effects executed by drought is a significant alternative. This study aimed to isolate and characterize PGPR strains from the *Opuntia Ficus-Indica* cactus plant's rhizosphere, cultivated in the semi-arid Shankargarh district of Uttar Pradesh, India. Tests for plant growth-promoting activity, such as the generation of indole acetic acid (IAA), phosphate solubilization, ammonia, carboxymethyl cellulase, and protease activity, were performed on all bacterial isolates. There were 246 bacterial strains isolated from the rhizospheric zone, and only 16.6 % showed drought resistance and various plant growth-promoting traits. The *Bacillus* sp. strain promoted the growth promotion of *Capsicum annum L.* under water stress (30 % field capacity). Additionally, *Bacillus sp.* isolates, with their potential for drought tolerance and plant growth promotion, could be applied in sustainable agriculture to enhance crop yield and resilience to water scarcity.

Introduction

Water is one of the most critical elements that restricts the growth of plants and all other living things. Drought is a complicated and natural phenomenon that affects many regions of the world and has detrimental effects on the environment, the economy, and society, leading to the development of various types of stress ***(Seleiman et al., 2021)***. Drought stress (DS) is disastrous for plant growth and directly impacts global food security and agricultural output. Since there are fewer water supplies and less rainfall, drought conditions are most frequently seen. It affects agriculture, economy, environment, deteriorates water quality and causes soil erosion. By 2050, similar trends will cause the area impacted by drought to double, with a 30 % decrease in water resources (Ingrao et al., 2023). Plants have developed various defence strategies against drought stress, including alterations to their cellular, molecular, and physiological makeup. All issues related to drought stress decrease soil fertility and microbial diversity and intensify nutrient competition. Recently, efforts have been made to implement Phyto beneficial soil bacteria to improve environmental rehabilitation and counteract the detrimental effects of drought (Kaushal et al., 2019). Various reports have shown the beneficial effects of PGPR on giving drought-stress-resistant crop varieties (Al-Turki et al., 2023). PGPR bacteria assist plant growth in low-water and high-metal environments, i.e., reducing stress. Additionally, these bacteria are used to synthesise biofilms and exopolysaccharides, which boosts soil aggregation, reduces microbial competition, offers exceptional protection against external stress, and benefits host plants (*Saeed et al., 2021*; *Carezzano et al., 2023*).

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Several PGPR taxa, such as *Klebsiella, Pseudomonas, Acinetobacter, Paenibacillus*, and *Bacillus*, have been shown in several studies to increase plant stress tolerance in dryland environments (*Getahun et al., 2020*; Kumar et al., 2020; *Xun et al., 2024*). The physiological and biochemical processes of plants can be changed by PGPR, which further lessens the consequences of drought. Rhizobacteria mostly employ direct or indirect processes to promote plant development (Chieb and Gachomo, 2023a). The synthesis of phytohormones like IAA, phosphate solubilization, siderophore formation and phytohormones promote nutrient intake and root development of plants (*de Andrade et al., 2023*). The predominant vegetation under water stress is cacti, home of xerotolerant bacteria in their rhizosphere. In drought-ridden environments, xerotolerant microorganisms offer a viable substitute for stimulating plant development. These bacteria have specific desired characteristics, such as producing exopolysaccharides, forming biofilms, and osmolytes that can endure dry conditions in an ecologically challenged setting and promote plant growth (*Kavamura et al., 2013*). Furthermore, by providing nutrients and hormones, fostering plant growth, and preserving a favourable wet environment for root development, microbes can shield plants against desiccants.

The present work aimed to isolate and molecularly characterize drought-tolerant bacteria isolated from the rhizospheric zone of cacti in the Shankargarh region and determine their traits that promote plant growth in drought-stressed environments. Shankargarh is an arid region with a high occurrence of heavy metals like silica and iron in the soil, making it a stressful environment for plant growth. Mainly in these regions, plants like *Opuntia Ficus-indica,* a cactus-like plant, are found. They generally host multiple types of root-associated microbes with mutual growth promotion activities. Samples are selected from this region due to its unique soil composition and climatic conditions.

Materials and methods

Sampling and isolation of root-associated bacteria

Rhizospheric soil was collected from healthy, bud bloom stage of growing 4–5 cacti from several locations in the semi-arid region of Shankargarh village (25.1908° N, 81.6117° E) Uttar Pradesh, India in January 2024 (Figs. 1 and 2). The climate in this region is semi-arid, with 900–950 mm of annual rainfall and dry, water-stressed soil. Rhizospheric soil samples were sealed in sterile plastic bags, brought cold to the laboratory, and kept at 4 °C. The surplus dirt was gently brushed off, and the remaining root soil was collected for serial dilution to extract bacteria associated with the roots. The bacterial isolation was performed using 1 gram of rhizospheric soil in 10 mL of saline buffer (NaCl 0.8 %; KCl 0.02 %; Na_2HPO_4 0.14 %; KH_2PO_4 0.024 %) under aseptic

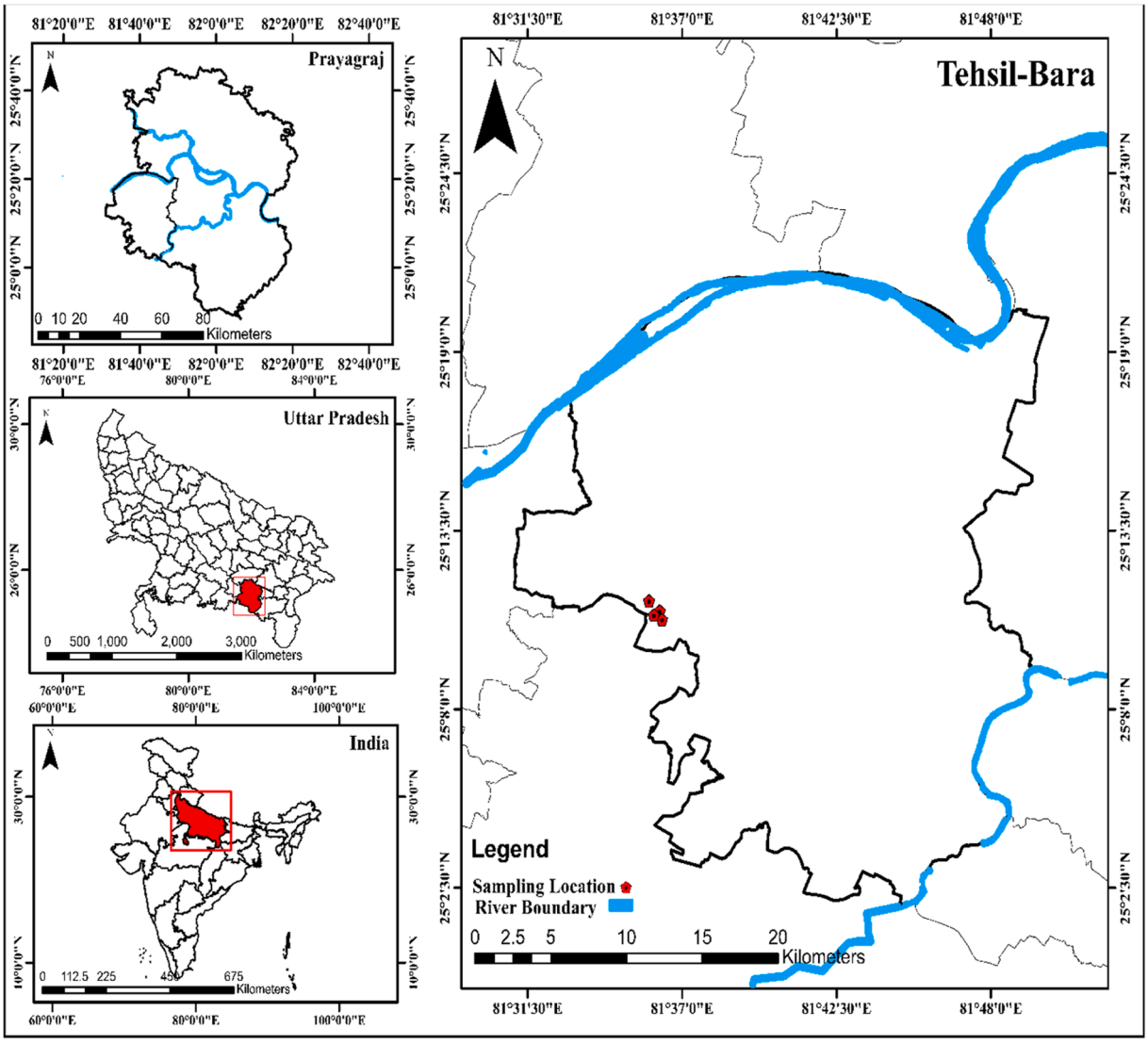

Fig. 1. Map of India and Uttar Pradesh, showing the sampling site in the semi-arid Sankargarh region of Prayagraj, India.

Fig. 2. Cacti plants are grown in stony soil and semi-arid Shankargarh region of Uttar Pradesh, India, used for the isolation of drought-resistant plant growth promoting rhizobacteria.

conditions (Abedinzadeh et al., 2019). The suspension (10^{-2} to 10^{-4}) after serial dilution inoculated in Tryptone soy agar (TSA) medium (10 %). Following a 24-hour incubation period at 28 °C, distinct morphological (colour, shape, growth rate, and colony morphology) bacterial colonies were selected for transfer to new bacterial inoculum and stored at -80 °C for further analysis. Further experiments were conducted using fresh bacterial cultures.

Screening of drought-tolerant bacteria

The drought-resistant bacteria were chosen based on their ability to thrive in the appropriate growth media at different sorbitol concentrations of 0 gL^{-1}, 85 gL^{-1}, 285 gL^{-1}, 520 gL^{-1}, and 660 gL^{-1} (Lasudee et al., 2018). The water activity will decrease as the sorbitol content rises. It can simulate drought and induce water stress. In this experiment, 48 to 72 h were spent incubating each bacterial isolate produced on the sorbitol-added medium of their growth mediums, which included varying amounts of active sorbitol. By adding sorbitol to the medium, the equivalent water (Aw) generated is 0.998, 0.986, 0.957, 0.897, and 0.844, in that order (Hallsworth et al., 1998). Bacterial isolates that showed signs of growth on TSA medium containing 520 g/l of sorbitol, respectively, were considered drought tolerant.

Characterisation of the bacteria for PGP traits

Exopolysaccharide production

A qualitative assessment of the exopolysaccharide synthesis by a subset of bacterial isolates was conducted by Paulo et al. (2012). Each strain was inoculated onto 5-mm diameter paper discs in the medium (2 % yeast extract, 1.5 % K_2HPO_4, 0.02 % $MgSO_4$, 0.0015 % $MnSO_4$, 0.0015 % $FeSO_4$, 0.003 % $CaCl_2$, 0.0015 % NaCl, and 1.5 % agar) was adjusted by adding 10 % saccharose, resulting in a pH of 7.5. The production's standout elements were the size of the generated halo and its slime-like texture. EPS generation by bacterial isolates was verified by combining mucoid material with 2 mL of 100 % ethanol and observing precipitate formation.

Indole 3-acetic acid (IAA) production

To assess the production of IAA, the colourimetric method described by Gordon and Weber (1951) was used. Each bacterium was injected with 100 μL at a concentration previously adjusted to 10^8 cells mL-1 (OD550 nm = 0.1) in 10 mL of Tryptone Soya Broth (TSB) and Nutrient Broth (NB) medium (10 %) modified with 5 mM of L-Tryptophan. At the same time, the mixture was incubated in the dark. After 48 h at 28 °C, the bacteria were separated by centrifugation at 10,000 rpm for 5 min. The supernatant, which included 750 μL of Salkowski reagent (50 mL of 35 % perchloric acid and 1 mL of 0.5 M $FeCl_3$), was then incubated for 30 mins in the dark. The IAA production (μg/mL) was observed by the appearance of a pink colour after 30 min of incubation. At the same time, the quantitative test was done by measuring the absorbance value using a spectrophotometer at a wavelength of 530 nm.

Phosphate solubilization

The capacity of the bacterial isolates to solubilize inorganic phosphate was used to assess their phosphate solubilisation. In the experiment, calcium phosphate, the inorganic form of phosphate, was added to a Pikovskaya agar media. This insoluble phosphate was necessary for testing the microorganism's ability to solubilize phosphate. After being streaked onto plates, bacterial cultures were cultured for four to five days at 30 °C. The bacterial colony's ability to dissolve phosphate was demonstrated by forming a translucent halo surrounding it. (Pikovskaya et al., 1948**).**

Zinc solubilization

The zinc solubilisation test was determined by Saravanan et al. (2007). The isolates were grown on the medium containing dextrose: 10.0; $(NH_4)_2SO_4$: 1.0; KCl: 0.2; K_2HPO_4: 0.1; $MgSO_4$:0.2; pH: 7.0; insoluble Zinc compounds (i.e., $ZnCO_3$): 0.1 %; and agar after incubation at 30 °C for 48 h. The formation of the clearance zone around the colonies indicates Zinc solubilization (Saravanan et al., 2007).

HCN production

HCN synthesis was identified using a 24 h-grown culture on LA medium (0.5g/l NaCl, 10 g/l tryptone, 5 g/l of yeast extract, and 15 g/l agar) supplemented with 4.4 g/L glycine. Whatman filter paper No 1 was soaked in a 2 % sodium carbonate solution in 0.5 % picric acid between the culture plate's base and lid. The filter paper's colour changed from yellow to orange-brown to signal the production of HCN; no colour changes suggested a negative result for this experiment (Lukkani et al., 2014).

Ammonia production

The rhizospheric isolates were cultivated in a peptone water medium for 48 h at 30 °C to identify ammonia production. After incubation, 1 mL of each culture was applied to each microtube along with 50 μL of Nessler's reagent (10 % HgI_2, 7 % KI, and 50 % aqueous solution of NaOH (32 %)). The appearance of a light-yellow hue indicated a small quantity of ammonia production. In contrast, the appearance of a deep yellow to brownish colour indicated the maximum amount of ammonia production (Dye et al.,1962).

Siderophores production

The capacity of the rhizobacterial isolate to generate siderophores was examined using a solid CAS agar medium [10 ml of Fe (III) solution (27 mg $FeCl_3$–$6H_2O$ and 83.3 μl of concentrated HCl in 100 ml ddH_2O) along with 72.9 mg hexadecyl trimethyl ammonium bromide (HDTMA) were dissolved in 40 ml ddH_2O]. The isolates were streaked on CAS (Chrome Azurol S) agar plates and incubated for five days at 30 °C in an incubator after being streaked. Regarding the siderophore's elimination of iron from the CAS, the bacterial colonies surrounding the orange halo area showed favourable outcomes (Alexander et al., 1991). The following formula was used to calculate the siderophore index (SI).

Siderophore Index = (Total diameter (colony diameter + halo zone diameter/ colony diameter)

Protease activity

The qualitative protease activity of the bacterial isolates was evaluated using the skim milk agar (SMA) medium [5 g/l of Casein enzymatic hydrolysate, 1 g/L of dextrose, 2.5 g/L of yeast extract, 1 g/L skim milk powder, 15 g/L of agar]. After being cultured in an LB medium, the bacterial strain was streaked over SMA media. The formation of a halogen zone showed that the bacterial strain had protease activity

(Masi et al., 2021).

Cellulase activity

Cellulase metabolic activity can be observed by performing it on carboxymethyl cellulose (CMC) medium (0.2 % $NaNO_3$, 0.1 % K_2HPO_4, 0.05 % $MgSO_4$, 0.05 % KCl, 0.2 % carboxymethyl cellulose salt, 0.02 % peptone, 1.7 % agar). Five microlitres of the bacterial isolate were inoculated into the CMC media plate and incubated at 28 °C for 48 h Subsequently, each plate was stained with iodine (0.666 % KI; 0.33 % iodine) for 5 min, and positive cellulase-producing strains were shown in the halo region. (Bera et al., 2014)

Biosurfactant production

Biosurfactant production was tested using the Oil Spreading assay described by Morikawa et al. (2000). The cultures were grown on LB broth (0.5 g/l NaCl; 10 g/l tryptone; 5 g/l of yeast extract) for 24 h. The freshly grown cultures were then centrifuged, and 10 µl of culture supernatant was added to the thin surface of 40 ml of distilled water and 10 µl of oil. The displaced and clearing zone formation indicates biosurfactant production (Morikawa et al., 2000).

Biochemical and molecular identification of selected bacterial isolates

Due to their drought resistance and PGP traits, six bacterial isolates were selected for biochemical and molecular characterisation. Several biochemical tests were performed according to the standard protocols, including catalase, citrate, oxidase, urease, starch hydrolysis, glucose, lactose, mannitol, and arabinose. The bacterial DNA isolation was done using the HiPurA Bacterial Genomic DNA Purification Kit. The 16S rRNA gene was amplified for molecular identification of bacterial isolates using bacterial-specific primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) (Hyder et al., 2020). PCR amplification was performed with 10 μl^{-1} Takara master mix (Takara Bio Inc. Japan) and 1.0 µM of each primer. The PCR amplification was performed in the Eppendorf Master cycler Nexus PCR Cycler consisting of Pre-denaturation at 94 °C for 3 min, then initial denaturation at 90 °C for 30 s, followed by 35 cycles of primers at 50 °C for 30 s, an extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. After purification, amplified PCR products were transferred to Himedia Pvt., Ltd. in Mumbai for Sanger sequencing. Nucleotide sequences were uploaded to the NCBI database and annotated using the NCBI BLAST service following DNA sequencing. All six sequences were aligned with similar sequences through MEGA6 software (Tamura et al., 2013) for constructing a phylogenetic tree.

Plant growth promotion experiment

In this study, Chilli (*Capsicum annum L.*) Pusa Sada bahar variety was selected as a model for the plant growth promotion test because it is of substantial economic importance, and these plants are known for their sensitivity toward environmental stressors (Kumar, 2019). Chilli plant seedlings (14-days-old) were surface sterilised and inoculated with each one-day-old bacterial inoculum. Control treatment was obtained by mixing the seed with sterile distilled water. The soil was placed into each pot once the sodium hypochlorite solution had sterilised them. Thirty per cent (30 %) of the field's capacity was maintained by alternate irrigation due to the dry conditions. The overnight growth culture of individual bacterial isolates was adjusted to 10^8 CFU/mL for weekly inoculation in the Chilli plants. Each pot contained a 10 ml dilution of bacterial strain that infected the plants. Plants were grown with 30 % of the water field capacity to obtain water stress conditions. Every experiment aimed at promoting plant development was conducted in triplicate. The average wet weight, shoot length, root length, leaf surface area, number of leaves and chlorophyll content were determined.

Statistical analysis

The data generated during the pot experiments were subjected to analysis of variance (ANOVA) by Dunnett's test multiple range test at $p < 0.05$ in order to compare the treatments with the control (uninoculated plants). Experimental data obtained from this study were statistically analysed using Microsoft Excel 2016.

Results

Isolation and in vitro assay of drought tolerance

A total of 246 bacterial isolates with different appearance, colour and sizes were obtained from the rhizospheric area of *Opuntia Ficus-Indica* cactus plants at four distinct locations in the Shankargarh region shown in Fig. 1. To evaluate the traits of several bacterial isolates resistant to water stress, bacterial growth was anticipated up to a concentration of 585 g/L sorbitol in TSA media. In the TSA medium, the bacterial isolates under osmotic stress concentration (585 g/L sorbitol), 12.6 % (19/150) and 23 % (22/96) isolates were shown to be highly tolerant, 14 % (21/150) and 19.7 % (19/96) were tolerant, and 73.4 % (120/150) and 59 % (55/96) were sensitive. The forty-one bacterial isolates that showed high tolerant activities against 585 g/L sorbitol were selected for further experiments.

Screening of bacterial isolates for the in vitro plant growth promotion activities

A total of 41 bacterial isolates were chosen for plant growth promotion characteristic analysis. A total of 25 % of bacterial isolates were able to produce the mucoid substance and showed a positive exopolysaccharide production test. Most isolates exhibited indole compound production at 50 to 100 µg/mL concentrations, but six bacterial isolates produced a high amount of indole compound production (>100 µg/mL). The indole compound production ability of all bacterial isolates was checked in the presence of l-tryptophan. Approximately 27.66 % of bacterial isolates showed phosphate solubilisation, 11 % of these isolates showed the highest ammonia production, 13 % of isolates were able to produce siderophores, and none of the isolates were able to produce HCN. However, 41.6 % of isolates had protease activity, 40.04 % exhibited cellulase activity, 13.09 % were able to produce biosurfactants, and 15.47 % of isolates observed zinc solubilization activity. Based on these results, six bacterial isolates showing positive PGP traits were selected for molecular characterization and identification.

Biochemical and molecular characterization of bacterial isolates

The identification and characterization of selected rhizospheric bacterial isolates were based on the morphological, physiological, biochemical and PCR amplification of the 16S rRNA gene. The biochemical, physiological, and PCR amplification results are summarized in Table 1. These bacterial strains were selected because of their potential drought resistance and positive PGP traits. The sequence similarity search of 16S rRNA sequences revealed that all six bacterial isolates belonged to the *Bacillus subtilis, Bacillus velezensis, Bacillus* sp., *Pseudomonas bubulae* and *Priestia megaterium*. The 16S rRNA gene sequences of all the bacterial isolates were submitted to the NCBI database. The BLAST analysis of the bacterial isolates with the available NCBI database showed that bacterial isolates (CR1-CR6) shared similarities with 97–100 % of the 16S rRNA gene sequences of the *Bacillus* and *Pseudomonas* genus. Strains CR1, CR2, CR3 and CR6 showed identity with *Bacillus* spp. with 97–100 % similarity. Isolate CR4 showed 100 % identity with *Pseudomonas bubulae,* and CR5 showed 99 % identity with *Priestia megaterium.* A phylogenetic tree, constructed based on the 16S rRNA gene sequences, indicated that all strains were closely related to the *Pseudomonas bubalus, Bacillus velezensis, Priestia megaterium, Bacillus licheniformis* and *Bacillus subtilis* bacterial isolates (Fig. 3).

Table 1
Morphology, physiology, biochemical test, PGP traits of rhizospheric bacterial isolates isolated from the rhizosphere region of Cactus plants. The symbols indicate the following: +, slightly positive; ++, highly positive; -, negative.

SN	Isolate code	Bacterial name	Morphology, physiology and biochemical tests	PGP traits
1	CR1	*Bacillus subtilis* (Accession No: PP766893)	Rod, motile, *G*+, Endospore formation (+), Catalase (+), Citrate (+), Oxidase (-), Urease (-), Starch hydrolysis (+), Glucose (+), Lactose (+), Manitol (+), Arabinose (+)	Sorbitol (520 gm/L, ++), IAA (105.10 µg/mL), HCN (++), Siderophore (+), Phosphate solubilizing (++), Ammonia production (++), Protease k (++), cellulose activity (-), Exopolysaccharide (+), Biosurfactant (-), Zn solubilizing (+)
2	CR2	*Bacillus velezensis* (Accession No: PP766887)	Rod, motile, *G*+, Endospore formation (+), Catalase (+), Citrate (+), Oxidase (-), Urease (-), Starch hydrolysis (+), Glucose (+), Lactose (+), Manitol (+), Arabinose (+)	Sorbitol (520 gm/L, ++), IAA (139.60 µg/mL), HCN (++), Siderophore (++), Phosphate solubilizing (++), Ammonia production (+++), Protease k (++), cellulose activity (-), Exopolysaccharide (+), Biosurfactant (+), Zn solubilizing (+)
3	CR3	*Bacillus* sp. (Accession No: PP766886)	Rod, motile, *G*+, Endospore formation (+), Catalase (+), Citrate (+), Oxidase (-), Urease (-), Starch hydrolysis (+), Glucose (+), Lactose (+), Manitol (+), Arabinose (+)	Sorbitol (520 gm/L, +), IAA (69.22 µg/mL), HCN (++), Siderophore (++), Phosphate solubilizing (++), Ammonia production (++), Protease k (-), cellulose activity (++), Exopolysaccharide (++), Biosurfactant (+), Zn solubilizing (+)
4	CR4	*Pseudomonas bubulae* (Accession No PP766889)	Rod, motile, G-, Endospore formation (-), Catalase (+), Citrate (+), Oxidase (+), Urease (+), Starch hydrolysis (-), Glucose (+), Lactose (-), Manitol (-), Arabinose (-)	Sorbitol (520 gm/L, ++), IAA (64.20µg/mL), HCN (++), Siderophore (-), Phosphate solubilizing (++), Ammonia production (-), Protease k (++), cellulose activity (++), Exopolysaccharide (++), Biosurfactant (-), Zn solubilizing (-)
5	CR5	*Priestia megaterium* (Accession No PP766892)	Rod, motile, G-, Endospore formation (-), Catalase (+), Citrate (+), Oxidase (-), Urease (+), Starch hydrolysis (-), Glucose (+), Lactose (+), Manitol (-), Arabinose (-)	Sorbitol (520 gm/L, ++), IAA (66.04 µg/mL), HCN (-), Siderophore (+), Phosphate solubilizing (+), Ammonia production (+++), Protease k (++), cellulose activity (++), Exopolysaccharide (++), Biosurfactant (-), Zn solubilizing (-)
6	CR6	*Bacillus velezensis* (Accession No PP766888)	Rod, motile, *G*+, Endospore formation (+), Catalase (+), Citrate (+), Oxidase (-), Urease (-), Starch hydrolysis (+), Glucose (+), Lactose (+), Manitol (+), Arabinose (+)	Sorbitol (520 gm/L, +), IAA (62.79 µg/mL), HCN (++), Siderophore (++), Phosphate solubilizing (++), Ammonia production (+++), Protease k (++), cellulose activity (-), Exopolysaccharide (+), Biosurfactant (+), Zn solubilizing (+)

Plant growth promotion experiment

The six bacterial strains identified as the most effective in drought-stress tolerance and *in vitro* plant growth promotion (PGP) were selected for further experimentation on plant growth (Fig. 3). We evaluated various parameters for Chilli plants, including leaf count, wet weight, shoot length, root length, leaf area, chlorophyll content, and dry biomass. The results were encouraging, showing a significant increase in all measured parameters compared to control plants. Specifically, drought-stressed plants inoculated with the six bacterial strains exhibited a significant ($p < 0.05$) enhancement in growth parameters relative to the control group (Fig. 4). The PGPR-treated Chilli plants demonstrated increases of 1.83 % to 43.11 % in root length and 11.11 % to 34.18 % in shoot length compared to control plants.

Additionally, chlorophyll content in treated plants was found to be 19.97 % to 71.88 % higher than that of control plants. These findings suggest drought-resistant bacterial isolates can substantially improve plant growth metrics such as leaf number, wet weight, shoot and root lengths, and leaf surface area under drought conditions. This confirms the potential of these bacteria to enhance plant health and yield even in water-limited environments.

Discussion

Drought is a major environmental problem that lowers plant growth and yields while damaging the agriculture and food industries. This stress generates reactive oxygen species (ROS), initiating osmotic stress in crops (Asghari et al., 2019). PGPR exhibit several mechanisms to enhance drought tolerance in plants, primarily through their interactions with plant roots and ability to adapt to water-limited environments. One significant mechanism involves the production of exopolysaccharides, which help retain soil moisture and improve soil structure, thus creating a more favourable environment for root growth. Also, drought-tolerant bacteria can synthesize phytohormones such as IAA, promoting root development and enhancing nutrient uptake under stress conditions. These bacteria also play a crucial role in phosphate solubilization, making essential nutrients available to plants and improving their overall health and resilience. Furthermore, some bacteria produce siderophores that chelate iron, increasing its availability in the soil, which is vital for plant metabolism. Forming biofilms by these bacteria can protect plant roots from desiccation and reduce competition with other microorganisms. Moreover, specific bacterial strains can induce the production of osmolytes, which help stabilize proteins and cellular structures under osmotic stress. Collectively, these mechanisms enhance the drought tolerance of plants and contribute to sustainable agricultural practices by promoting plant growth in challenging environmental conditions. This research aims to isolate and characterize the drought-tolerant rhizobacteria from the semi-arid Shankargarh region of Uttar Pradesh, India. The Shankargarh region is of particular interest due to its unique combination of deficient average rainfall and soil containing heavy metals, making it an ideal location for understanding the behavior of microbes under drought conditions.

A total of 256 bacteria were isolated on TSA media, which were obtained from the rhizospheric region of the cacti; only 41 were able to tolerate the drought stress created by the 585 gm/L concentration of sorbitol as its act osmotic agent, which reduces the water activity. The ability of these bacteria to grow in these concentrations indicates their resilience to drought conditions. This ability confirms the sustainability

Table 1 (*continued*)

SN	Isolate code	Bacterial name	Morphology, physiology and biochemical tests	PGP traits
			Manitol (+), Arabinose (+)	(+), Biosurfactant (+), Zn solubilizing (+)

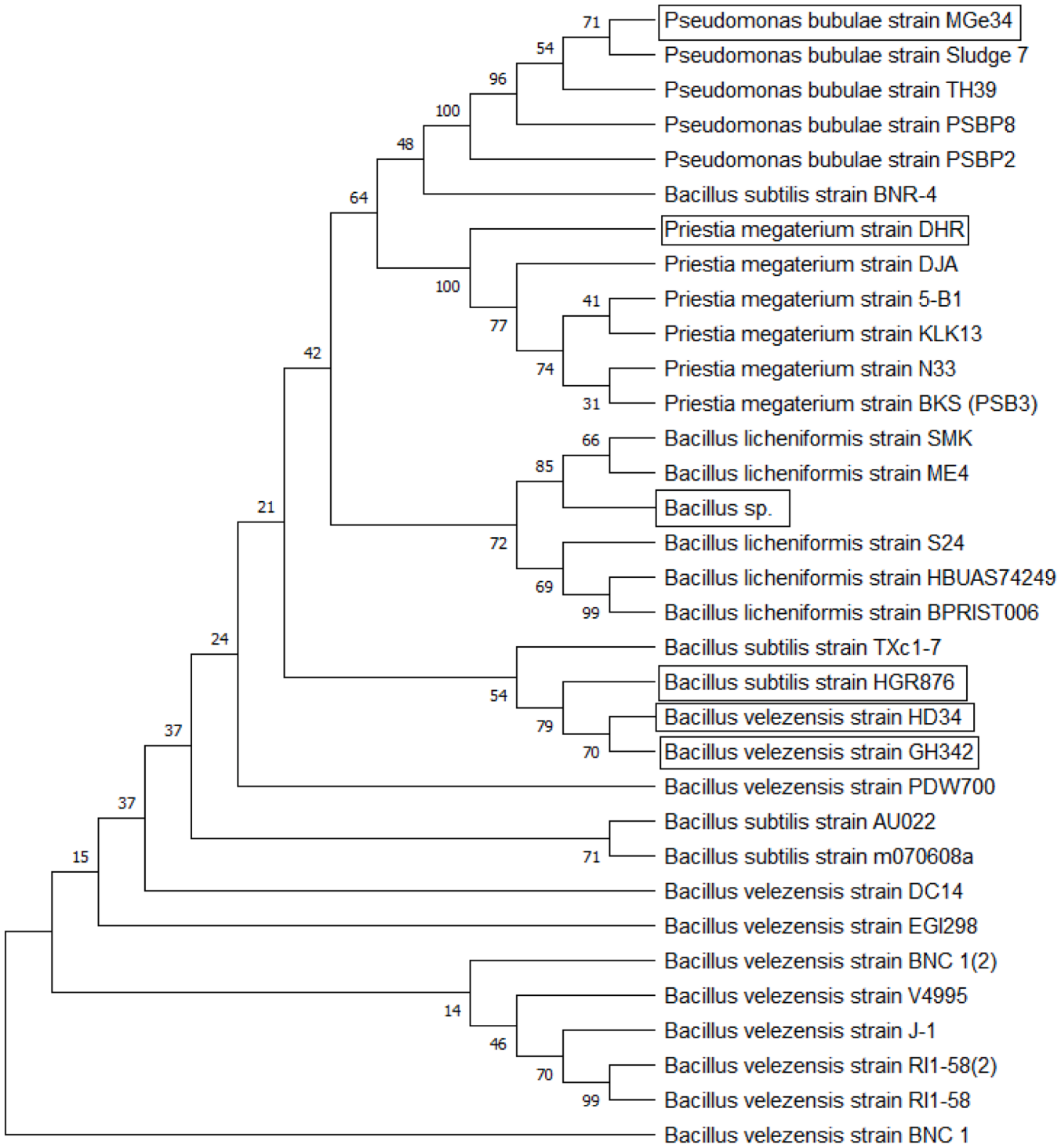

Fig. 3. Phylogenetic analysis of all six isolated bacterial strains based on 16S rRNA gene sequences. The phylogenetic tree was constructed through the Neighbor-Joining algorithm with a Bootstrap value of 1000. The tree is drawn to a scale of 0.05 substitutions per nucleotide position.

of Xero-tolerant bacteria, a term used to describe bacteria that can thrive in arid conditions to enhance plant growth under drought. Patel et al. (2022) showed that osmotic stress-tolerant bacteria strains *Bacillus tequilensis* and *Pseudomonas stutzeri* enhanced plant properties like root length, shoot length, chlorophyll content and antioxidant properties of the wheat and brinjal under the drought. Similarly, Curá et al. (2017) explained that these drought-resistant bacteria enhanced the maize plant's total biomass and carbon content under drought. The PGPR strains isolated in this study also enhanced the root length, chlorophyll content and shoot length.

These bacteria isolates have multiple PGP properties like Indole compounds production, exopolysaccharides production, and zinc and phosphate solubilization, which all help in the overall development of plants under stress conditions through various mechanisms like phyto-hormone, osmolytes, and antioxidant production, including modulation of gene expression and ion homeostasis (Gupta et al., 2022). In this research, 25 % of bacteria were able to produce exopolysaccharides. According to Cheng et al., 2020 exopolysaccharide production helps

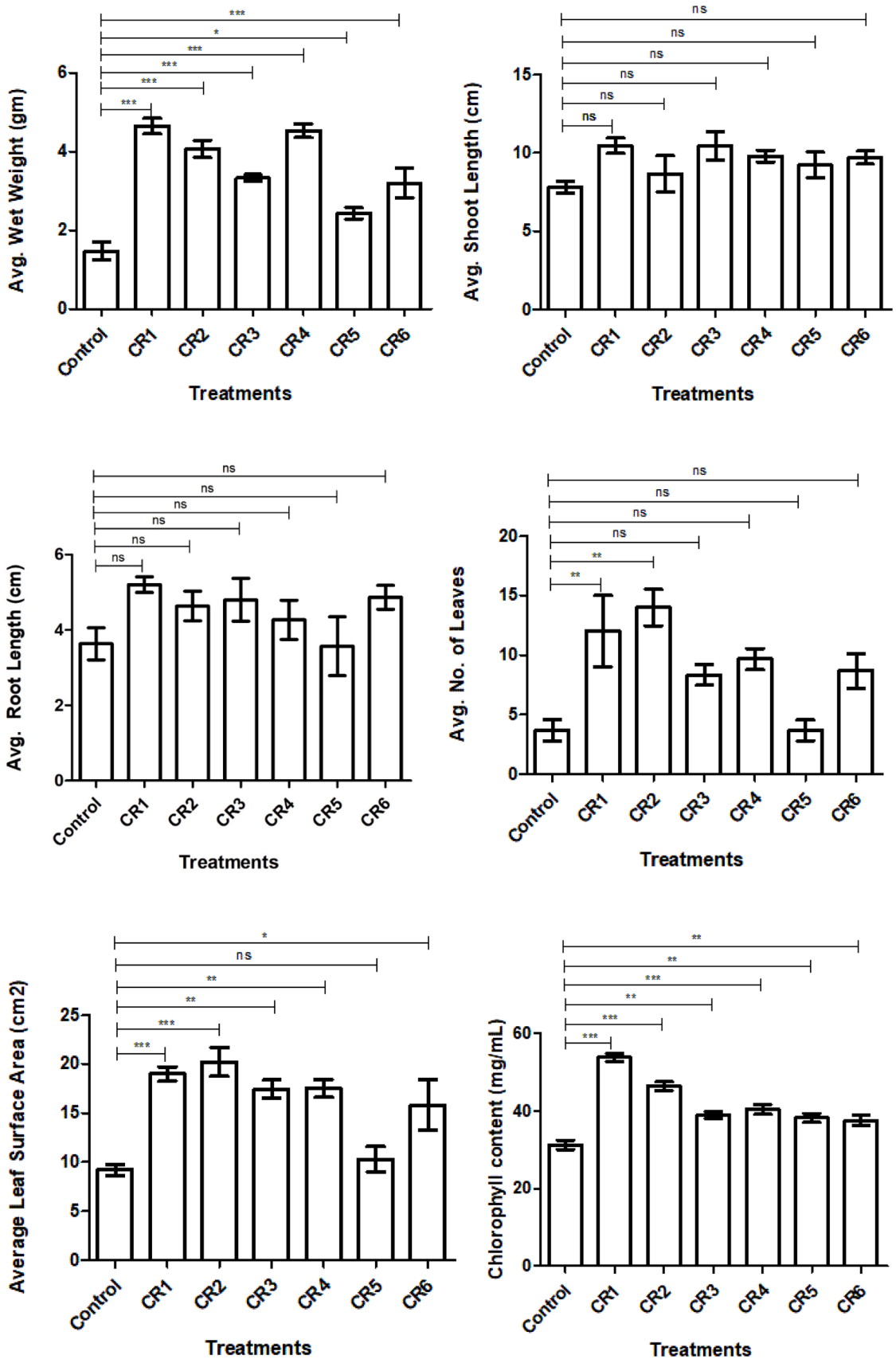

Fig. 4. *In-vivo* test of drought-tolerant rhizospheric isolates for plant growth promotion. Data shown are mean ± standard deviation of fresh weight, shoot length, root length, number of leaves, leaf surface area and Chlorophyll content. Each value is the mean of triplicates. Data were analyzed using a two-way analysis of variance, and treatment means were compared. Strick sign (*) indicated a significant difference ($P < 0.05$).

improve soil structure and aggression by retaining the water. Bacterial exopolysaccharides also play an important role in improving abiotic stress due to heavy metals in the soil, enhancing plant growth and crop yield (Shreshtha et al., 2024; Bhagat et al., 2021). For drought stress conditions, Indole compound production plays a critical role in root development and elongation as they act synergistically to control the primary roots and root hairs but function antagonistically in lateral root formation, and more extensive roots will help in accessing more water and nutrients (Hu et al., 2021a). The isolates in this study are tested to produce at different levels, even crossing 100 μg/ml. These signify their potential to develop the growth of roots even under stressful conditions. Hu et al. (2021b) showed how auxin and nitrate signaling pathways interact with local and systematic root system architecture, which is required to improve nitrogen usage and agricultural productivity. Phosphate and zinc are the essential nutrients generally immobilized in the soil. A total of 27.66 % of isolates and 15.47 % of isolates could solubilize phosphate and zinc, respectively. This solubilization is vital in drought conditions as the presence of nutrients has been limited in solubilization activities (Breitkreuz et al., 2020). Siderophores are used for important strategies by microbial organisms as they form complexes with Fe and enhance their solubilities required for the uptake under iron-deficient conditions (Rajkumar et al., 2017). A total of 13 % of bacterial isolates produced siderophores. Drought-resistant bacteria are generally observed to produce high amounts of siderophores (Arzanesh et al., 2010). Additionally, 11 % of the isolates are associated with necessary ammonia production as they provide nitrogen for plant growth. Ammonia-producing bacteria and nitrogen content in the soil also improves soil fertility, enhance root development, alkaline micro-environment required for phosphate solubilization, and indirect pathogen suppression (Alori et al., 2017).

The current plant growth promotion experiment using *Capsicum annum L.*(chili) showed the potential of the isolates to enhance plant growth under drought conditions. PGPR-treated plants exhibited up to 43.11 % higher root length and 34.18 % higher shoot length than the control length. These bacteria improve the nutrient uptake rate and stimulate root elongation required for water absorption under drought stress. Similarly, these bacteria enhanced 71.88 % of the chlorophyll content in the treated plants, confirming improved photosynthetic efficiency, which is involved in nutrient availability and stress mitigation by PGPR. Along with this, enhanced photosynthesis causes better biomass accumulation under drought stress. PGPR is a cost-effective and important approach to mitigating the negative impact of drought stress and improving agricultural productivity in the dryland (Gowtham et al., 2022).

The expression of plant-promoting activities in bacteria under drought stress can vary significantly among different strains, reflecting their adaptive mechanisms to environmental challenges. Research indicates that PGPR enhances drought tolerance in plants through multiple biochemical pathways. For instance, PGPRs such as *Bacillus, Pseudomonas*, and *Klebsiella* produce indole-3-acetic acid (IAA), which has been linked to improved root development and nutrient uptake during water scarcity (Chieb et al., 2023). Additionally, these bacteria can solubilize phosphates and produce exopolysaccharides, aiding in moisture retention in the soil and bolstering plant resilience against drought conditions (Ahmad et al., 2022). Studies have shown that the physiological responses of plants to PGPR inoculation under drought stress include increased antioxidant enzyme activity and osmotic adjustment through the synthesis of compatible solutes (Khan et al., 2019; Al-Turki et al., 2023). For example, inoculation with IAA-producing PGPR enhances root architecture, crucial for water absorption during drought (Chieb et al., 2023). Furthermore, the expression of beneficial traits may be influenced by environmental factors, leading to variability in performance among different bacterial strains under drought conditions (Rafedzi et al., 2024). Understanding these specific responses is vital for selecting effective PGPR for sustainable agricultural practices to enhance crop resilience in arid environments. This variability underscores the importance of continued research into the mechanisms by which PGPR interacts with plants to mitigate the adverse effects of drought stress.

The findings of this study have significant implications for sustainable agriculture, particularly in arid and semi-arid regions. The identified bacterial isolates, with their potential to be developed into biofertilizers, offer a promising outlook for enhancing crop productivity and resilience. This emphasis on crop productivity instils a sense of optimism about the future of their agricultural yields. Future research should focus on large-scale field trials to validate these findings in natural conditions and assess the long-term impact on soil health and plant productivity. Moreover, exploring the genomic and proteomic profiles of these bacterial strains could provide deeper insights into the mechanisms underlying their drought tolerance and plant growth-promoting activities. This knowledge could lead to the development of more effective bio-inoculants tailored to specific crops and environmental conditions. In conclusion, the successful isolation and characterization of drought-tolerant, plant growth-promoting bacteria from *Opuntia Ficus-Indica* cactus plants present a promising avenue for sustainable agriculture. Applying these bacteria as bio-inoculants can significantly mitigate the adverse effects of drought, ensuring food security and promoting sustainable farming practices in arid and semi-arid regions.

Conclusions

This study successfully isolated and characterized plant growth-promoting rhizobacteria (PGPR) from the rhizosphere of *Opuntia ficus-indica* cactus plants in the semi-arid Shankargarh region of Prayagraj, India. The identified bacterial strains exhibited significant drought tolerance and various plant growth-promoting (PGP) characteristics, such as ammonia production, phosphate solubilization, indole-3-acetic acid (IAA) production, and exopolysaccharide synthesis. Notably, genera like *Bacillus* and *Pseudomonas* dramatically enhanced growth, including the number of leaves, wet weight, shoot length, root length, and leaf surface area in pot trials with chilli plants compared to control plants. These findings underscore the potential of these drought-tolerant PGPR as bio-inoculants to improve crop resilience and production in water-stressed environments, presenting a promising approach for sustainable agriculture in arid and semi-arid regions. However, several limitations should be acknowledged. The research was conducted in a specific semi-arid region of Uttar Pradesh, India, which may restrict the generalizability of the findings to other geographical areas with different soil compositions and climatic conditions. Although 246 bacterial strains were isolated, only 16.6 % exhibited drought resistance and growth-promoting traits, raising questions about the potential diversity of PGPR in other plant species or environments not explored in this study.

Furthermore, the experiments were conducted under control conditions that may not fully replicate field scenarios where multiple environmental factors can influence PGPR efficacy. While various biochemical tests assessed the traits of the isolated strains, further studies are needed to elucidate the specific mechanisms through which these PGPR exert their beneficial effects on plant growth under drought stress. Future research should focus on broader geographic sampling to identify additional PGPR with effective drought resistance traits across diverse environments. Conducting large-scale field trials is essential to validate the effectiveness of these identified PGPRs in real agricultural settings while assessing their impact on crop yield and resilience under varying drought conditions. Investigating the molecular mechanisms underlying the interactions between PGPR and plants will provide deeper insight into how these bacteria promote growth and enhance stress tolerance. Additionally, exploring the potential application of these PGPR in sustainable agricultural practices could be particularly beneficial in developing drought-resistant crop varieties that thrive in water-scarce environments. This study contributes significantly to understanding and applying PGPR in enhancing plant resilience against

drought stress by addressing these limitations and outlining future perspectives.

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CRediT authorship contribution statement

Kumar Shreshtha: Data curation, Methodology, Writing – original draft. **Aman Prakash:** Data curation, Methodology. **Prashant Kumar Pandey:** Data curation, Methodology. **Arun Kumar Pal:** Data curation, Methodology. **Jyotsna Singh:** Data curation, Methodology. **Pooja Tripathi:** Conceptualization, Formal analysis, Supervision. **Debasis Mitra:** Validation. **Durgesh Kumar Jaiswal:** Writing – review & editing. **Sergio de los Santos-Villalobos:** Writing – review & editing. **Vijay Tripathi:** Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

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Data availability

Data will be made available on request.

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