RESEARCH ARTICLE

Isolation and Characterization of Drought-tolerant Rhizobacteria from Arid Regions of Central Kanpur, Uttar Pradesh, and its Growth-promotional Effects on *Phaseolus vulgaris*

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Abstract

Abiotic elements, including drought, have a significant impact on agricultural productivity worldwide. Bioinoculants consisting of rhizobacteria have been proposed as an environment friendly, beneficial method to strengthen crop resistance to drought stress. The current study involves the isolation of rhizobacteria from arid and semi-arid regions of Kanpur Dehat, Uttar Pradesh and tested for drought tolerance at different concentrations of polyethylene glycol (PEG)-6000. Three bacterial isolates with maximum drought tolerance were characterized and identified molecularly, which include *Pseudomonas sp.* strain FD-37/23, *Exiguobacterium aurantiacum* strain GBRS02 and *Microbacterium paraoxydans* strain HZLJC2-1. Bacterial strains were screened for PGPR traits such as siderophore, indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, ammonia production, hydrogen cyanide production, and phosphate solubilization. *Exiguobacterium aurantiacum* strain GBRS02 and *Pseudomonas sp.* strain FD-37/23, exhibited maximum drought tolerance of (0.81 ± 0.09) and (0.82 ± 0.08) while *Microbacterium paraoxydans* strain HZLJC2-1 showed drought tolerance of (0.69 ± 0.03) at -0.73 Mpa water potential. These strains were selected for bio-inoculation for bean plant. The bacterial inoculation resulted in significant increment in plant height, Relative water content (RWC), proline and other growth parameters. These findings may provide insight into the strains' potential application as rhizobacteria that stimulate plant development, either alone or in combination, which might aid local crops grow in drought-stressed environments.

Highlights

- Drought tolerant plant growth promoting rhizobacteria (PGPR) were isolated from semi- arid and arid regions.
- Key isolates identified were *Pseudomonas sp.* strain FD-37/23, *Exiguobacterium aurantiacum* strain GBRS02 and *Microbacterium paraoxydans* strain HZLJC2-1
- Strains exhibited key PGPR traits, including siderophore production, IAA synthesis, ACC deaminase activity, HCN production, and phosphate solubilization.
- *Exiguobacterium aurantiacum* and *Pseudomonas sp.* showed the highest drought tolerance of 0.81 ± 0.09 and 0.82 ± 0.08 at -0.73 MPa water potential, respectively.
- Bioinoculation of these strains in bean plants significantly enhanced plant height, RWC, proline levels, and other growth parameters.

Keywords: Polyethylene glycol, Siderophore, Phytohormone, Indole acetic acid, Drought tolerant bacteria.

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Introduction

It is imperative that the effects of abiotically caused water stress on plant development and crop productivity receive immediate attention on a global scale in order to prevent future food shortages. Severe droughts are caused by the constant reduction in the amount of water needed for plant consumption in drought regions of the world. Water stress reduces the growth of plants, development of roots, photosynthetic rate, and nutrient assimilation by adversely affecting the biochemical, physiological, and morphological processes of the plant (Ghadirnezhad *et al.,* 2023). Reduced water availability as a consequence of climate change has been connected to a decrease in plant production. The second biggest obstacle to the production of common beans (*Phaseolus vulgaris L.*), after disease, is drought (Sofi *et al.,* 2021). Researching plant-growth-promoting rhizobacteria (PGPR) that can withstand drought appears to be a promising method for improving plant growth in regions with stunted water regimes. The potential of PGPR in relation to inducing drought resistance of tomato, soybean, wheat, maize, and common bean has been confirmed by numerous studies (Gowtham *et al.,* 2020, Danish *et al.,* 2020,

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Camaille *et al.,* 2021). PGPRs exhibit the rhizospheric regions of the soil along with the surfaces of roots, and surrounding of the plant roots. They can either directly or indirectly improve the crop yield and quality. The close connection that exists between rhizospheric microorganisms and host plants may impact the response of plants to abiotic or biotic stimuli. The small area of the soil environment known as the rhizosphere is directly impacted by the secretions of root exudates. Direct mechanism entails either enabling specific plant nutrients to be taken up from the soil environment or sensitizing plants with substances synthesized by bacteria, while indirect mechanism involves PGPRs to reduce or prevent the negative effects of phytopathogens (Bouremani *et al.,* 2023). The bacteria also facilitate the alteration of root architecture during droughts, which contributes to the cycle of nutrients along with helping with the intake of nitrogen and phosphorus. Moreover, one of the remarkable effects of PGPR on crops has been shown to be the inhibition of leaf senescence during drought conditions and the regulation of plants' ethylene levels through the formation of ACC deaminase (Shahid *et al.,* 2023).

There are several PGPRs that can synthesize phytohormones and stimulate plant growth and proliferation as well as promote tolerance against abiotic stresses (Etesami and Maheshwari, 2018). Several crop plants face extreme stress due to the severe lack of water, and one of the main causes of significant losses in crop productivity worldwide (Junaid and Gokce, 2024). The utilization of PGPRs has been the best technique of inducing drought stress resistance in plants due to its exceptionally low input costs and environmentally friendly nature, thus it is gaining attention from all over the world to manage drought stress in arid regions. Our research offers a fresh perspective of using three potent rhizobacteria, *Pseudomonas sp.* strain FD-37/23, *Exiguobacterium aurantiacum* strain GBRS02 and *Microbacterium paraoxydans* strain HZLJC2-1 isolated from arid regions, by evaluating their capacity to tolerate drought and to promote plant growth, and by offering suggestions for further research aimed at reducing the negative impacts of water stress on plant growth and productivity.

Materials and Methods

Isolation of Indigenous rhizobacteria from drought lands

A saline solution (0.9% NaCl) 9.0 ml was added to 1.0 gram of soil to isolate rhizospheric bacteria. Dead bacterial cells, stones, dirt, and debris were removed from the soil suspension by vortexing it for five minutes. Fresh roots were cleaned with distilled water to isolate the bacteria that adhere to roots followed by surface sterilization of roots in 70% ethanol for three minutes to isolate endophytic bacteria. After that, roots were washed with 2% sodium hypo chlorate for two min and then with distilled water. Homogenized the root sample to make the solution. The serial dilution of root washing solutions, soil suspension and homogenized roots solution was done in a sequential manner (10^{-1}–10^{-6}). Inoculated media were incubated at a suitable temperature with 100 µL of the supernatant from each dilution of admixture (Tsegaye *et al.,* 2019)

Screening drought stress tolerance of rhizobacterial isolates

The isolated rhizobacteria were tested for drought resistance at various water metric potentials (-0.05, -0.15, -0.30, -0.45, and -0.73 MPa) in Tryptone Soya Broth (TSB) obtained by adding PEG-6000 in an adequate amount (5, 10, 15, 20, and 25%) (w/v) accordingly (Sandhya *et al.,* 2009). The overnight-grown cultures of rhizobacterial isolates with a cell density of 1×10^7 cells mL^{-1} was deployed as primary inoculum. Upon incubation for 24 hours at 30 ± 2˚C and 120 rpm, the absorbance was taken at 600 nm through a UV-visible spectrophotometer (117, Systronic, Ahmedabad, India) to measure rhizobacterial growth at different PEG concentrations (Grover *et al.*, 2014).

Morphological and biochemical characterization of drought-tolerant rhizobacterial isolates

Morphological characterization of each isolated strain was accomplished on a suitable media, after streaking all the isolates on appropriate media, followed by incubation for 24 to 48 hours. The distinctive colonies were purified and observed for identification and further study. The pure bacterial cultures were identified following Bergey's Manual of Determinative Bacteriology based on morphological characteristics and further evaluated for various PGPR attributes (Kloepper *et al.*, 1988). Gram staining of all the isolates was performed of and the Catalase test was done using the method of Taylor and Achanzar (1972), while the oxidase test was performed using the Gaby and Hadley (1957) method.

Plant growth-promoting attributes of the drought-tolerant rhizobacterial isolates through differential characterization method

Pikovskaya's medium was used to evaluate bacterial isolates for their ability to solubilize phosphate *in-vitro*. For seven days, the cultures were incubated at 30°C after being spot-inoculated on Pikovskaya's medium plates. A favorable outcome for phosphate solubilization was demonstrated by the clean zone surrounding the bacterial growth (Alaylar *et al.*, 2020). The isolated bacterial strains were inoculated in a minimal salt medium (MM9) and allowed to stand at 30°C for 2 to 3 days to perform the organic acid production test. The presence of pink color in the media is attributed to the synthesis of organic acid using methyl red as an indicator (Tsegaye *et al.*, 2019). Formation of indole acetic acid (IAA) was detected following the procedure of Bric *et al.,* (1991). The precursor of IAA, 100 mg/l tryptophan, was added to Luria Bertani (LB) broth to cultivate bacterial cultures, followed by incubation at 30°C for 5 days at 250 rpm. Completely populated cultures were centrifuged for 10 minutes at 10,000 rpm. Over 4 mL of the Salkowski reagent (35% of $HClO_4$, 50 mL and 0.5 M $FeCl_3$ of 1-mL) was added to the 2 mL of the supernatant. The appearance of pink color in the reaction mixture indicated the production of IAA. Quantification of IAA was done by following the method of Gutierrez *et al.*, (2009). Ammonia production in peptone water was measured according to Hayat *et al.*, (2013). The bacterial isolates (fresh culture) were incubated at 30°C for 48 hours in 10 mL of peptone water then 0.5 mL of Nessler's reagent was mixed in every tube and a positive outcome for ammonia production was confirmed by the appearance of a

brown to yellow color in the test tubes. HCN production was done by screening bacterial isolates through the methodology described by Castric, (1975). The nutritional medium plate with 4.4 g of glycine/L^{-1} was used to inoculate the isolates by placing a filter paper (Whatman, number one) soaked in a solution (2% sodium carbonate and 0.5% picric acid) on top of the plate and sealed with parafilm. After 4 days of incubation at 30°C, the filter paper's color changed from yellow to brown, signifying the formation of HCN (Schippers *et al*., 1987). Siderophore production by PGPRs was checked by the method given by Schwyn and Neilands, (1987). Spot inoculation of the fresh cultures onto Chrome azurol S (CAS) agar plates and incubated for 3 to 5 days at 30°C. Orange haloes surrounding bacterial colonies verified the production of siderophores. To test for ACC deaminase activity, the methodology described by Gupta and Pandey (2019) was followed. Every isolate was cultured for 24 hours at 28° C in 5 mL of TSB while shaking at 120 rpm. The cultures underwent a 5 minutes centrifugation at 3,000 rpm followed by washing the pellet with Tris-HCl twice (0.1 M, pH 7.5), then the same solution (1-mL) was used to resuspend the pellet, and spot-inoculated onto Petri plates that were supplemented with modified Dworkin and Foster (DF) minimal salt medium (KH_2PO_4, 4.0 g, $MgSO_4$.$7H_2O$, 0.2 g; Na_2HPO_4, 6.0 g, glucose 0.2 g, gluconic acid 0.2 gram citric acid 0.2 g, micronutrient solution (10 mg H_3BO_3, 1 mg $FeSO_4.7H_2O$, 124.6 mg $ZnSO_4.7H_2O$, 78.22 mg $CuSO_4$, 11.19 mg $MnSO_4.H_2O$, 10 mg MoO_3 in 1,000 mL distilled water) (pH 7.2) with 3 mM ACC as the only nitrogen source (Dworkin and Foster, 1958; Penrose and Glick, 2003). Plates containing 0.2% w/v of $(NH_4)_2SO_4$ served as the (+) control, whereas plates containing only DF minimum salts medium served as the (-) control. Incubation of the plates lasted 3 days at 28°C. The bacterial growth on ACC-supplemented plates was examined in addition to the negative and positive controls.

16 S rRNA gene amplification, sequencing and phylogenetic analysis

Whole genomic DNA was extracted from selected isolates using the CTAB (cetyltrimethylammonium bromide) procedure, and the results were confirmed by 1% agarose gel electrophoresis. The 16S-rRNA fragment was amplified using high–allegiance PCR polymerase. For the amplification of the 16S rRNA region, forward primer (5′ GGATGAGCCCGCGGCCTA 3′) and reverse primer (5′ CGGTGTGTACAAGGCCCGG 3′) were used (Weisburg *et al*., 1991). Each 1-µL of the forward and reverse primers, 2 µL of the cell lysate template, 1-µL of DI water, and 5 µL of the master mix ($MgCl_2$, buffer, and Taq) make up approximately 10 µL of the PCR reaction. The conditions under which the PCR was conducted were as follows: a 5-minute initial denaturation at 94°C; 40 cycles of 1 minute at 95°C, 1-minute at 55°C, 2 minutes at 72°C, and a 10-minute final cycle at 72°C. By using 1% agarose gel electrophoresis in TAE buffer and UV gel documentation, the end product's amplification was examined. Sequencing of 16S rRNA gene was carried out by Biokart India Pvt. Ltd. (Bangalore). Maximum Likelihood method and Tamura-Nei model were used to infer the evolutionary history (Tamura and Nei, 1993). MEGA 11 software was used to conduct the evolutionary analyses (Tamura *et al*., 2021). The NCBI sequences of organisms that are closely related to the newly discovered species were then retrieved and compared. Consequently, the sequences were sent to the NCBI, and every organism was assigned an accession number.

Pot Experiments

Selected bacterial strains were cultured on 100 ml of nutrient broth for 24 hours at 37°C (pH 7.3), (Hi Media, India). Fresh bacterial culture was centrifuged at 8000 rpm for 10 minutes and the pellet was resuspended in sterile distilled water (SDW) to get 1×10^8 colony-forming unit. 25 mL of bacterial culture was added with 100 mg of carboxy methyl cellulose (CMC) to mediate the adherence of bacteria on seed surface. The surface-sterilized seeds with 1% sodium hypochlorite (NaOCl) solution were soaked in bacterial cultures containing CMC and incubated 24 hours on a shaker at 150 rpm. Thereafter, seeds had been aseptically dried in laminar air flow (LAF) and used for the experiment. Bean seeds inoculated with three isolated drought-tolerant strains and uninoculated control seeds were sown in the plastic pots (5 inches) containing 150 g of sterilized potting soil. Drought conditions were maintained by providing water up to 30% of the field capacity. Three treatments with three different strain coated seeds were T1, T2, and T3, and one treatment was with control plants under well-water (WW) and water-stressed (WS) conditions. T1 is coated with bacterial strain BK S2, T2 is coated with BK S3, and T3 is coated with UK S1. The soil utilized throughout the experiment was collected from the garden of Babasaheb Bhimrao Ambedkar University, Lucknow.

Assessment of plant growth properties under drought stress

The growth parameters of bacteria coated seeds of bean plants such as plant height (cm), relative water content (RWC), proline and lipid peroxidation, catalase and electrolyte leakage under well- water and water-stress condition were carried out using different techniques. The RWC of the test samples were conducted according to the method of Wang *et al.,* (2015). The proline accumulation in fresh bean leaves were tested following the acid-ninhydrin method given by Bates *et al.,* (1973). The lipid peroxidation was tested in terms of malondialdehyde (MDA) in the samples as an end product according to the method given by Davenport et al. (2003). Electrolyte leakage (EL) and catalase activity were determined as described in Haque et al. (2020).

Data Analysis

ANOVA (Analysis of variance) was used to test the significant differences of measurements at $p \leq 0.05$. Values are presented as Mean ± Standard Deviation (SD)

Results

The soil used for bacterial isolation was collected from root-free soil of arid regions of Kanpur Dehat, Uttar Pradesh, India. The soil characteristics were pH (8.17 ± 0.03), EC (240 ± 10.6 µS/cm), available N (23.5 ± 1.5 mg/kg), available P (19.3 ± 0.3 mg/kg), available K (17.17 ± 0.15 mg/kg), organic C (0.74 ± 0.03%). The bacteria were isolated from soil samples on a nutrient agar media (NAM) plate. All the isolates were tested for drought tolerance following 24 h incubation. A total 6 non-identical isolates were tested at different PEG concentrations, out of which 3 isolates revealed maximum drought tolerance at –0.73

Table 1: Drought stress tolerance of different bacterial strains at- 0.05–0.15MPa, -0.30–0.45 MPa and -0.73MPa water stress tolerance, (Mean ± standard deviation, n=3)

OD (600 nm) at PEG conc.		*Control*	*5% PEG*	*10% PEG*	*15% PEG*	*20% PEG*	*25% PEG*
S. no	*Bacterial strain*						
1	BK S2	2.06 ± 0.06	1.86 ± 0.09	1.70 ± 0.10	1.1 ± 0.1	1 ± 0.17	0.81 ± 0.09
2	BK S3	1.72 ± 0.02	1.37 ± 0.05	0.91 ± 0.07	0.88 ± 0.01	0.73 ± 0.04	0.69 ± 0.03
3	AK S3	1.72 ± 0.11	1.37 ± 0.12	0.99 ± 0.09	0.73 ± 0.06	0.56 ± 0.034	0.32 ± 0.08
4	UK R5	1.12 ± 0.04	0.82 ± 0.06	0.61 ± 0.06	0.53 ± 0.03	0.48 ± 0.02	0.44 ± 0.05
5	UK S1	2.09 ± 0.09	1.91 ± 0.15	1.43 ± 0.15	1.17 ± 0.11	0.92 ± 0.06	0.82 ± 0.08
6	BU R3	2.06 ± 0.1	1.7 ± 0.27	0.80 ± 0.13	0.71 ± 0.16	0.58 ± 0.07	0.49 ± 0.08

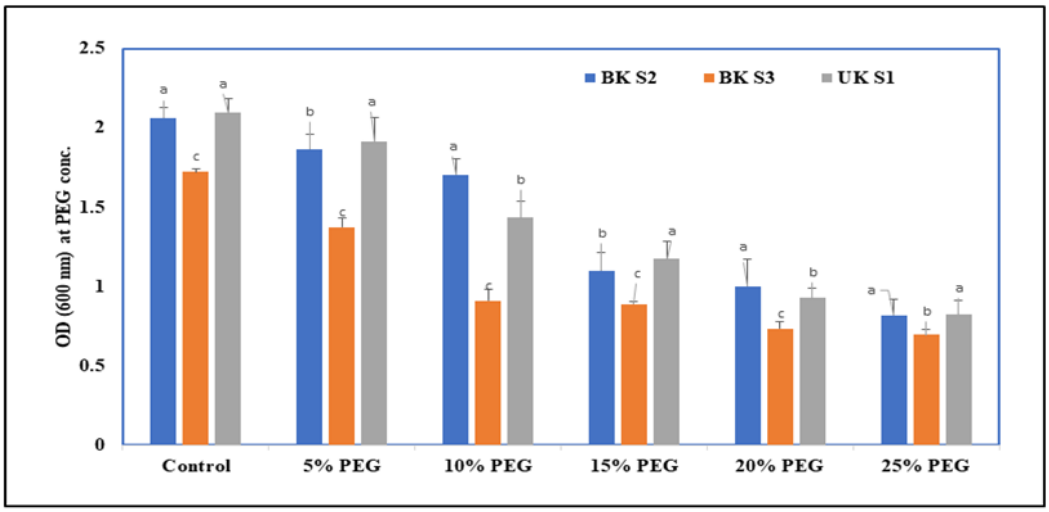

Fig. 1: Growth of the three most tolerant strains under non-stressed (control) and drought-stressed conditions (at 5%, 10%, 15%, 20% and 25% PEG concentrations)

MPa water potential (25% PEG). Their drought stress tolerance is ascribed in Table 1. The drought stress tolerance plot of 3 best strains (BK S2, BK S3, UK S1) is given in Fig 1.

The biochemical and morphological characterization of all the drought-stress tolerant stains were carried out. Two strains were found Gram's positive; one isolate was gram's negative. All strains were catalase positive, two were oxidase positive and one was negative. Their biochemical and morphological characteristics are summarized in Table 2. All eight strains were further characterized for their PGPR traits and were marked positive for phosphate solubilization, organic acid, ammonia production, HCN production and siderophore. Two strains were positive for ACC deaminase synthesis. Quantitative analysis of IAA production showed that bacterial strains that were incubated in LB broth with and without L-tryptophan at 30°C for 48 hours exhibited a difference in IAA production. A low amount of IAA was produced by all three strains without the addition of tryptophan, whereas IAA production was enhanced after adding 200 µg/mL tryptophan to the nutrient broth.

The three most drought-tolerant strains were chosen for 16S rRNA sequencing and data analysed by the BLAST search tool and the non-redundant database. The identity of the tested isolates was validated by phylogenetic analysis of the 16 S rRNA gene sequence of all three bacterial isolates from related bacterial species, and clustering at the genus level was detected. The sequences of identified bacterial isolates were submitted to the NCBI database. The 16S rRNA gene sequence of Bacterial strain BK S2 had maximum similarity to the sequence of *Exiguobacterium aurantiacum* strain GBRS02. Bacterial strain BK S3 showed maximum similarity with *Microbacterium paraoxydans* strain HZLJC2-1 and bacterial strain UK S1 showed maximum similarity with *Pseudomonas sp.* strain FD-37/23. The GenBank/EMBL/DDBJ accession number for the isolates are MT373550.1., MT605416.1 and PP906200.1, respectively (Fig 2).

Bacterized bean seedlings recorded significant enhancement in plant growth parameters under drought stress conditions compared with uninoculated controls, such as plant height, relative water content (RWC), proline, and catalase (Fig. 3). A corresponding significant decrease in MDA content and electrolytic leakage (EL) was also observed. An increase of approx. 55- 60% in plant height was recorded for bacteria-treated (T1, T2, and T3) bean plants, as compared to untreated

Table 2: Morphological, biochemical and PGPR characteristics of drought-tolerant rhizobacterial isolates

Bacterial strains		*BK S2*	*BK S3*	*UK S1*
Colony morphology	Form	Circular	Circular	Circular
	Color	Yellow	Orange	Cream
	Appearance	Rough	Slimy	Slimy
Cell-morphology	Gram's stain	+	+	_
	Shape	Rod	Rod	Rod
Biochemical characteristics	Catalase	+	+	+
	Oxidase	_	+	+
Phosphate solubilization		+	+	+
Organic acid production		+	+	+
IAA produced without tryptophan (µg/ml)		3.36 ± .04	1.2 ± .08	7.8 ± .1
IAA produced with tryptophan (µg/ml)		21.1 ± .1	13.4 ± 1.3	33.8 ± 0.9
Ammonia production		+	+	+
HCN production		+++	++	++
Siderophore		+	+	+
ACC deaminase		+	_	+

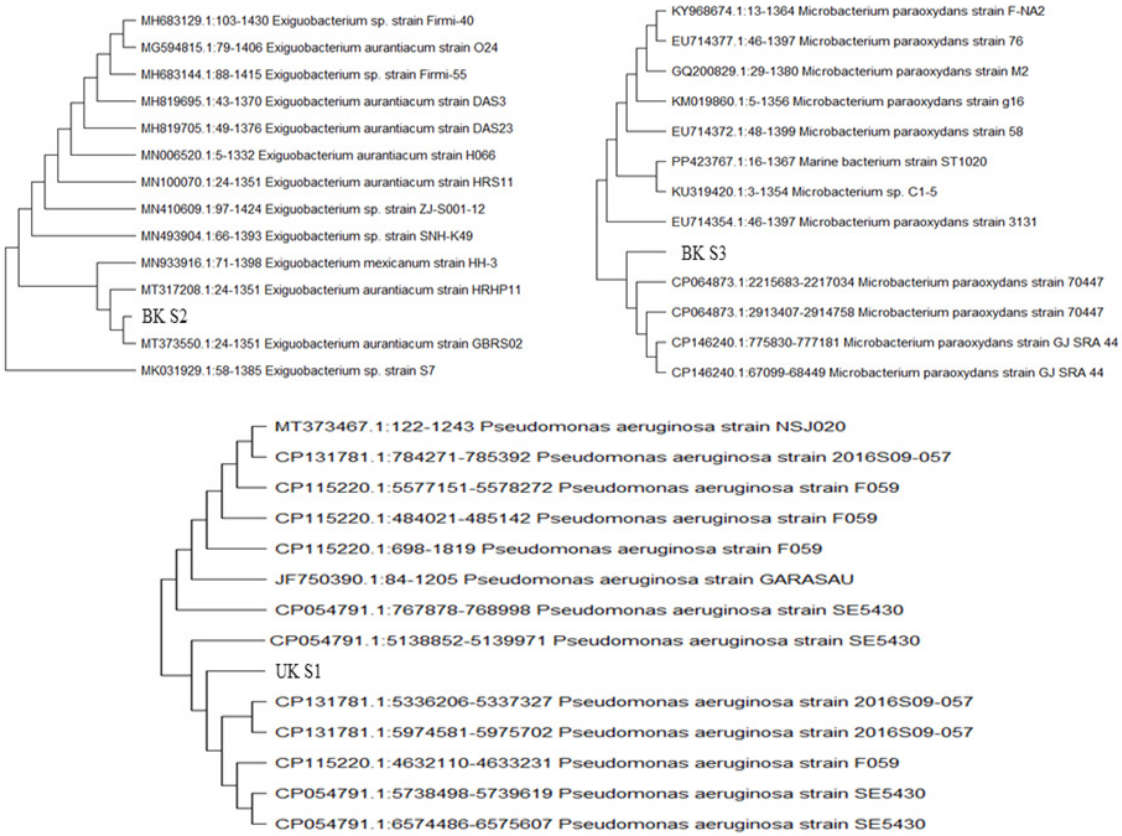

Fig. 2: The phylogenetic tree of three most tolerant PGPR stains, BK S2, BK S3, UK S1. Evolutionary analysis was conducted by using the software MEGA 11

controls under stress conditions. In comparison to control plants, the plants treated with bacteria showed enhanced RWC irrespective of the imposition of drought. Comparing treated bean plants (T1, T2, T3) grown under drought stress to their control plants, an approximate 20–30% increase in RWC was seen. Plant leaves frequently accumulate proline in response to

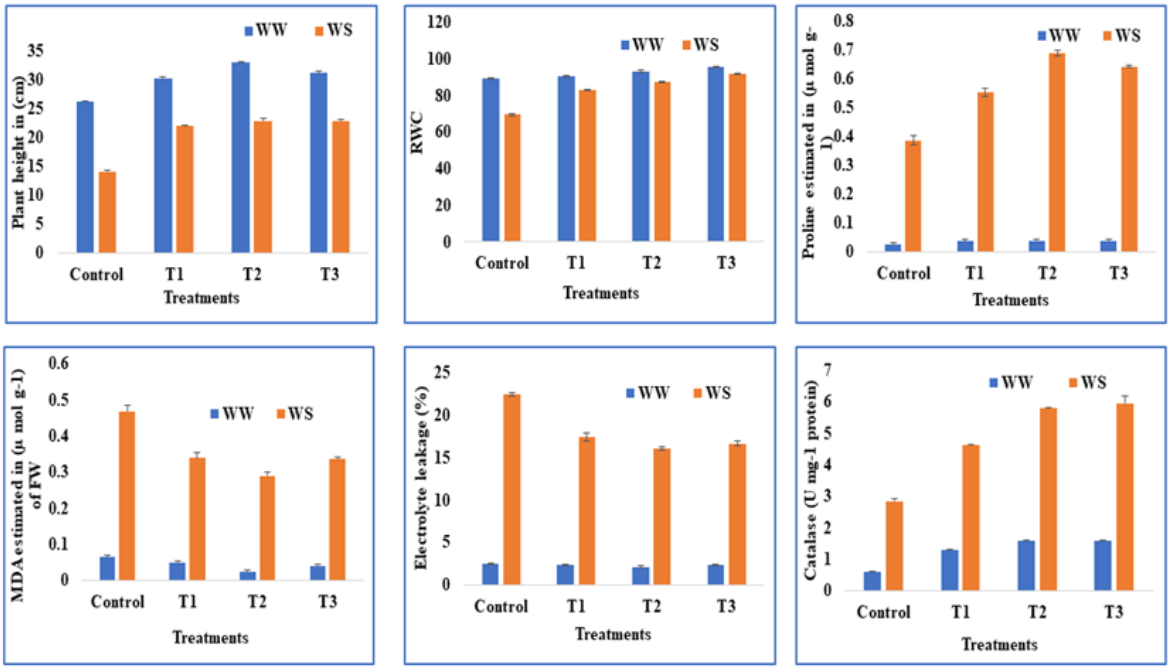

Fig. 3: Growth parameters of bean plants with and without bacterial treatments (Control, T1, T2, T3) under well water (WW) and water stress conditions (WS)

water stress in order to regulate the osmotic pressure. Proline also functions as an antioxidant and enzyme stabilizer. The proline content increased significantly by about 40 to 80% in bacterial treatments (T1, T2, and T3), compared to control plants, under drought stress conditions. The amount of MDA serves as a measure of oxidative damage and illustrates the lipid peroxidation of membrane lipids. Regardless of the treatments, the MDA accumulation was considerably increased under water stress conditions compared to well water conditions. But a significant decrease of about 20 to 36% in MDA content in treated bean plants was observed compared to stress-induced control plants. Another plant-protecting enzyme that was significantly altered by rhizobacteria treatment is catalase (CAT). Catalase activity was found to increase by 40 to 56% with inoculation of bacterial treatments T1, T2, and T3. A measurement of the amount of dead cells is called electrolyte leakage (EL), which increases as the severity of drought increases, irrespective of treatments. The EL in uninoculated plants under water stress conditions was very high, but a decrease of 20 to 25% was found in inoculated plants, respectively. The outcomes confirmed the advantage of treating seeds with drought-tolerant rhizobacteria in terms of controlling oxidative stress.

Discussion

The discrete areas of microbial interactions with the roots of the plants are represented by the rhizosphere which is a home to a large number of PGPR, and research has shown how to best utilize these microbes for crop production and sustainable agriculture. One major factor influencing rhizobacteria's ability to promote plant growth is their capacity to survive and establish a successful colonization of the plant root. Moreover, effective plant root association and colonization by PGPR is essential for stimulating growth irrespective of their mechanism of action. Thus, it is necessary to screen for drought tolerance of PGPR and ensuring its abundance in stress condition to assess their effectivity and reduce water stress in plants. *Pseudomonas, Microbacterium paraoxydans* and *Exiguobacterium aurantiacum* have been discovered with PGPR properties in several researches (Nawaz *et al.*, 2020, Kaur and Sharma, 2013, Mandal *et al.,* 2024). Thus, more research is required to establish the possible benefits of rhizobacteria strains being introduced to plants in both greenhouse and field experiments, both alone and in combination. The identification of PGPR with plant-growth-promoting characteristics from the genera *Bacillus, Microbacerium*, and *Pseudomonas* has been reported, and it has demonstrated a high potential for encouraging plant development in bean plants (Vejan *et al.,* 2016). The results of the current study also confirm the biostimulatory ability of rhizobacteria to improve plants in a greenhouse during drought stress. All three strains have demonstrated strong growth at 25% PEG-mediated high drought resistance, reducing the impact of water stress on the bacterial-treated bean plants and promoting growth. Arshad *et al.,* (2007) reported that ACC deaminase containing PGPR helps plants to resist oxidative stress by hydrolysing ACC into ammonia and α-ketobutyrate and reducing the amount of ethylene (stress hormone) produced. It is already known that these ACC-deaminase-producing bacteria promote plant growth, especially under stressful circumstances including heavy metal exposure, flooding, excessive salinity, and drought. In this investigation, two discovered bacteria tested positive for ACC deaminase. It is commonly recognized that the PGPR promotes plant growth through symbiotic

relationships. Rhizobacteria inoculation generally improves plant development through a variety of processes, including nitrogen fixation, ACC deaminase activity, the synthesis of Indole Acetic Acid, siderophores, and other growth-promoting characteristics (Oleńska *et al.,* 2020). Plants can increase their uptake and retention of water and nutrients by changing the structure of their roots through the synthesis of phytohormones (Jia *et al.,* 2022). Pot experiments have demonstrated that the growth features displayed by the isolates from the genera *Exiguobacterium, Microbacterium*, and *Pseudomonas* contributed significantly to the plant growth in comparison to the control, in the *in-vitro* growth assessment. The method depends on the interchange of substances between the microorganisms and the plant, which includes improved nutrient releases, cycling, organic matter breakdown, and the development of systemic tolerance against abiotic and biotic challenges. Previous studies by Kour et al. (2020) and Chandra et al. (2020) have shown how rhizosphere microorganisms may be used to induce drought tolerance in plants. The improvement in bean plants was seen in line with previous findings that advocates the utilization of rhizobacteria alone or in combination with other agents to influence physiological alteration and enhance crop yield. Therefore, especially in semi-arid or dry environments, plant treatment with drought-resistant rhizobacteria that have several growth-enhancing traits can increase the efficacy of the plant growth. Numerous PGPR strains have been reported to promote plant growth and resilience to various abiotic and biotic stress conditions such as *Azotobacter, Pseudomonas, Azospirillum, Bacillus,* and *Klibsiella* (Meena *et al.,* 2020). Sandhya *et al.,* (2010) reported using rhizobacteria from *Pseudomonas* species as biostimulants to enhance maize growth through nutrient absorption and bioavailability under drought stress. Dastager *et al*., (2010) characterized *Exiguobacterial* strain and reported its plant growth promotion in cowpea. A different investigation, shown a considerable increase in bean growth and production by freshly identified B. thuringiensis from Pakistan (Siddiq *et al.,* 2018). The rhizobacterium *Azospirillum brasilense* HM053 was found to boost maize growth, nutrients, and chlorophyll content by Scott *et al.,* (2020). The current study reported the benefits of rhizobacteria strains *Pseudomonas sp.* strain FD-37/23*, Exiguobacterium aurantiacum* strain GBRS02 and *Microbacterium paraoxydans* strain HZLJC2-1 for the growth of bean plants against water stress, which supports the findings of Steiner *et al.,* (2020). The study's findings demonstrated the coexistence of a bacterial inoculant with beans and the production of increased growth under drought stress. These findings suggested that *Pseudomonas sp*. strain FD-37/23, *E. aurantiacum* strain GBRS02, and *Microbacterium paraoxydans* strain HZLJC2-1 could potentially alleviate the effects of drought stress on bean plants grown in areas with limited water resources. The capacity of bacterial strains to endure and adapt to hostile environments may have contributed to the increased crop output following the seed inoculation.

Conclusion

The study's conclusions provide credence to the theory that certain plants, like beans, can demonstrate significant input in drought-tolerant plant adaptation. Thus, we suggest *that Pseudomonas sp., Exiguobacterium aurantiacum, Microbacterium paraoxydans,* and other drought-tolerant rhizobacteria be investigated further as bioinoculants for the management of water stress in bean and other food crops. The findings obtained can provide deeper insights into the effects of rhizobacteria on bean growth and offer theoretical support for the practical application of these strains in field experiments.

This bio-rational approach can significantly benefit the semi-arid and arid regions of developing nations worldwide, helping to alleviate or reduce food insecurity issues. However, further research on these bacterial strains is essential to evaluate their efficacy as potential candidates for biofertilizer formulation. Additionally, omics research holds great promise for elucidating the identity and precise mechanisms of these strains in a natural plant environment. This could pave the way for further exploration into strategies to enhance agricultural productivity, promote plant growth in drought-stricken areas, and ensure food security.

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Declaration of interest statement

There are no conflicts of interest between the work disclosed in this publication and the personal or financial relationships of any of the authors, as all the authors have confirmed.

Author Contribution

Shivangi Awasthi (First author)- Conceptualization, methodology, formal analysis, writing of original draft, investigation and visualization. Ashutosh Tripathi- Writing, review and editing, Devesh Vishwakarma- Writing, review and editing, Deepa Kannaujiya- Writing, review and editing, Dr. Rajeeva Gaur (Corresponding author)- Writing-review, editing and correction, Dr. Shikha (Corresponding author)- Writing-review, editing and correction.

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