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Enhancing Germination of Wheat Seeds under Drought Conditions by *Bacillus cereus* DS1 Isolated from the Rhizospheric Soil of *Celosia argentea*

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Abstract

Drought is a prolonged period of insufficient rainfall, leading to water scarcity that adversely affects both the natural environment and human endeavours. Water scarcity disrupts photosynthesis, resulting in underdeveloped, smaller plants, wilting leaves, reduced root growth, and, under severe conditions, plant death. Drought remains one of the most significant challenges to global food security, causing crop failures, rising food prices, and widespread malnutrition. The key objective of the present investigation was to isolate bacterial species capable of surviving drought conditions and to evaluate their plant growth-promoting potential. The *Bacillus cereus* DS1 strain was isolated from a soil sample collected from the rhizospheric region of the drought-tolerant plant *Celosia argentea*. Among the various tested osmotic pressure conditions, *Bacillus cereus* DS1 not only significantly tolerated 5% PEG 8000 (−0.47 MPa osmotic pressure) but also exhibited promising plant growth-promoting properties. The in vitro plant growth-promoting activity of the isolate was evaluated using various tests, including hydrogen cyanide, indole acetic acid, ammonia, and siderophore production; ACC deaminase activity; and phosphate solubilization and nitrogen fixation under both stress and non-stress conditions. Additionally, the effect of *Bacillus cereus* DS1 on seed germination was assessed, and a notable enhancement of 88% seed germination

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was observed in bioprimed seeds compared to the control (seeds primed with distilled water). Overall, the local isolate *Bacillus cereus* DS1 has the potential to withstand various levels of drought stress and enhance seed germination under drought conditions, indicating that it could serve as a valuable bioinoculant for improving crop yield in arid and semi-arid regions.

Abbreviations

PGPR	Plant Growth-Promoting Rhizobacteria
FAO	Food and Agriculture Organization of the United Nations
NB	Nutrient Broth
CSIR	Council of Scientific and Industrial Research
BLAST	Basic Local Alignment Search Tool
NCBI	National Centre for Biotechnology Information
MEGA	Molecular Evolutionary Genetics Analysis
PEG	Polyethylene Glycol
ACC	1-aminocyclopropane-1-carboxylic acid
HCN	Hydrogen Cyanide
ACS	1-aminocyclopropane-1-carboxylate synthase

Introduction

Anthropogenic activities and global warming have increased drought, which creates a serious challenge to worldwide food production.[1] Drought stress is a major abiotic factor that negatively impacts crop productivity, varying from brief and mild episodes to severe and extended conditions that significantly restricting crop yields.[2,3] Water stress impacts a plant's water potential and turgor pressure, disrupting normal cellular functions and leading to significant changes in its morphological and physiological characters.[4,5]

Water content is a key factor influencing plant growth and is largely impacted by drought conditions and the level of nutrients present in the soil. The transport and diffusion of water-soluble nutrients, carried out by water through the roots, are also impacted.[6,8] The drought stress in plants leads to an increased production of free radicals, which triggers oxidative damage such as lipid peroxidation, degradation of cellular membranes.[9,11] Such physiological impairments may drastically reduce crop productivity, with yield losses varying between 30% and 90% based on the plant species and the specific growth stage exposed to drought stress.[12]

In recent times, to keep up with the increasing demand for food and enhance agricultural productivity, a wide range of chemical pesticides and fertilizers have been employed.[13,14] However, the intensive use of chemicals can cause significant environmental damage, posing major ecological threats and creating serious risks to human health.[15,16]

Current strategies to enhance plant growth and productivity include traditional breeding techniques, genetic engineering, and priming approaches.[17,20] While these methods have shown considerable promise, each comes with its own set of challenges and constraints, ranging from lengthy development times and regulatory hurdles to inconsistent results under field environment.[21,22] Nowadays, Plant Growth-Promoting Rhizobacteria (PGPR) have arisen as a fascinating and effective alternative to traditional inputs, gaining recognition as biofertilizers in modern agriculture.[23] PGPRs are a diverse group of microorganisms includes *Bacillus*, *Pseudomonas, Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, and *Serratia*.[24] They display host specificity, indicating that their effectiveness can vary across different plant species or cultivars.[25] Selecting the appropriate species of PGPR tailored to each plant type is essential to maximize their positive impacts on plant growth and resilience to stress. Only the identified species can then be artificially inoculated in the rhizosphere of that plant along with the existing flora. Inoculating plants with PGPR in the rhizospheric

soil is one solution to counter the problem of drought stress for sustainable farming.[26] They can enhance plant development by either producing beneficial phytohormones—like gibberellins, auxins, and cytokinins—or by facilitating improved nutrient acquisition from the soil, including nitrogen, phosphorus, and essential micronutrients. These mechanisms not only boost root and shoot growth but also help plants better withstand environmental stresses. In addition to their direct growth-promoting effects, PGPR also support plant health indirectly by producing antagonistic compounds—such as antibiotics, siderophores, and hydrogen cyanide—that suppress harmful phytopathogens.[27,28] Numerous studies suggest that bacterial strains can promote plant growth even in drought-stressed conditions.[29,31] These bacterial strains enhance a plant's drought tolerance, making them particularly beneficial for cultivation in arid and semi-arid regions.[32]

This study aims to isolate a PGPR strain from the rhizosphere of *Celosia argentea*, which can enhance plant growth and increase survival rates under drought conditions. The *Celosia argentea* is an ornamental plant belonging to the Amaranthaceae family. *Celosia argentea* shows fast growth, is strongly adaptable, and has a high propagation rate,[33] but it is sensitive to drought stress. Drought significantly damages the cellular membrane of *Celosia argentea*,[34] but the plant selected for this study was well adapted to drought conditions and thrived in a dry area, drawing significant attention.

Materials and Methods

Soil Sample

The rhizospheric and surrounding soil from *Celosia argentea* was collected from Shelgaon village in Barshi tehsil, Solapur district, India. The plant was uprooted, and the adhered soil was removed and collected in a sterile container. The soil sample was subjected to analysis for various parameters: pH, electrical conductivity, organic carbon, calcium, magnesium, chloride, carbonate, bicarbonate, and other metals using standard methods of the FAO (2020).[35]

Isolation of Bacteria

The collected rhizospheric soil sample was inoculated in presterilized NB and then transferred to a nutrient agar plate. Morphologically distinct colonies were selected and transferred to nitrogen-free mineral agar plates with the following composition (gL^{-1}): glucose, 20.0; $FeCl_3 \cdot 6H_2O$, 0.025; $MgSO_4 \cdot 7H_2O$, 0.5; $Na_2MoO_4 \cdot 2H_2O$, 0.005; $CaCl_2$, 0.05; K_2HPO_4, KH_2PO_4, 0.2; 0.8; agar, 15; pH 7.0. The inoculated petri-plates were incubated at 30°C for 2 days. Bacterial colonies showing growth on the nitrogen-free medium were selected for further study.

Biochemical and Morphological Characterisation

The bacterial strain was identified based on basis of morphological and biochemical properties, determined by observing the bacterial colony and cell size, shape, spore formation, and Gram characteristics using Gram staining under 100x oil immersion on a Micron Optic MONO CXL microscope. Several biochemical tests were performed following Bergey's Manual of Determinative Bacteriology.

Identification of Bacteria

The bacterial isolate was identified through its morphological and biochemical properties. Molecular identification was achieved by sequencing the 16S rDNA, which was carried out at the National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory, Pune, India. The obtained sequence was analysed against database sequences using BLASTn, accessible through the NCBI server. Sequences showing the maximum similarity as determined by the BLAST analysis were retrieved and utilized for constructing a phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA11). Additionally, the nucleotide sequence of the isolate has been submitted to the GenBank database.

Assessment of Bacterial Drought Stress Resistance

The bacterial strain was isolated and subsequently transferred to a nutrient broth. Polyethylene Glycol (PEG) 8000 was added to the nutrient broth to mediate osmotic pressure. The concentrations of PEG 8000 used were as follows (in g/g): 0.023, 0.037, 0.042, 0.051, and 0.062. These concentrations corresponded to osmotic pressures of -0.15, -0.30, -0.36, -0.48, and -0.73 Mpa, respectively. According to Michel (1983),[36] the osmotic pressure at a temperature 25°C was calculated using the equation given below.

$$OP=1.29\times C^2\times T-140\times C^2-4.0\times C$$

In the 100 ml of the above-prepared stress medium, 2 ml of a 24-hour-old bacterial culture from the nutrient broth was inoculated and kept at 25°C for 2 days. After 2 days, bacterial growth was measured by the optical density at 600nm against uninoculated broth using a spectrophotometer (Bioera single beam spectrophotometer). The culture growth in normal conditions (without PEG 8000) was used for comparison.

ACC Deaminase

The ACC deaminase-producing ability of an organism was confirmed as per protocol suggested by Patil *et al*. (2016),[37] for this minimal ACC medium (in g/L, Glucose 2.0, Sodium citrate 2.0, $CaCO_3$ 4.0, $C_6H_{11}KO_7$ 2.0) with final concentration of ACC was 3.0 mmol l^{-1}, prepared as per Penrose and Glick (2003),[38] containing 0.005% of bromothymol blue. After 24 hours of incubation at 30°C, the colony developed a blue colouration, suggesting its potential to produce ACC deaminase. This method offers only a qualitative assessment of ACC deaminase activity.

Phosphate Solubilization Index

The bacterial strain selected based on drought tolerance was evaluated for phosphate solubilization index. A bacterial colony from Nutrient agar was inoculated on a Pikovskayas agar plate and incubated at 30°C for 3 days. Following incubation, the diameters of both the colony and the zone were recorded, and the phosphate solubilization index was calculated with the formula below.[39]

Phosphate Solubilization Index=(Colony Diameter + Halozone Diameter)/(Colony Diameter)

Siderophore Production and Nitrogen-Fixing Ability

The siderophore production of the bacterial strain was performed and analysed by spot inoculation of an overnight-grown culture on Chrome Azurol-S agar plates, and the plates were incubated for 2 days at 30 °C.[40]

The nitrogen-fixing ability of the selected bacterial strain was analyzed by inoculating it on nitrogen-free Jensen's medium with composition g/L: Sucrose 20, $MgSO_4$ 0.5, NaCl 0.5, K_2HPO_4 1, $FeSO_4$ 0.1, Na2MoO_4 0.005, $CaCO_3$ 2, Agar 15.

HCN and Ammonia Production

HCN production test was done by using a Nutrient agar medium with glycine (4.4 g/L). The isolated colony of the strain was inoculated on the Nutrient agar plate. A Whatman No. 1 filter paper saturated with picric acid (0.5%) and sodium carbonate (2%) solution was placed on the inner surface of the Petri dish lid, and the plates were incubated at 30 °C for 4 days.[41] After incubation, a shift in the color of filter paper from yellow to orange, red, and brown corresponds to low, medium, and high levels of HCN production, respectively.[42]

The bacterial colony was inoculated in peptone broth g/L: 10.0 g peptone; 5.0 g NaCl; and 7.0 pH [43] incubated at 30°C for 2 days. Following incubation, Nessler's reagent (0.5 ml) was added, and observed for faint yellow, yellow, and brown colours, indicating the lowest to high production of ammonia.[44]

Indole-Acetic Acid

The selective bacterial strains' ability to secrete Indole-acetic acid was analyzed by inoculating a colony in 100 ml Luria broth with 10 mg of tryptophan. After incubation, the broth was centrifuged at 10733 X G. The 1 ml of supernatant was added with 2 ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$), and the reaction mixture was incubated in dark for 30 min. The absorbance was recorded at 530nm. All the above-mentioned microbiological tests were performed in triplicate.

Seed Germination Study

The seed germination study was performed in vitro using the Petri plate method with sterilized blotting paper, and each experiment was carried out in three replicates to ensure accuracy and reproducibility. To eliminate contaminants, wheat seeds were treated with 0.1% mercuric chloride for five minutes, then thoroughly rinsed with double-distilled water to ensure effective surface sterilization. For biopriming, the seeds were immersed in a bacterial suspension containing 10^7 CFU/mL for 2 hours, whereas control seeds were soaked in distilled water under identical conditions. The bacterial suspension was prepared by inoculating 5 µL of bacterial culture into 100 mL of nutrient broth, then incubating at 30°C for 24 hours to allow bacterial growth. Post-soaking, the seeds were placed onto sterilized blotting paper within

Petri plates, moistened with 5 mL of either double-distilled water (control) or the bacterial suspension, as described by.45 Drought stress was artificially induced by adding 5 mL of a 5% PEG 8000 solution, equivalent to an osmotic potential of -0.47 MPa, into the Petri plates. Notably, the isolated bacterial strain exhibited robust growth under osmotic pressures as extreme as -0.48 MPa, demonstrating its adaptability.

Each Petri plate contained 15 seeds, and four distinct experimental groups were established: (1) water control, (2) bacterial control (bacterially primed seeds), (3) seeds treated with 5% PEG 8000 alone to simulate drought stress, and (4) seeds treated with both 5% PEG 8000 and bacterial suspension. The plates were kept at 25°C in a controlled growth chamber to assess germination under the specified conditions. Statistical analysis was done using MS-Excel software. Seed germination percentage was assessed after 4 days using the standard formula

Relative Germination Percentage (%)=(Number of seed germinated in treatment)/(Number of seeds germinated in control)×100

Results and Discussion

Soil Sample And Bacterial Isolation

The rhizospheric and surrounding soil from *Celosia argentea* was collected from Shelgaon village of Solapur district (India) (Fig. 1). The district is considered drought-prone because of inadequate rainfall, with an annual average rainfall of 545mm. Solapur is one of 72 drought-prone districts in India.

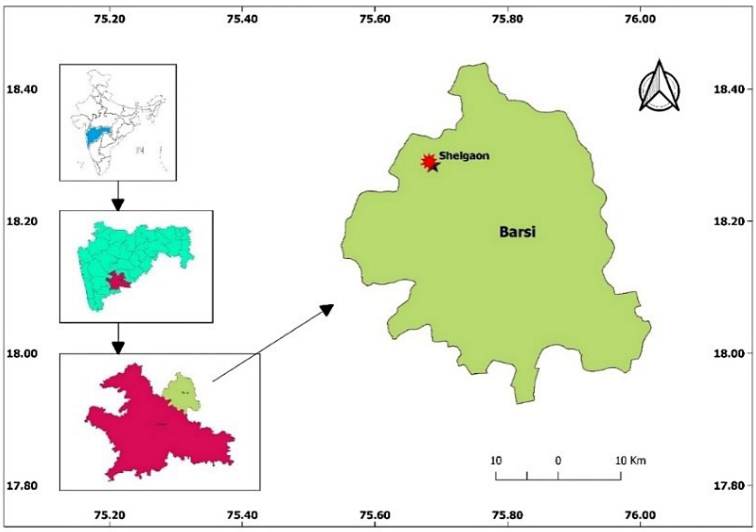

Fig. 1: Map of the sample collection site

The soil sample was collected and analyzed for different physical and chemical characteristics (Table 1). In the soil, a reservoir of nutrients is available and required for crop plants, but not at optimum levels. Soil analysis is needed to determine the level of available nutrients and soil quality for specific crop plants. Analysing soil can yield valuable insights that help optimise nutrient utilisation and enhance overall agricultural productivity. The sample was subjected to the isolation of drought stress-tolerant PGPR. The five morphologically distinct colonies were selected and inoculated on a nitrogen-free medium. Among five isolates, one that could fix nitrogen and was able to tolerate stress induced by 5% PEG 8000 (-47MPa) was selected for further study. The bacteria's stress tolerance was verified by observing the optical density of their growth after incubation. For comparison, bacteria cultured in nutrient broth without PEG 8000 were the control (data not shown).

Table 1: Physicochemical analysis of soil sample

Soil Analysis	Result
Calcium (mEq%)	0.4
Organic carbon (%)	0.9
pH	7.08
EC (mili-siemens cm-1)	0.885
Magnesium (mEq%)	ND
Nitrogen kh-1	210
Phosphate kh-1	4.3
Potassium kh-1	270

ND- Not Determined

Identification of Bacterial Strain

The isolated bacterium was characterized as Gram-positive, rod-shaped, motile, and endospore-forming. It exhibited growth in a pH range of 5 to 8, with optimal growth at pH 7. The temperature range for growth was 15°C to 40°C, with the optimal temperature at 30°C, and it was inhibited at 50°C. The bacterium tolerated salt concentrations up to 7.5%. Biochemical tests and 16S rDNA molecular identification confirmed its identity. The partial 16S rDNA sequence of the bacterial isolate was acquired from the National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory, Pune, India. Following its acquisition, the sequence was submitted to GenBank under the accession number PQ772196; the bacterial strain was identified as *Bacillus cereus* DS1 (PQ772196). Phylogenetic tree was subsequently created utilizing MEGA 11 software to analyse its evolutionary relationships (Fig. 2).

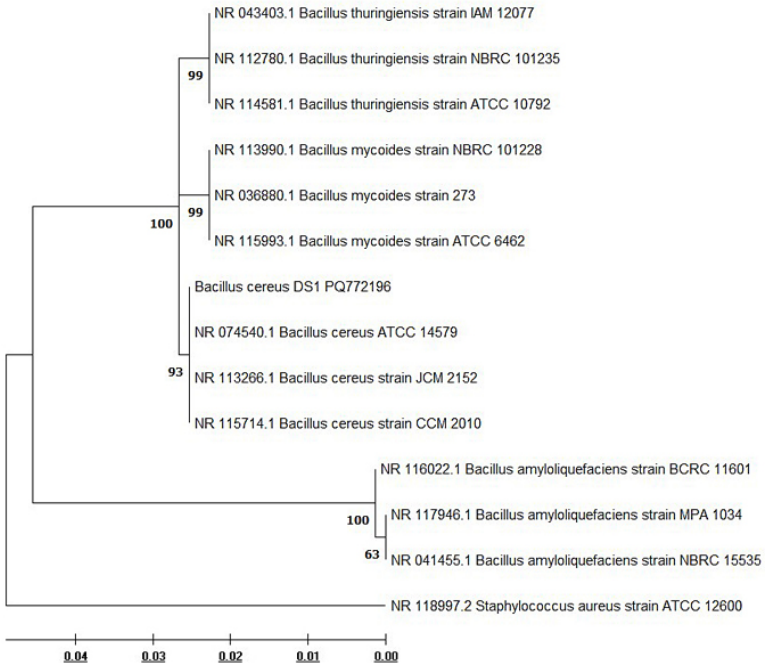

Fig. 2: Phylogenetic tree of Bacillus cereus DS1 PQ772196

Bacterial Drought Stress Tolerance

Ethylene levels in plants are influenced by both biotic and abiotic stresses, which in turn play a key role in modulating various physiological processes,[46] reducing root and shoot length under stress conditions. In ethylene biosynthesis, S- adenosylmethionine (S-AdoMet) is converted by 1-aminocyclopropane-1-carboxylate synthase (ACS) to 1- aminocyclopropane-1-carboxylate (ACC), which is the precursor of ethylene. The ACC is degraded by ACC deaminase, decreases ethylene-induced damage, ameliorates plant stress, and promotes plant growth.[47]

The bacteria was identified as *Bacillus cereus* (PQ772196) was able to produce ACC deaminase enzyme confirmed by plate assay, the blue color zone around the bacterial colony in the ACC minimal agar plate with pH indicator dye (bromothymol blue) indicative of ACC deaminase production. The isolated bacteria also effectively produce plant growth-promoting traits in normal and stress-mediated conditions. It solubilised phosphate, with a solubilization index of 2.57 under non-stress conditions and 2.42 under stress conditions. The isolate also produced indole-3-acetic acid, ammonia, hydrogen cyanide, and siderophores, and it was capable of nitrogen fixation in both stress and non-stress environments (Table 2).

Table 2: Plant growth-promoting factors producing ability of Bacillus cereus DS1 PQ772196 under stress and non-stress conditions

Trait	Non-stress condition	Stress condition
Phosphate	+	+
Indole acetic acid	+	+
HCN	+	+
Ammonia	+	+
Nitrogen fixation ability	+	+
Siderophore	+	+
ACC deaminase	+	+

"+" Positive, "-" Negative

Seed Germination Study

Drought stress hampers seed germination, although the severity and adverse effects differ across species.[48] The PGPR enhances plant growth by producing different growth regulators, stimulating the growth directly, aiding in nodulation, or indirectly stimulating nodulation.[49,50] However, PGPR solubilizes the minerals by producing organic acids, mineralizes and enhances nutrient uptake.[51] In the study on seed germination under drought conditions, wheat seeds were bioprimed using a suspension of the isolated strain *Bacillus cereus* DS1, and the germination percentage was subsequently evaluated. When wheat seeds were bioprimed with *Bacillus cereus* DS1 in the absence of PEG 8000, the germination rate matched the control experiment, with 100% of the seeds successfully germinating. A 5% PEG 8000 solution, inducing drought stress at -0.47 MPa, was applied, and the seed germination rate was observed. Under this stress condition, germination occurred at a reduced rate of 14%. However, when the stress was combined with the bacterial suspension, germination significantly improved, reaching 88%. This evidence indicates that the bacteria enhance seed germination under drought conditions.

Fig. 3: wheat seeds were treated with A) 5% PEG 8000, B) Distilled water, C) Only bacterial suspension, D) 5% PEG 8000 in combination with bacteria.

Table 3: Germination performance of seeds under control, bacterial, PEG, and combined treatments

Sr. No.	Treatment	Replicate 1	Replicate 2	Replicate 3	SD	Total seeds germinated
1	Control	14	14	14	0	42
2	Bacterial suspension	14	14	14	0	42
3	5% PEG	2	3	1	0.82	6
4	Bacteria + 5% PEG	12	12	13	0.47	37

Germination in the control set was considered as 100% for comparison.

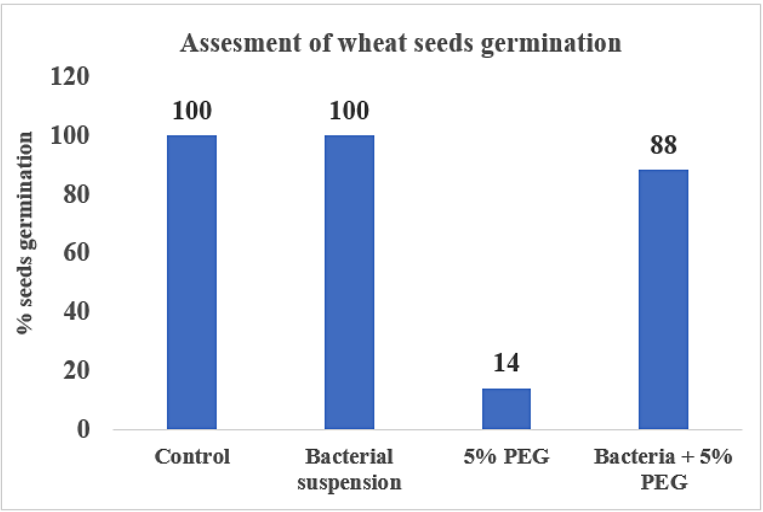

Fig. 4: Assessment of wheat seeds germination by various treatment

Bacillus cereus, as reported by De Oliveira *et al*. (2024),[52] has been effectively utilised to enhance popcorn plants' growth under drought conditions. This bacterial strain promotes notable morphological changes and enhances several key growth parameters—such as elevated chlorophyll content, improved chlorophyll fluorescence and maximum fluorescence intensity, greater stem diameter, expanded leaf area, longer and denser roots, increased biomass accumulation, and more efficient water use. Additionally, multiple studies[28,53–55] highlight the *Bacillus* genus as a promising candidate among plant growth-promoting bacteria, offering considerable potential for improving plant resilience and productivity. *Bacillus cereus* is widely recognised as a pathogenic species; however, some isolated strains have been reported to be environmentally safe and able to produce some plant growth-promoting traits, including IAA and ACC deaminase.[56]

The bacteria identified as *Bacillus cereus* DS1 may have the potential to reduce the severity of drought stress, enhance nutrient uptake, and make plants more stress-tolerant under drought conditions. This study primarily examined seed germination. However, a comprehensive exploration of *Bacillus cereus* DS1 for plant growth and crop yield improvement is also necessary.

Conclusion

To address water scarcity and reduce dependence on harmful agrochemicals, researchers are investigating an eco-friendly approach to activate plants' innate defence mechanisms against abiotic stresses. PGPR are essential for boosting the

growth of plants and increasing productivity under drought conditions. The *Bacillus cereus* DS1 isolated in this study has the potential to produce various plant growth-promoting traits under stress and non-stress conditions. *Bacillus cereus* DS1 has the potential to stimulate nutrient assimilation under water-deficient environments, as evidenced by a seed germination study. The outcomes of the present research suggest that *Bacillus cereus* DS1 could be useful in developing bioinoculants to boost crop production in arid and semi-arid areas. Consequently, this bacterium could be a valuable resource for enhancing global food security.

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Conflict of Interest
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Ethics Statement
This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement
This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from other Sources
Not Applicable

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- **Snehal Maruti Patil:** Data Collection, Methodology, Analysis,
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- **Shubham Chandrakant Kavade:** Data Collection, Analysis, Writing
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