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Isolation And Characterization Of Plant Growth-Promoting Rhizobacteria (PGPR) From Rhizospheric And Endophytic Soil Under Drought Conditions

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Abstract

Soil microorganisms play a crucial role in enhancing soil fertility and plant growth, particularly under drought conditions. This study aimed to isolate and characterize plant growth-promoting rhizobacteria (PGPR) from the rhizosphere and endosphere of Cicer arietinum (chickpea) grown in the Rewa district of Madhya Pradesh, India. A total of 36 non-rhizospheric soil samples were collected and analyzed for their physicochemical properties, including pH, electrical conductivity (EC), organic carbon, and macronutrient content (N, P, K). The study identified 62 phosphate-solubilizing bacterial (PSB) isolates based on halo formation on Pikovskaya's agar medium [13]. The isolates were further screened for plant growth-promoting (PGP) traits, including indole-3-acetic acid (IAA) production, zinc solubilization, hydrogen cyanide (HCN) production, ammonia production, siderophore production, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. Eleven bacterial strains exhibited significant drought tolerance when tested under polyethylene glycol (PEG6000)-induced osmotic stress. These isolates demonstrated robust exopolysaccharide production, phosphate and zinc solubilization, and IAA production, highlighting their potential for use as biofertilizers in drought-prone regions. Molecular characterization using 16S rRNA sequencing confirmed their taxonomic identities. The findings of this study provide insights into the potential application of these bacteria in sustainable agriculture for improving soil fertility and plant resilience under water stress conditions.

Keywords

Drought stress, Phosphate solubilization, Rhizobacteria, Cicer arietinum, Plant growth-promoting bacteria, Sustainable agriculture.

Introduction

Drought stress significantly affects global agricultural productivity by limiting water availability and reducing soil fertility. It is one of the most widespread and detrimental abiotic stressors impacting crop growth, yield, and quality, especially in semi-arid and arid regions.[1]The lack of adequate soil moisture alters plant physiological processes, reduces nutrient mobility, and restricts microbial activity, collectively leading to reduced biomass and impaired reproductive success in crops. With climate change intensifying the frequency and severity of drought episodes, it becomes imperative to develop sustainable and biologically efficient strategies to mitigate these effects.[2,3]

Soil microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), play a critical role in enhancing soil nutrient availability and plant stress tolerance. PGPR are beneficial bacteria that colonize the rhizosphere or internal plant tissues and confer advantages to their host plants.[4,5]These bacteria are known for their multifunctional traits, including nitrogen fixation, phosphate solubilization, zinc mobilization, siderophore production, and synthesis of phytohormones like indole-3-acetic acid (IAA). These traits not only enhance plant growth but also support resilience against drought, salinity, and pathogen attack. Moreover, many PGPR strains exhibit 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which helps modulate ethylene levels in stressed plants, promoting root elongation and shoot development under water-deficit conditions.[6,7]

Chickpea (*Cicer arietinum*), an important leguminous crop in India and many developing countries, is particularly vulnerable to drought stress. As a staple source of protein, its production is crucial for food security and soil health through nitrogen fixation. However, drought severely limits its yield potential and nutrient uptake, especially during the flowering and pod-filling stages.[8] Enhancing the rhizosphere and endosphere microbial composition with drought-resilient PGPR may offer a viable, eco-friendly approach to sustain chickpea productivity under changing climate conditions.[9]

This study was designed to isolate and characterize culturable drought-tolerant PGPR strains associated with chickpea grown in the Rewa district of Madhya Pradesh, a region characterized by its semi-arid climate and variable rainfall. Rhizospheric and endophytic bacterial communities were targeted for their potential to withstand osmotic stress and contribute to plant growth. The selected bacterial isolates were evaluated for key functional traits, including phosphate and zinc solubilization, indole-3-acetic acid (IAA) and exopolysaccharide (EPS) production, as well as ACC deaminase activity.[10]The broader aim of this work was to identify potent microbial strains that could serve as bioinoculants or biofertilizers, supporting sustainable chickpea cultivation in drought-prone regions.[11]

MATERIALS AND METHODS

Study Design and Scope

This study focused on the isolation, molecular characterization, and evaluation of drought-tolerant plant growth-promoting rhizobacteria (PGPR) associated with Cicer arietinum (chickpea). The bacterial strains were screened for their ability to solubilize phosphate, produce exopolysaccharides, synthesize siderophores, and withstand drought stress. Further molecular characterization was conducted using 16S rRNA sequencing to identify bacterial strains at the genetic level.[12]

Study Location

Geographical and Climatic Description

The study was conducted in Rewa district, Madhya Pradesh, India, which is located at 24°32′17″N, 81°17′43″E, with an elevation of 259 meters above sea level. The region experiences a semi-arid to subtropical climate, with an average annual rainfall of 1039.8 mm.[13] The temperature varies significantly across seasons, with winter temperatures dropping as low as 0.6°C to 6°C, while summer temperatures can reach up to 46°C. The monsoon season, from July to October, brings significant rainfall, contributing to soil fertility. The presence of the Vindhya Mountain range and Kaimur Hills influences the soil composition and climatic conditions, making it an ideal location for studying the diversity of drought-tolerant bacteria.[14]

Sampling Sites

A total of 12 different locations were selected for soil and root sample collection within Rewa district. These locations were chosen based on variations in soil composition, altitude, and agricultural activity. The sites included urban, peri-urban, and rural agricultural areas to ensure a diverse microbial population. The altitude of these locations ranged from 205 meters to 340 meters, which provided insight into how elevation might influence microbial diversity in rhizosphere soil.

Sample Collection and Processing

Collection of Soil and Root Samples

Samples were collected from four major locations within the district: Rewa City, Gurh, Simaria, and Mangawan. Each of these locations was divided into three different sampling sites that were at least 2–3 km apart to capture a diverse bacterial population. From each site, three independent samples were taken, resulting in a total of 36 rhizospheric soil samples. Additionally, 36 non-rhizospheric soil samples and 36 root samples were collected, amounting to a total of 108 samples. Sampling was conducted during the flowering stage (May 2024) to ensure the presence of an active rhizosphere microbiome.[15]

Sample Handling and Storage

The collected soil samples were **air-dried, sieved (2 mm), and stored at -20°C** before further analysis. Root samples were **placed in sterile centrifuge tubes (50 mL) and transported in an icebox at 4°C** to prevent microbial degradation. All samples were properly labeled and stored under **aseptic conditions** until further processing.[16]

Physico-Chemical Analysis of Soil Samples

To assess soil fertility and microbial habitat conditions, **pH, electrical conductivity (EC), organic carbon content, and macronutrient levels (nitrogen, phosphorus, potassium)** were analyzed.

Soil pH Measurement

Soil pH was determined using **a calibrated pH meter** following standard procedures. Approximately **5 g of soil** was mixed with **25 mL of distilled water**, and the solution was stirred thoroughly before measuring pH. This test provided insights into soil acidity or alkalinity, which influences microbial activity and nutrient availability.

Electrical Conductivity (EC) Measurement

Electrical conductivity was measured to determine the ionic concentration of the soil solution, which affects plant growth and microbial interactions. 10 g of soil was suspended in 40 mL of distilled water, shaken for one hour, and left undisturbed before measuring EC using a conductivity meter.

Soil Nutrient Analysis

Soil samples were analyzed for available nitrogen (N), phosphorus (P), and potassium (K) using standardized protocols:

- Kjeldahl Method for nitrogen estimation
- Olsen Method for phosphorus determination
- Ammonium Acetate Extraction Method for potassium analysis

These analyses helped determine soil fertility levels and their impact on microbial communities.

Isolation of Plant Growth-Promoting Bacteria (PGPB)

Isolation of Rhizospheric Bacteria

Bacterial isolation was performed using serial dilution and spread plating techniques. Soil samples were diluted (10^{-2} to 10^{-4}) and plated on Nutrient Agar Medium (NAM). After 24 hours of incubation at 37°C, morphologically distinct bacterial colonies were selected for further characterization.[17]

Isolation of Phosphate-Solubilizing Bacteria (PSB)

To identify PSB, bacterial isolates were cultured on Pikovskaya's Agar Medium [13]. Colonies producing halo zones around them were considered phosphate-solubilizing strains. These isolates were further purified and stored at -80°C in 30% glycerol for long-term preservation.

Isolation of Endophytic Bacteria

Endophytic bacteria were isolated from surface-sterilized root samples. The roots were washed with 95% ethanol (1–2 min), followed by 1% sodium hypochlorite (3 min), and rinsed in ethanol (30s). To ensure proper sterilization, the final wash was plated on NAM and incubated for 24–48 hours at 37°C. The sterilized root samples were then macerated in phosphate-buffered saline (PBS), centrifuged, and stored.[18]

Morphological and Biochemical Characterization

Morphological Examination

Morphological characteristics such as colony shape, size, pigmentation, and Gram staining were analyzed under a microscope.

Screening for Drought-Tolerant Bacteria

To evaluate bacterial tolerance to water stress, isolates were grown in Tryptone Soy Broth (TSB) at 28°C for 48 hours. Polyethylene glycol (PEG6000) was added to create an osmotic stress of -1.5 MPa, mimicking drought conditions. Bacterial growth was quantified using spectrophotometric analysis (OD600). Strains that survived under these conditions were selected for further characterization.

Molecular Characterization (16S rRNA Sequencing)

The most promising bacterial isolates were genetically identified using 16S rRNA sequencing. Bacterial DNA was extracted using the HiPurA Bacterial Genomic DNA Kit, and PCR amplification was performed using universal primers 27F and 1492R. Amplified products were sequenced using Sanger sequencing, and sequence alignment was conducted using MEGA software for phylogenetic analysis.[19]

Characterization of Bacteria for PGP Traits

Exopolysaccharide (EPS) Production

Bacteria were grown in TSB medium containing 15% PEG 6000 for 72 hours to stimulate EPS production under drought stress. EPS extraction and quantification were performed following Fett et al. (1986, 1989) using the phenol-sulfuric acid assay.

Indole-3-Acetic Acid (IAA) Production

IAA production was detected using the Salkowski reagent method. Isolates were grown in Luria-Bertani (LB) broth with and without L-tryptophan (200 μg/mL), followed by optical density measurement at 535 nm.[20]

Results and Discussion

Physicochemical Properties of Soil

The physicochemical properties of the collected soil samples were analyzed to determine their fertility, nutrient availability, and suitability for microbial growth. The pH of the collected soil samples ranged from 6.7±0.12 to 8.3±0.05, indicating that most of the soils were slightly alkaline. Electrical conductivity (EC) varied between 0.20±0.01 to 0.26±0.09 dS/m, reflecting differences in soil salinity, which can affect plant nutrient uptake and microbial activity. The organic carbon content, an essential parameter for soil fertility, ranged between 1.007±0.008% to 2.648±0.01%, suggesting that the soil has a suitable level of organic matter to support microbial communities and enhance nutrient cycling.

Isolation of Phosphate-Solubilizing Bacteria (PSB)

A total of 80 phosphate-solubilizing bacterial isolates were identified based on their ability to form clear halos around their colonies on phosphate agar media (PAM). Among these, 62 isolates maintained their solubilization ability consistently during multiple sub-culturing processes. The bacterial isolates were further tested for plant growth-promoting traits, including IAA production, zinc solubilization, ammonia production, siderophore production, and ACC deaminase activity.

Drought Tolerance of Bacterial Isolates

The ability of bacterial isolates to tolerate drought stress was assessed using polyethylene glycol (PEG6000). The highest drought tolerance was observed in 11 bacterial strains, which showed significant growth even at PEG concentrations as high as 50%. These strains exhibited strong exopolysaccharide production, phosphate solubilization, and indole-3-acetic acid (IAA) production, indicating their potential role in mitigating drought stress in plants.

Molecular Characterization of Bacterial Isolates

The 16S rRNA gene sequencing of the most promising bacterial isolates confirmed their taxonomic identities, revealing their close relatedness to well-known plant growth-promoting bacterial genera, including Bacillus and Pseudomonas. Phylogenetic analysis showed a strong evolutionary relationship among the identified strains, suggesting their potential role in improving soil fertility and plant growth under water-deficient conditions.

Physicochemical Properties of Soil Samples in Rewa District, Madhya Pradesh

Soil fertility and productivity are governed by its physicochemical properties, which influence nutrient availability, microbial activity, and crop adaptability. This study assessed essential parameters including pH, electrical conductivity (EC), available macronutrients (nitrogen, phosphorus, potassium), and organic carbon across 12 locations in Rewa district. These parameters form the foundation for developing sustainable, location-specific agronomic strategies and improving the microbial habitat for beneficial rhizobacteria.

Detailed Evaluation of Soil Characteristics

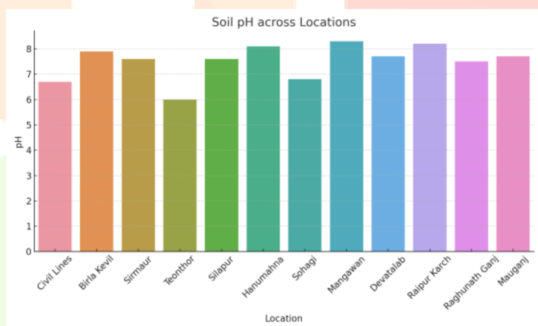

Figure 1: Soil pH Across Locations

Soil pH is a master variable that governs the solubility of nutrients and activity of microbial communities. In this study, the pH ranged from 6.0 (Teonthor) to 8.3 (Mangawan), representing a transition from slightly acidic to moderately alkaline conditions. Most agricultural crops thrive in the range of 6.5 to 7.5. Acidic soils may favor micronutrient solubility but reduce phosphorus availability, while alkaline soils may precipitate essential nutrients like iron and zinc, leading to deficiencies. Hence, site-specific pH management such as lime or sulfur amendments may be advised to optimize nutrient uptake.

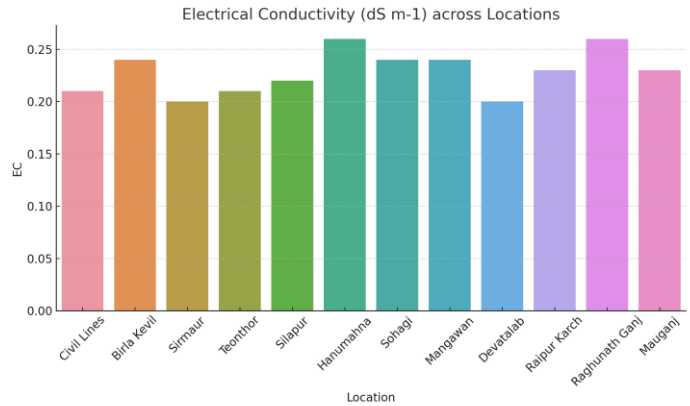

Figure 2: Electrical Conductivity (ds m⁻¹) Across Locations

Electrical Conductivity (EC) is indicative of the total soluble salts in the soil and directly impacts osmotic potential affecting water absorption by roots. EC values across locations were low (0.20–0.26 ds/m), suggesting minimal salt stress and excellent conditions for seed germination and root elongation. These values fall well below the critical salinity threshold for most crops (0.8 ds/m), affirming the non-saline nature of the studied soils.

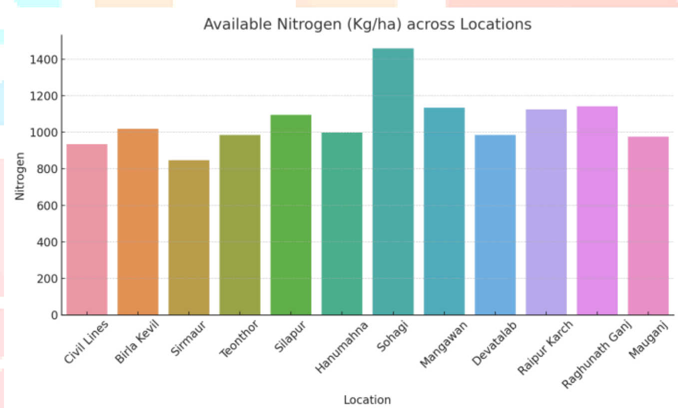

Figure 3:Available Nitrogen (Kg/Ha) Across Locations

Nitrogen (N) is the most dynamic and limiting macronutrient, essential for vegetative growth, chlorophyll synthesis, and protein production. The nitrogen content varied significantly, with Sohagi recording the highest levels (~1459 kg/ha) and Sirmaur the lowest (~847 kg/ha). These differences may arise from varied organic matter levels, mineralization rates, cropping intensity, and fertilizer usage. While most sites exceeded the critical level of 1000 kg/ha, locations with lower nitrogen might benefit from integrated nutrient management combining organic manures and biofertilizers.

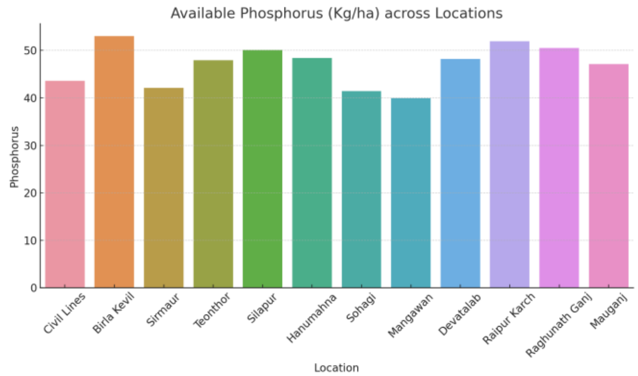

Figure 4: Available Phosphorus (Kg/Ha) Across Locations

Phosphorus (P) supports energy transfer, root proliferation, and reproductive development in plants. Phosphorus values ranged from 39.94 (Mangawan) to 53.01 (Birla Kevil), suggesting that all sites are above the critical threshold (>15 kg/ha) for adequate crop growth. Excess phosphorus can lead to antagonism with zinc, hence the values observed also align with balanced nutrient uptake scenarios. These values indicate effective P-cycling possibly due to organic residue decomposition or microbial phosphate solubilization.

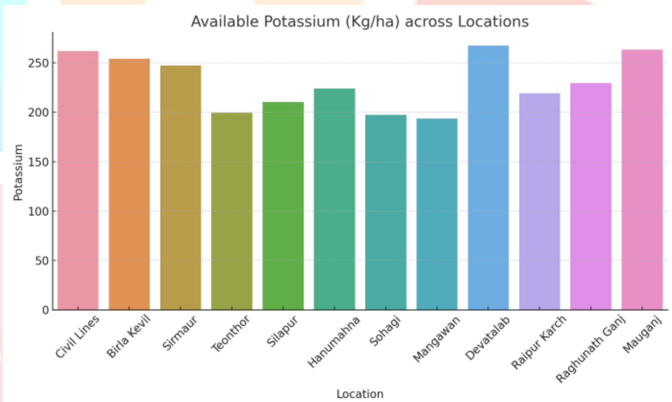

Figure 5: Available Potassium (Kg/Ha) Across Locations

Potassium (K) is essential for osmotic regulation, photosynthesis, and resistance to abiotic stress. Ranging from 193.74 (Mangawan) to 267.59 (Devatalab), potassium levels are well within the medium to high fertility range (>150 kg/ha), signifying sufficient natural reserves. K availability depends on mineral composition and CEC. High values in Devatalab and Mauganj suggest higher feldspar or mica content in soil parent material. This status ensures good root health and tuber or fruit quality in crops like potatoes and tomatoes.

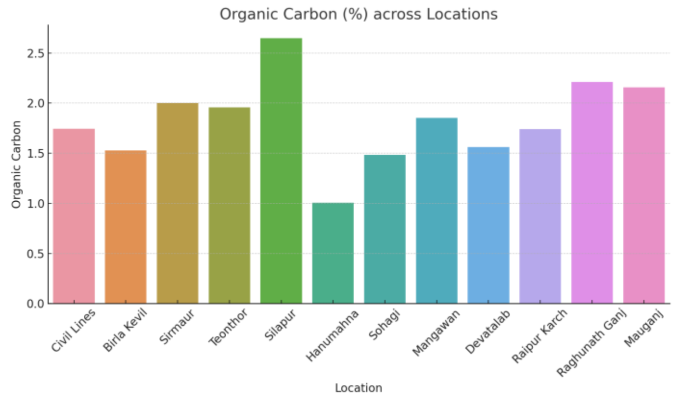

Figure 6: Organic Carbon (%) Across Locations

Soil organic carbon (SOC) reflects the biological health and nutrient-holding capacity of the soil. SOC values ranged from 1.01% (Hanumahna) to 2.65% (Silapur), with the majority falling in the optimal 1–3% range. SOC enhances microbial diversity, nutrient buffering, and physical structure, promoting better aeration and moisture retention. The high SOC in Silapur may result from compost application or residue retention, while lower levels in Hanumahna may necessitate organic amendments or cover cropping. These observations suggest varied biological potential and carbon management needs across sites.

Future Agronomic Recommendations

In light of the observed variability and the need for improved site-specific interventions, several future agronomic strategies are recommended. Firstly, it is advisable to apply lime in acidic soils and gypsum in alkaline zones to maintain optimal pH levels, which are critical for nutrient solubility and microbial efficiency. Secondly, the promotion of organic matter inputs such as green manures, composts, and retained crop residues should be emphasized, particularly in locations with low soil organic carbon, such as Hanumahna. This will aid in improving soil structure, moisture retention, and microbial activity. Precision nitrogen management, including customized fertilization plans for nitrogen-deficient areas like Sirmaur, should be implemented based on periodic soil testing to avoid over- or under-application. The integration of biofertilizers, particularly phosphate-solubilizing bacteria (PSB), can enhance phosphorus availability and contribute to reduced reliance on chemical fertilizers. Lastly, establishing a routine soil health monitoring system will be essential for tracking nutrient dynamics and adapting inputs based on real-time data. Collectively, these practices will foster sustainable soil management, boost resilience against climate stressors, and support enhanced crop productivity throughout the Rewa region.

Conclusion

The comprehensive physicochemical evaluation of soils across various locations in the Rewa district of Madhya Pradesh reveals that the region's soils are broadly suitable for intensive and sustainable agricultural practices. The majority of soil samples demonstrated balanced macro-nutrient levels, moderate to high organic carbon content, and minimal issues related to salinity. These characteristics affirm a strong potential for productive crop cultivation. However, the analysis also uncovered variability in nitrogen and organic carbon content across sites, underscoring the need for region-specific soil management approaches. This heterogeneity suggests that while the overall soil health is favourable, targeted strategies must be employed to address nutrient deficiencies and optimize soil resource utilization. These findings emphasize the necessity for tailored agronomic planning that aligns with the specific needs of each agroecological zone within the district to sustain long-term agricultural productivity.

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