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Original Article

Characterization and isolation of 1-aminocyclopropane1-carboxylate (ACC) deaminase-producing, plant growth-promoting rhizobacteria from the rhizosphere of Egyptian wheat cultivars for improved drought resilience

Caracterização e isolamento de rizobactérias promotoras do crescimento de plantas produtoras de 1-aminociclopropano-1-carboxilato (ACC) desaminase da rizosfera de cultivares de trigo egípcio para melhor resiliência à seca

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Abstract

Drought stress severely damages the growth and development of wheat and leads to lower crop production. The application of plant growth-promoting rhizobacteria (PGPR) is a sustainable approach that enhances plant tolerance to drought. This study investigated whether different bacterial isolates could promote wheat growth under drought conditions. A total of 73 bacterial isolates were obtained from the rhizosphere of Egyptian wheat cultivars, 30 of which exhibited ACC deaminase activity. The isolates were selected based on various PGPR characteristics, including phosphate solubilization, siderophore production, nitrogen fixation, indole-3-acetic acid production, biofilm formation, and antagonistic abilities. The active ACC colonies were screened for these traits and based on in vitro promotion of wheat plant growth, root inoculum from four wheat plants was used and grown under drought conditions. The percentage yield of wheat plants increased in the weight of wheat plants, while in total biomass it was found that the treatments showed significant differences compared to the control. The most effective ACC was from the wheat isolate B. subtilis. The bacterial types were identified at the genus level by sequencing the 16s rRNA gene. In conclusion, this research suggests PGPR such as *V. paradoxus* and *K. oxytoca* have the potential to reduce the effects of drought stress in Egyptian wheat cultivars.

Keywords: ACC deaminase, PGPR, wheat, drought, Rhizosphere bacteria.

Resumo

O estresse hídrico danifica severamente o crescimento e o desenvolvimento do trigo, e leva à redução da produção agrícola. A aplicação de rizobactérias promotoras do crescimento de plantas (PGPR) como uma abordagem sustentável aumentou a tolerância das plantas à seca. Este estudo investigou se diferentes isolados bacterianos poderiam promover o crescimento do trigo em condições secas. Um total de 73 isolados foram identificados da rizosfera de cultivares de trigo egípcio, dos quais 30 exibiram atividade de ACC desaminase. Os isolados foram então selecionados com base em várias características de PGPR, incluindo solubilização de fosfato, produção de sideróforos, fixação de nitrogênio, produção de ácido indol-3-acético, formação de biofilme e habilidades antagônicas. As colônias ACC ativas foram rastreadas para essas características e, com base na promoção *in vitro* do crescimento da planta de trigo, o inóculo de raiz de quatro plantas de trigo foi usado e cultivado em condições de seca. A produção percentual de rendimento aumentou no peso das plantas de trigo, enquanto na biomassa total foi descoberto que os tratamentos mostraram uma diferença significativa do controle. O ACC mais eficaz foi do isolado de trigo *B. subtilis*. Os tipos bacterianos foram identificados no nível de gênero por sequenciamento do gene 16s rRNA. Em conclusão, esta pesquisa sugere que PGPR, como *V. paradoxus* e *K. oxytoca*, têm o potencial de reduzir os efeitos do estresse por seca em cultivares de trigo egípcio.

Palavras-chave: ACC desaminase, PGPR, trigo, seca, bactérias da Rizosfera.

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1. Introduction

One of the biggest challenges facing the world today is the impact of climate change, particularly drought stress, on agricultural productivity. Drought stress alters various plant traits at physiological, biochemical and molecular levels and leads to significant crop losses (Saleem et al., 2007). Drought stress has already led to a 10% decline in cereal production over the last four decades, and projections indicate that more than half of all cultivated land will be affected by 2050 (Mickky et al., 2020). Wheat, a staple food that provides 20% of the world's calories, is particularly vulnerable to drought stress during the flowering phase, resulting in reduced grain quantity and quality (Saini and Westgate, 1999).

Efforts to mitigate the effects of drought stress on crop productivity include the development of drought-resistant crop varieties and the use of water-saving irrigation techniques (Zhang et al., 2021). However, some of these approaches face challenges in practical implementation due to technological limitations and high resource investments (Langridge et al., 2022). One promising strategy is the use of beneficial microbes such as plant growth-promoting rhizobacteria (PGPR), which have been shown to improve drought resistance and water-use efficiency of plants (Danish and Zafar-ul-Hye, 2019).

PGPR, such as *Bacillus amyloliquefaciens* and *Agrobacterium fabrum*, have been found to promote root elongation, mitigate the effects of drought and improve nutrient mobilization, leading to a significant increase in plant growth (Zafar-ul-Hye et al., 2019). These beneficial bacteria produce exopolysaccharides that bind to plant roots and form biofilms that protect against drought stress (Carezzano et al., 2023). In addition, PGPR containing ACC deaminase have been shown to convert ACC, a precursor of ethylene synthesis, into compounds that do not trigger stress responses, thus contributing to drought tolerance (Danish & Zafar-ul-Hye, 2019). Recent studies have demonstrated the efficacy of PGPR in alleviating drought stress in various crops, including wheat, maize, tomato, grapevine and Arabidopsis (Al-Turki et al., 2023).

Phosphate solubility is an important process for the promotion of microorganisms. It is also of central importance for plant growth and soil fertility. The ability of phosphate-solubilizing bacteria to convert insoluble phosphate into a soluble form, in this case dihydrate, through processes such as acidification and chelation reactions is well established (Walpola et al., 2012; Liu et al., 2023). In addition to the secretion of organic acids, microorganisms capable of phosphate dissolution release enzymes such as phosphatase and many other external agents to break down the insolubility of phosphate and improve its availability for uptake by plants (Liu et al., 2020). The production of organic acids and enzymes such as phosphatase and phytase by phosphate-solubilizing bacteria is as important a cause in the process of solubilization as any other that can be named (Walpola et al., 2012).

Microorganisms offer several approaches to stimulate plant growth, including phosphate dissolution. Microbes produce siderophores that help plants absorb iron and promote healthy growth (Nithyapriya et al., 2021). They also concluded that nitrogen-fixing bacteria such as Rhizobium not only fix atmospheric nitrogen and solubilize phosphate but can also provide plants with essential nutrients. Moreover, they added that microbes that produce indole-3-acetic acid (IAA) stimulate root development and growth, increasing the efficiency of nutrient uptake. Finally, the development of biofilms by beneficial rhizobacteria promotes the colonization of plants and improves their nutrient uptake. It also helps them defend against diseases and provides life-saving protection against environmental stresses such as droughts. Therefore, the diverse activities of plant growth-promoting rhizobacteria, including phosphate solubilization, siderophore production, nitrogen fixation, IAA production and biofilm formation, hold great promise for increasing plant productivity and resilience to challenging environmental conditions.

The present study aims to isolate PGPR strains producing ACC deaminase from the rhizosphere of drought tolerant wheat cultivars and to investigate their influence on the development of Egyptian wheat under drought stress conditions. This research will elucidate the mechanisms by which PGPR improve drought tolerance in wheat and thus provide valuable insights into new strategies for sustainable agriculture in the face of climate change.

2. Materials and Methods

2.1. Rhizosphere samples collection

The rhizospheric soil samples were collected from different Egyptian wheat cultivars in January 2022: Giza 168, Misr 1, Gemiza-9, Gemiza-11, and Shandweel 1 being the most promising wheat cultivars developed by Agricultural Research Center (ARC), Giza, Egypt. The experiment was carried out in the experimental field station of the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt, with coordinates 30°01'25" N, 31°12'11" E. Five plants of each wheat cultivar were aseptically uprooted and brought to the laboratory in sterile bags. The roots were carefully shaken mechanically, using the clumps of soil loosely adhering to the roots and the soil particles firmly adhering to the roots. The soil from the rhizospheres was suspended and vortexed in 10 ml of sterile 1% NaCl solution, and the suspension was used to isolate the bacteria from the rhizospheres.

2.2. Isolation of ACC deaminase-producing bacteria

The rhizobacteria were isolated from the rhizosphere suspension on a nutrient agar medium using the serial dilution method. Appropriate dilutions of the samples were plated on Luria-Bertani nutrient agar medium and then incubated at 28ºC for 48 hours. The morphologically distinguishable colonies were then inoculated into a sterile minimum DF salt medium with an infrared heater, using 3 mM ACC instead of (NH4)2 SO4 as the nitrogen source. The plates were then incubated for 3 days at a temperature of 28°C and checked daily for bacterial growth. Those that grew were classified as ACC deaminase producers and subcultured to obtain purified isolates.

2.3. Phosphate solubilization assay

ACC-positive isolates were spot inoculated on Pikovaskya's agar medium (Pikovskaya, 1948) containing 0.5% tricalcium phosphate as a source of recalcitrant Phosphate. The inoculated plates were incubated at 28°C for 7 days and growth was monitored until the seventh day. Bacterial colonies that showed clear halo zones around bacterial growth were "phosphate solubilizers".

2.4. Siderophore production assay

The siderophore production assay was performed on a Chrom Azurol S (CAS) agar plates using a chemically defined medium (Schwyn and Neilands, 1987). The *A. brasilense* strains were grown for two days at 28 °C in a rotary shaker at 140 rpm in NB. Then 0.05 ml of the cultured bacteria were spotted in triplicate onto CAS agar plates and incubated at 28 °C for 7 days. The formation of orange halos around the colonies on the blue CAS agar shows the ability of the bacterial strains to produce siderophores. The ratio of halo diameter to colony diameter was determined for five different replicates and the data were expressed as mean ± standard deviation (SD).

2.5. Identification of isolates with nitrogen fixing activity

The nitrogen-fixing ability of the bacterial isolates was qualitatively assessed by growing the bacteria in nitrogen-free (NF) medium containing (g/L) 20g mannitol, 0.2g K2 HPO4, 0.2g NaCl, 0.2g MgSO4.7H2O, 0.1g K2 SO4, 5g CaCO3 and 20g agar (Khoury et al., 2015). After 48 hours of incubation at 28°C, the growth of the isolates was measured.

2.6. The activity of isolates against fungal pathogen

In brief, a 0.5 cm 2 disc containing active fungal mycelia of *Rhizoctonia solani* was placed in the center of a 90 mm disposable plastic Petri dish containing PDA medium. Bacterial isolates aseptically skimmed from this overnight culture were arranged in a square at equal distances around the fungal disc. For the untreated control, a fungal disc was placed on PDA medium as previously shown and only sterile water was sprinkled on top. Growth was measured every day while all plates were incubated at 28 °C for 8 days. For the untreated control, a fungal disc was placed on a PDA medium as previously shown and sprinkled with sterile water only. Growth was measured every day while all plates were incubated at 28 °C for 8 days.

2.7. Indole acetic acid production

The rhizobacterial isolates were inoculated in Luria Broth LB medium supplemented with 5 Mm tryptophan and then incubated for 7 days at 28°C and 200 rpm on an orbital shaker. Salkowski reagent 0.5M FeCl3 70% perchloric acid was used to quantify IAA formation using the colorimetric technique Salkowski reagent 0.5M FeCl3 70% perchloric acid. The development of a red color, indicating the synthesis of indolic compounds, when the Salkowski reagent is added 4:1 to the cell-free culture supernatant was measured at 530 nm using a UV-vis spectrophotometer (Gordon & Weber, 1951). This allowed quantification of IAA using pure indole-3-acetic acid as the standard. A standard curve was prepared using pure IAA in the range of 0 to 100 g mL 1, one can calculate the concentration of yield Five different replicates of each sample were taken.

2.8. Biofilm formation assay

The bacterial isolates were cultivated aerobically overnight in a nutrient broth medium. To achieve an optical density of 0.02 at 600 nm, the cultures were diluted with fresh NB medium. A 96-well polystyrene plate was used for the bacterial cultures. 160 µl of the culture was injected into each well. The cultures were left at 28 °C for 48 hours. At the end of the incubation, the amount of biofilm developed was measured using a crystal violet staining method (O'Toole, 2011). The wells of the microtiter plate were pumped and gently washed at least five times with dd H2O to remove weakly bound bacterial cells and allowed to stand at room temperature for 30 minutes. The samples were then stained by adding 200 µl of 0.1 percent crystal violet to each well. After 20 minutes of incubation at 28 °C, the plates were washed. The wells of the plates were cleaned. An amount of 70 percent ethanol was used to determine the amount of crystal violet stain added to each dry well. After incubation for 20 minutes, the absorbance was determined using a plate reader at 590 nm. Five replicates were performed for each sample.

2.9. In vitro assay for stress tolerance in response to drought

Bacterial isolates were grown in triplicate on NB medium modified with 0%, 10%, 15% and 20% PEG6000 at 28°C for 24 hours at 160 rpm in a shaking incubator to evaluate desiccation tolerance. At different stress levels, the growth of the isolates was determined using a UV-Vis spectrophotometer to measure optical density (O.D.) at 600 nm. Five different replicates were performed for each sample.

2.10. Genomic DNA isolation and Molecular characterization

Bacterial DNA genomes were extracted during molecular characterization (Chen and Kuo, 1993). The isolates were genotyped using BOX-PCR with BOX-A1R primer (5′-CATACGGCAAGGCGACGCT-3′) as described by Li et al. (2015). The PCR contained 1µM of the primer reaction from the 2µM stock, 1 X PCR buffer containing 1.5 mM Mg 2+, 10 mM of each dNTP, and 2 units of Taq DNA polymerase (DreamTaq) Hot Start DNA polymerase. PCR was performed by initial denaturation for 5 minutes at 95 °C and then 35 cycles of denaturation at 94 °C for 1 minute, annealing at 50 °C for 60 seconds, extended at 72 °C for 60 seconds, and then a synthesis cycle at 72 °C for 10 minutes. Amplification was performed using the ABI 2720 Applied Biosystem thermal cycler. Agarose (2%) in Tris Acetic acid EDETA buffer was used for gel electrophoresis to separate the DNA.

The 16S rRNA gene-specific primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) were used for PCR amplification. PCR was performed as described above, except that the annealing temperature was 55 °C for 60s.

The PCR products of 1500 bp were later purified using QIA quick PCR kit from QIAGEN and sent to Macrogen, Korea for sequencing. The obtained sequence was compared with the GenBank databases using the BLAST (Basic Local Alignment Search Tool) algorithm available through the National Center for Biotechnology Information (NCBI) (National Center for Biotechnology Information, 2023) The sequences were deposited in NCBI GenBank and assigned accession numbers)168/1, accession number OR680806; 168/2, accession number OR680807; 168/4, accession number OR680808; 168/7, accession number OR680809; Shd5, accession number OR680812; G11/5, accession number OR680810; and M8, accession number OR680811) and further analyzed using MEGA X software by a neighbor-joining method to obtain the evolutionary relationship (Kumar et al., 2018).

2.11. The effect of potential bacteria on wheat under field conditions

Wheat seeds (Gemmeiza 9 cultivar) were surface sterilized with 70% alcohol for 1 min and then with 3.5% hypochlorite for 5 min and rinsed 5 times with sterile distilled water (Long et al., 2008). Selected potential PGPR were cultured overnight at 28°C on nutrient broth medium to produce the inoculum. Cells developed in the exponential phase were separated by centrifugation (14,000 rpm for 7 minutes) and then washed twice to obtain pure cells. The collected cells were resuspended in nutrient broth and carefully adjusted to a final concentration of 108 CFU/ml.

Surface-sterilized wheat was dipped into the inoculated nutrient solution and incubated for 1 hour at 28°C with a potential PGPR isolate. The treated seeds were spread on sterilized filter paper and air-dried in a laminar flow hood. The control seeds were treated in the same way as the PGPR-treated seeds, with the difference that sterilized deionized water was used instead of the possible PGPR isolate. Treated and controlled seeds were grown under field conditions. After three months, the wheat grown from seed was subjected to a drought treatment by not applying water for two months. The growth and yield parameters (plant height, ear length, number of shoots, number of grains, weight of 100 grains and total biomass) were measured on the wheat plants after the drought stress treatment.

The trial was designed in a randomized complete block design (RCBD) with 4 blocks and 4 plots. In each block, there was one plot for the control wheat plants and the remaining three plots were for wheat plants treated with different potential plant growth-promoting rhizobacteria (PGPR) treatments. A total of ten plants were selected from the two middle rows of each plot to evaluate plant growth and various growth-related parameters. In this study, the effects of 3 PGPR treatments on the morphological characteristics of wheat plants were investigated, with T1 (treatment 1) representing the control group, T2 (treatment 2) wheat plants inoculated with isolate 168/4, T3 (treatment 3) wheat plants inoculated with isolate 168/7, and T4 (treatment 4) wheat plants inoculated with isolate M8.

2.12. Statistical analysis

The statistical analysis was performed using the analysis of variance (ANOVA) function of the Statistical Package for Social Sciences (SPSS) version 19 software. A one-way ANOVA followed by a Duncan´s Multiple Range Test at the 0.05 level of significance to compare the means between the different groups. Finally, the differences between group means were statistically analyzed using this test. The results were presented as mean ± standard deviation (SD), which is a measure of central tendency and dispersion. We also used the MS Office 365 suite, more specifically Microsoft Excel, to calculate the standard error and mean, which allowed us to analyze the variability and precision data. To illustrate all these features and display the actual data in a graph, Excel was used to create several charts and graphical outputs for a clear visual interpretation of the results. These included bar charts, line graphs, and scatter plots to illustrate the comparisons of trends and relationships between data sets.

3. Results

3.1. Identification of ACC deaminase-producing bacteria and qualitative assessment of PGR characteristics

A total of 73 microbial isolates were successfully isolated from the rhizosphere of different wheat cultivars. Of these isolates, 30 showed the ability to produce ACC (1-aminocyclopropane-1-carboxylate) deaminase. The microbial isolates obtained from the rhizosphere of different wheat varieties showed pronounced plant growth-promoting properties (Table 1). The results of the tests for phosphate dissolution, siderophore production and nitrogen fixation showed variability between isolates (Figure 1). In the antifungal assay, G168/4 and G168/7 showed significant antifungal activity (Figure 2). Certain strains, namely G168/1, G168/2, G168/4, G168/7, G11/5, M8 and Shd5, were selected for qualitative PGPR assays (IAA production, biofilm formation and desiccation tolerance) as they had shown remarkable performance and favorable results in previous quantitative PGPR assays.

3.2. Quantitative evaluation of PGR characteristics

The potential of selected PGPR isolates was investigated for their ability to produce indoleacetic acid (IAA). The results showed significant differences in IAA production between the different isolates (Figure 3). Among the tested isolates, M8 showed the highest IAA concentration and recorded a significant value of 206.53 µg/ml. In contrast, G168/4 and G168/2 had relatively low I.A.A. concentrations, with values of 2.40 µg/ml and 2.72 µg/ml respectively. Isolates G168/1, G168/7, and Shd5 had moderate I.A.A. concentrations, with values of 3.96 µg/ml, 4.21 µg/ml, and 3.81 µg/ml, respectively. These isolates exhibited moderate IAA production capacity. Interestingly, G11/5 had a relatively high I.A.A. concentration of 9.33 µg/ml.

The biofilm production capacities of different PGPR (plant growth-promoting rhizobacteria) isolates were evaluated based on their optical density (OD) measurements at 570 nm. Among the tested isolates, G168/4 showed the highest optical density at 570 nm, recording a

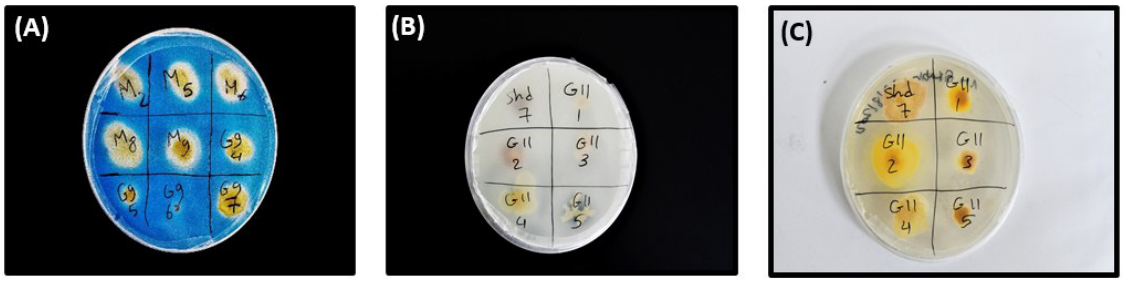

Figure 1. Qualitative assays performed to assess various plant growth-promoting traits in different bacterial isolates. The assays were conducted on agar plates, with each section of the plate representing a different isolate. (A) Siderophore Production Assay: siderophore production by various bacterial isolates on Chrome Azurol (CAS) agar plates. Siderophore production is indicated by the formation of yellow-orange halos around the bacterial colonies, signifying the chelation of iron from the blue dye. Representative isolates showing prominent yellow-orange halos include G9/7, G9/4, and M8; (B) Phosphate Solubilization Assay; represents the phosphate solubilization capacity of different isolates on Pikovskaya's agar plates. The formation of clear zones around the colonies indicates the solubilization of inorganic phosphate. Representative isolates such as G11/1 and Shd7 demonstrate clear zone, indicating their ability to solubilize phosphate, and (C) Indole-3-Acetic Acid (IAA) Production Assay; representing the IAA production by different isolates on agar plates supplemented with L-tryptophan. The development of pink to red zones around the colonies indicates the presence of IAA. Representative isolates with significant pink to red zones include G11/2 and Shd5, indicating higher levels of IAA production

remarkable value of 2.93 (Figure 4). Shd5 and M8 also showed relatively high optical densities of 1.47 and 1.49, respectively, indicating their considerable potential for biofilm production. G168/7, with an optical density of 1.05, falls in a medium range of biofilm production. On the other hand, G168/1 and G11/5 had lower optical densities of 0.78 and 0.45, respectively. G168/2 had the lowest optical density of 0.19 at 570 nm.

3.3. Assessment of ACC deaminase-producing bacteria for drought tolerance

Microbial isolates showed different growth patterns under different concentrations of polyethylene glycol (PEG) as shown in Figure 5. Interestingly, isolate 168/4 exhibited the highest growth at 0% PEG with an absorbance of approximately 1.4 OD, but its growth significantly decreased with increasing PEG concentrations. On the other hand, isolates M8 and Shd5 maintained relatively high growth across all PEG concentrations, suggesting better tolerance to drought stress. Nevertheless, growth of isolates 168/2 and G11/5 was notably reduced under higher PEG concentrations, indicating lower drought tolerance. Also, isolate 168/1 showed moderate growth at 0% and 5% PEG but experienced significant reduction at 15% and 20% PEG.

3.4. Molecular profiling and characterization of ACC deaminase-producing bacteria

Optimization of the BOX PCR amplification settings enabled the detection of different fingerprint patterns for each bacterial isolate. This analysis resulted in eight separate electrophoretic profiles corresponding to the eight isolates, demonstrating the uniqueness of each bacterial strain. A 1,500 base pair segment of the 16S rRNA gene was isolated and analyzed by sequencing. All 7 bacterial strains were assigned assigned to different phyla including α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, and Firmicutes.. Of the seven microbial isolates, two

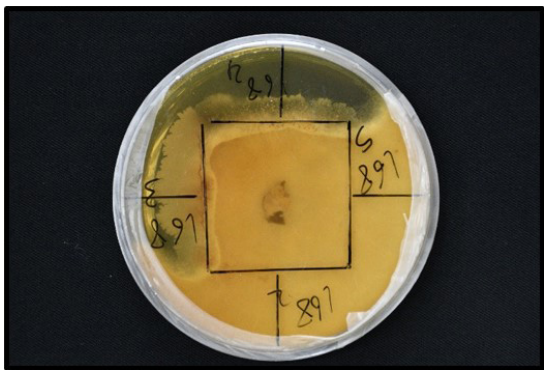

Figure 2. Antifungal activity of a representative plant growth-promoting rhizobacteria (PGPR) isolates against a pathogenic fungal strain. The assay was performed on a potato dextrose agar (PDA) plate. The plate is divided into four quadrants, each representing a different PGPR isolate. The central area contains the pathogenic fungal strain, and the antifungal activity is indicated by the inhibition zones around the bacterial colonies, indicating fungal growth suppression.

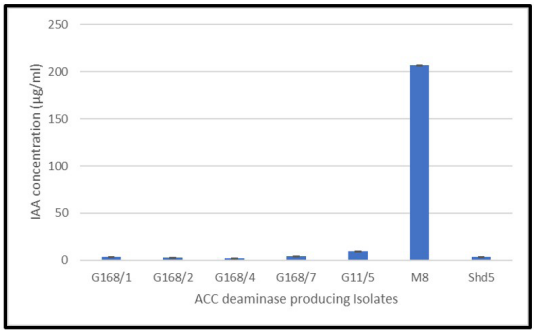

Figure 3. Indole-3-Acetic Acid (IAA) concentration (µg/ml) produced by various ACC deaminase-producing bacterial isolates. The x-axis represents the different selected isolates: G168/1, G168/2, G168/4, G168/7, G11/5, M8, and Shd5. The y-axis shows the IAA concentration (µg/ml). Each value represents the mean ± SD of five replicates.

Table 1. summary of the functional traits of various ACC deaminase-producing bacterial isolates, including their abilities in nitrogen fixation, siderophore production, and phosphate solubilization. These traits are key indicators of the isolates' potential to promote plant growth and enhance soil fertility.

Isolate	N2 fixation	Siderophore production	Phosphate solubilization
168/1	++	++	+
168/2	++	+++	-
168/3	+++	-	-
168/4	+++	++	-
168/5	++	+	-
168/6	+	++	-
168/7	+	++++	-
168/8	+	+	-
G9/4	+++	+	-
G9/5	+	+	-
G9/6	+	+	-
G9/7	+++	+	-
G9/8	++	+	-
G9/9	+++	+	-
G11/1	+	+	-
G11/2	++	+	-
G11/3	+	-	-
G11/4	++	+	-
G11/5	+	-	++
M2	++	+	-
M5	+	+	-
M6	++	++	-
M8	+	+	-
M9	+	++	-
S2	+	+	-
S7	+	-	-
Shd3	+	+	-
Shd5	+++	++	-
Shd6	++	+++	-
Shd7	++	+	-

- = No activity; + = Low activity; ++ = Moderate activity; +++ = High activity; ++++ = Very high activity.

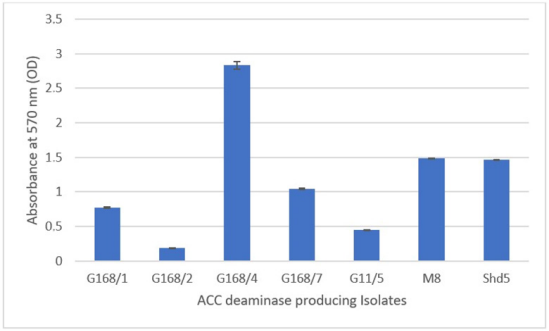

Figure 4. Assessment of the biofilm production capacities of various ACC deaminase-producing bacterial isolates, measured by absorbance (570 nm). The x-axis represents the different isolates: G168/1, G168/2, G168/4, G168/7, G11/5, M8, and Shd5. The y-axis shows the absorbance values (570 nm), which is indicative of the biofilm biomass produced. Each value represents the mean ± SD of five replicates.

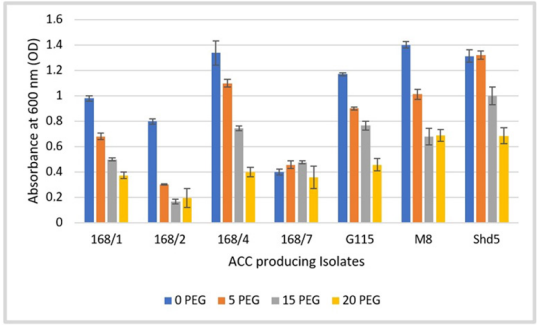

Figure 5. Growth patterns of different ACC deaminase-producing bacterial isolates under different concentrations of polyethylene glycol (PEG), measured by absorbance at OD =600 nm. The x-axis represents the selected isolates: 168/1, 168/2, 168/4, 168/7, G11/5, M8, and Shd5. The y-axis represents the absorbance values (600 nm), indicating bacterial growth. Each isolate's growth was tested under four different PEG concentrations: 0%, 5%, 15% and 20%. Each value represents the mean ± SD of five replicates.

strains belonged to the genus Pseudomonas, namely 168/1 (Pseudomonas sp.) and 168/2 (Pseudomonas sp.), one strain belonged to the genus Variovorax, namely 168/4 (*Variovorax paradoxus*), one strain belonged to the genus Bacillus, namely 168/7 (*Bacillus subtilis*), one strain belonged to the genus *Achromobacter*, namely G11/5 (*Achromobacter* sp.), one strain belonged to the genus Klebsiella, namely M8 (*Klebsiella oxytoca*), and one strain belonged to the genus Phyllobacterium, namely Shd5 (*Phyllobacterium phragmitis*). To illustrate the relationships between the tested isolates and other related bacteria, a phylogenetic tree was constructed using the 16S rDNA data (Figure 6).

3.5. Evaluation of agronomic traits in wheat inoculated with ACC deaminase-producing bacteria

Based on their PGP properties and their ability to tolerate higher PEG concentrations in an LB medium, the final isolates 168(4), 168(7) and M8 were selected for inoculation of Egyptian wheat seeds. Measurements of growth and yield traits were documented after the produced wheat plants were placed under drought stress. An analysis of variance (ANOVA) was performed to evaluate the differences in plant morphological traits among the different treatments, followed by a post-hoc least significant difference (LSD)

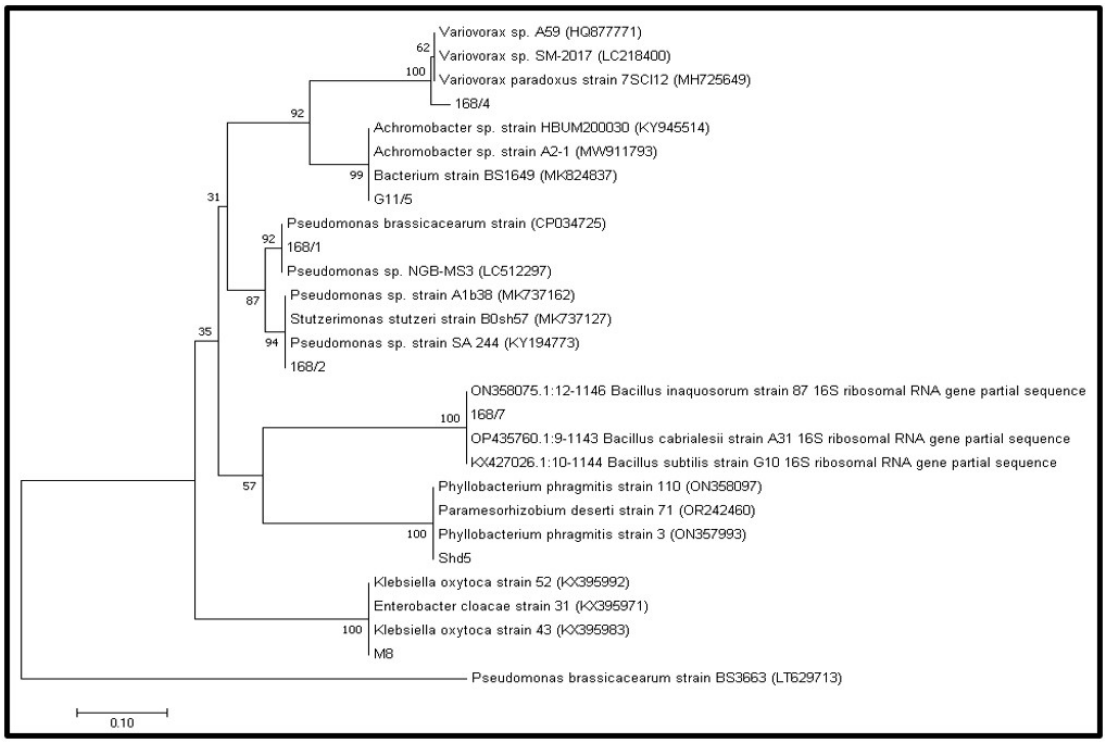

Figure 6. Phylogenetic tree illustrates the relationships among the seven selected ACC deaminase-producing bacterial isolates based on the analysis of their 16S rRNA gene sequences. The tree was constructed using the neighbor-joining method, with bootstrap values (expressed as percentages of 1000 replications) shown at branch points to indicate the reliability of each branch

test to further investigate these differences. The effects of the different treatments with different bacterial isolates on plant height were evaluated (Figure 7a).

Among these, wheat plants treated with *Bacillus subtilis* (T3) showed a remarkable and statistically significant increase in plant height of 27.59%. This was closely followed by *Variovorax paradoxus* (T2) with a significant increase of 24.68% compared to the untreated control plants (T1). In addition, plants inoculated with *Klebsiella oxytoca* (T4) showed a significant increase in height of 21.21% compared to the untreated control plants. Wheat ear length increased in all treatment groups (Figure 7b), but a significant improvement of 22.45% was observed only in wheat plants inoculated with *Bacillus subtilis* (T3) compared to the untreated control group. Wheat plants inoculated with *Variovorax paradoxus* (T2) also showed a significant increase in ear length with an improvement of 20.41%. On the other hand, wheat plants treated with *Klebsiella oxytoca* (T4) showed the smallest change in ear length compared to the control group, with a minimal change of 18.37%.

As far as the number of grains is concerned, the wheat plants inoculated with *Bacillus subtilis* (T3) showed the clearest and statistically significant improvement. They showed a remarkable 26.69% increase in grains compared to the untreated control group (Figure 7c). In second place were the plants treated with *Variovorax paradoxus* (T2), which showed a moderate increase in grains of 6.77. Although this increase was considerable, it was not statistically significant. On the other hand, the wheat plants treated with *Klebsiella oxytoca* (T4) also showed a considerable increase in the number of grains with an increase of 3.18%. However, as with T2, this increase was not statistically significant.

Regarding the number of tillers, the wheat plants inoculated with *Bacillus subtilis* (T3) showed the clearest and statistically significant improvements, with a remarkable increase of 70.83% compared to the untreated control group (Figure 7d). In second place, the plants treated with *Variovorax paradoxus* (T2) showed a marked increase in tillering of 62.5, which was also statistically significant. On the other hand, the wheat plants treated with *Klebsiella oxytoca* (T4) showed a remarkable increase in pollen count of 33.33%, which was not statistically significant.

In terms of 100-grain weight, treatment 3 showed a remarkable increase of 58.14% compared to the control group, indicating that the introduction of *Bacillus subtilis* (T3) significantly promoted the growth of the wheat plant (Figure 7e). This was closely followed by treatment 2 with a significant increase of 50.30%, indicating that *Variovorax paradoxus* (T2) also had a positive effect on plant growth compared to the untreated control plants. Treatment 4 with *Klebsiella oxytoca* (T4) still showed a remarkable increase in plant weight by 42.75 compared to the control group, albeit to a slightly lesser extent.

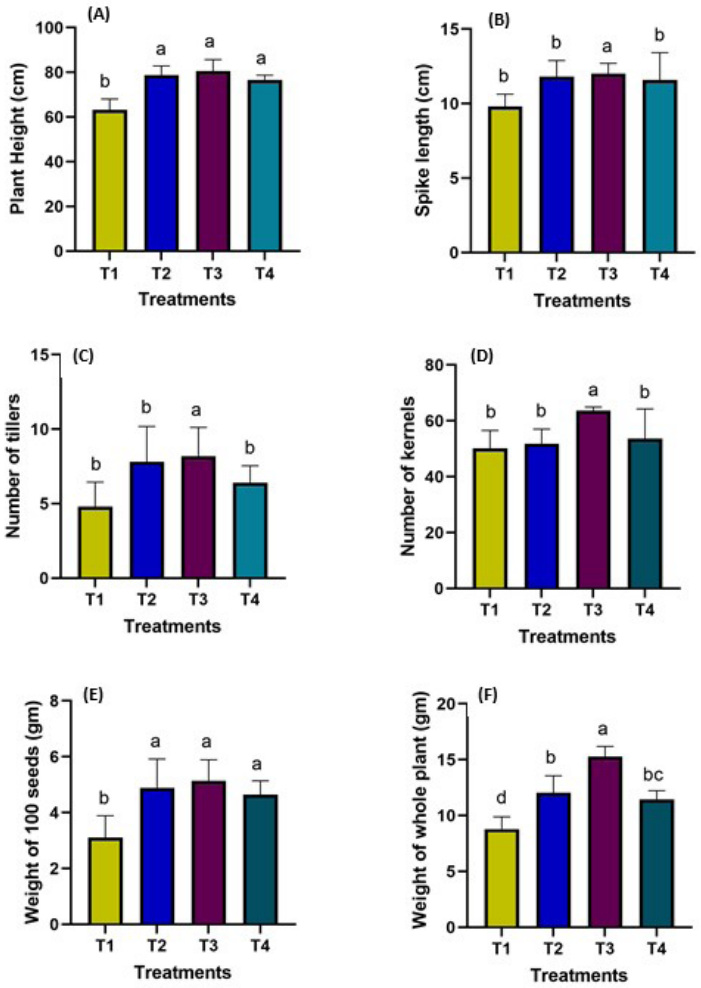

Figure 7. Comparative analysis of plant growth-promoting rhizobacteria (PGPR) traits in different wheat cultivars under drought stress conditions; Plant Height (A), Spike Length (B), Number of Tillers (C), Number of Kernels (D), Weight of 100 Seeds (E), and Whole Plant Weight (F). Each panel represents a different PGPR trait, measured under varying drought stress conditions (0% PEG, 5% PEG, 15% PEG, and 20% PEG). Data represents the mean values of five replications ± SD. Lowercase letters placed above the bars indicate statistically significant differences between the treatments at a significance level of $P < 0.05$

When evaluating the influence of the treatments on the total biomass of the wheat, treatment 3, in which the wheat plants were inoculated with *Bacillus subtilis*, proved to be the most remarkable performance (Figure 7f). It showed a significant increase, which was about 52.9% higher than that of the untreated control group. Followed by treatment 2, in which *Variovorax paradoxus* was inoculated, which showed a remarkable effect with an increase in total biomass of about 20.6 . Treatment 4, in which *Klebsiella oxytoca* was inoculated, showed a slightly lower effect, but still contributed to a considerable increase in total biomass of 14.7 compared to the control group.

4. Discussion

Plant Growth-Promoting Rhizobacteria (PGPR) have been widely investigated for their positive influence on wheat growth, especially under drought stress conditions. Salem et al. (2024) reported more growth parameters and stress tolerance indicators such as increased biomass production, better root and shoot/shoot length and development in wheat plants inoculated with PGPR isolates. Moreover, Sedri et al. (2022) and Upadhyay et al. (2012) documented the allelopathic effects of PGPR on wheat under water stress conditions and saline soil and showed an increase in grain yield, mineral uptake, and antioxidant activity. Moreover, the study by Hafez et al. (2019) has underlined that PGPR and salicylic acid have synergistic effects in increasing wheat productivity and nutrient uptake under water-deficit conditions. Cumulatively, these findings emphasize the potential of PGPR in enhancing the performance of wheat against stress through the mechanism of enhanced nutrient availability, root development, and further in-built features of stress resistance in wheat, posing a promising sustainable

approach through PGPR in resolving the negative impact of a given environmental stressful conditions to wheat crops.

PGPRs possessing ACC deaminase activity have the potential to attenuate the inhibitory effects of ethylene on plant growth and improve plant stress resistance via phytohormone signaling pathways. This in turn supports plant growth and mitigates abiotic stress factors such as drought (Gupta et al., 2022). In our study, we initially collected a total of 73 bacterial isolates, but only 30 of them were found to have ACC deaminase activity. Subsequently, all bacterial isolates were subjected to a comprehensive assessment of their plant growth-promoting rhizobacteria (PGPR), which included both qualitative and quantitative assessment.

Phosphate dissolution and the production of siderophores are crucial properties that contribute to improved plant growth. While most isolates in our study showed limited phosphate solubilization, isolates G11/5, G168/4 and G168/7 demonstrated remarkable abilities in both phosphate solubilization and siderophore production. These results are in line with previous studies emphasizing the importance of these traits in improving nutrient availability and iron uptake for plant health (Ahemad and Kibret, 2014). Isolates G168/4 and G168/7 exhibited strikingly high siderophore production, which may facilitate iron uptake by plants. This property is particularly important in nutrient-poor soils, where the availability of iron can limit plant growth (Pedraza, 2015).

Patten and Glick (2002) examining siderophore production and its correlation with drought resistance, found that the strain exhibiting higher siderophore production was associated with increased host plant drought resistance. In addition to their other traits, G168/4, G168/7 and G11/5 also showed marked antifungal activity. Antifungal properties in plant growth-promoting microbes are valuable for protecting plants from fungal pathogens and improving disease resistance (Omar et al., 2021).

The remarkable antifungal ability of G168/4 and G168/7 supports their potential as biocontrol agents for the control of fungal diseases in wheat cultivation. All isolates possessed the ability to fix nitrogen, an important property that can promote plant growth by providing an essential nutrient. This observation is consistent with the role of nitrogen-fixing PGPR in promoting plant health and productivity (Spaepen and Vanderleyden, 2011). The differences in the results of qualitative PGPR assessments could possibly be due to differences in the species studied, as found in previous studies (Kang et al., 2014).

The observed variation in IAA production between PGPR isolates emphasizes the importance of selecting appropriate strains for specific agricultural or bioremediation purposes. Isolate M8 showed the highest IAA concentration, clearly outperforming all other isolates with a value of 206.53 µg/ml. This high IAA production of M8 indicates that it could be an effective growth promoter in agriculture. In contrast, G168/4 and G168/2 had significantly lower IAA concentrations, indicating their limited ability to produce IAA. These isolates may have other beneficial properties and further studies are needed to explore their potential in different applications. Isolates G168/1, G168/7 and Shd5 exhibited moderate IAA concentrations, placing them in an intermediate range of IAA production capacity. These isolates could be valuable in situations where balanced promotion of plant growth without excessive hormone levels is required (Yarza et al., 2014). Interestingly, G11/5 exhibited a relatively higher IAA concentration compared to the mid-range isolates. This result suggests that G11/5 may be a promising candidate for promoting plant growth, especially under conditions where higher IAA content is desired.

Biofilm formation is an essential trait for PGPR as it can enhance their survival and colonization in the rhizosphere (Glick, 2014). Assessment of biofilm production ability based on optical density at 570 nm revealed considerable variability between the isolates tested. G168/4 showed the highest biofilm production potential with an optical density of 2.93. This result suggests that G168/4 may have a strong ability to form biofilms that can facilitate root colonization and subsequent promotion of plant growth (Compant et al., 2010). Shd5 and M8 also showed relatively high biofilm production capacities with optical densities of 1.47 and 1.49, respectively. These isolates may be suitable for applications where robust biofilm formation is desired. G168/7 falls in a medium range of biofilm production with an optical density of 1.05.

This intermediate ability indicates its potential for rhizosphere colonization and its ability to provide some biofilm-mediated benefit to plants. On the other hand, G168/1 and G11/5 showed a lower capacity for biofilm production. G168/2 showed the lowest optical density at 570 nm, indicating a limited potential for biofilm formation. These isolates may need further optimization or may be better suited for applications where extensive biofilm formation is not a primary requirement.

Our use of BOX-PCR to assess genetic diversity among ACC deaminase-producing bacteria is reminiscent of previous studies that have used similar molecular techniques. For example, Hardoim et al., 2012) used BOX-PCR to characterize bacterial isolates involved in plant-microbe interactions. Their results emphasized the importance of genetic diversity in shaping microbial functional potential, which is consistent with our observations. Sequencing of the 16S rRNA gene allowed us to classify the bacterial isolates into two distinct phyla: Alpha (α)-Proteobacteria and Beta (β)-Proteobacteria. This classification is consistent with the established taxonomic hierarchy of bacteria based on their 16S rRNA gene sequences (Etesami and Beattie, 2018).

The identification of Firmicutes and Gamma (γ)-Proteobacteria within the bacterial isolates analyzed further contributes to the diversity of the observed phyla. Within the alpha-Proteobacteria, two isolates were identified as belonging to the genus Pseudomonas (168/1 and 168/2), demonstrating their potential as effective ACC deaminase producers. Pseudomonas species are known for their plant growth-promoting abilities, making these isolates promising candidates for further investigation in agriculture (Beneduzi et al., 2012). The beta-proteobacterium *Variovorax paradoxus* (168/4) identified in our study is also known for its plant growth-promoting properties and ACC deaminase activity, further supporting the relevance of our

findings for agriculture (Saitou and Nei, 1987). Of the other genera identified, *Bacillus* (168/7), *Achromobacter* (G11/5), *Klebsiella* (M8) and *Phyllobacterium* (Shd5) were each represented by one isolate. These genera have different metabolic capabilities and have been associated with different plant-microbe interactions, demonstrating the potential versatility of ACC deaminase-producing bacteria in different ecological niches (Kumar et al., 2023).

Furthermore, our identification of the genera Pseudomonas, Variovorax, Bacillus, Achromobacter, Klebsiella and Phyllobacterium among ACC deaminase-producing isolates is consistent with previous reports (Kumar et al., 2023). These genera have been consistently associated with ACC deamination activity and promotion of plant growth. The presence of these genera in our study emphasizes their importance in different ecosystems and reinforces the idea that ACC deaminase-producing bacteria are widely distributed across different environments.

The construction of a phylogenetic tree to clarify the evolutionary relationships between our isolates is a widely used approach in microbial ecology. This methodological choice is consistent with the study by Saitou and Nei (1987). who laid the foundations of phylogenetic analysis using molecular data. Our phylogenetic tree showed a clear clustering of isolates within their respective genera and phyla, a pattern consistent with previous studies of bacterial taxonomic relationships (Etesami and Beattie, 2018).

Our results show that inoculation of wheat plants with plant growth-promoting rhizobacteria (PGPR) can significantly increase plant growth and biomass. Treatment 3, in which wheat plants were inoculated with *Bacillus subtilis*, was the most effective and resulted in a remarkable 64.28% increase in plant weight and 53.67% increase in total biomass compared to the untreated control group. This is consistent with previous studies that have shown that *Bacillus subtilis* is a highly effective PGPR that can promote plant growth and development through a variety of mechanisms, including nitrogen fixation, phosphate solubilization, and the production of phytohormones and other beneficial compounds (Pignata et al., 2016).

Treatment 2, in which the wheat plants were inoculated with *Variovorax paradoxus*, also resulted in a significant increase in plant growth and biomass, with a 38% increase in plant weight and a 14.54% increase in total biomass compared to the control group. *Variovorax paradoxus* is another known PGPR that has been shown to promote plant growth and development through a variety of mechanisms, including nitrogen fixation, phosphate solubilization and the production of phytohormones and other beneficial compounds (Caballero-Mellado et al., 2007; Marra et al., 2011).

Treatment 4, in which the wheat plants were inoculated with *Klebsiella oxytoca* , resulted in a smaller but still significant increase in plant growth and biomass, with a 30.44% increase in plant weight and a 2.89% increase in total biomass compared to the control group. *Klebsiella oxytoca* is a lesser known PGPR than *Bacillus subtilis* and *Variovorax paradoxus*, but has been shown to promote plant growth and development through similar mechanisms.

Overall, the results of this study suggest that inoculation of wheat plants with PGPRs can be an effective strategy to increase plant growth and biomass. Which PGPRs are most effective will likely depend on the plant species, soil conditions and other environmental factors. However, the results of this study suggest that *Bacillus subtilis*, *Variovorax paradoxus* and *Klebsiella oxytoca* are all promising candidates for use as PGPRs to promote wheat growth and biomass.

The discussion of the limitations of the study seems to be noteworthy. Firstly, variability in field conditions may affect the efficacy of PGPR in the real world. For instance, soil type, climate, and microbial diversity are subject to the need for in-depth research. It is necessary to explore the long-lasting effects of these PGPR on the balance of the soil microbiomes. Secondly, it is important to consider the ecological implications of using PGPR without detailed studies. Thirdly, there is a need to investigate the impact of the studied PGPR on other crops. Thus, a relevant topic is cross-crop studies.

Proposed future research directions include conducting extensive field trials across different geographical regions and soil types to validate the effectiveness of these PGPR under diverse agricultural conditions. Also, we need to investigate the long-term impacts of PGPR application on soil health, crop rotation, and soil microbial communities to ensure sustainable use. Finally, detailed mechanistic studies explore the molecular mechanisms by which these PGPR confer drought tolerance to further enhance their effectiveness through genetic or biotechnological interventions.

5. Conclusion

In summary, this research highlights the use of ACC deaminase-producing plant growth-promoting rhizobacteria (PGPR) to improve the resistance of wheat to drought. The practical applications cover many aspects. The first is sustainable agriculture. The identified PGPR strains, such as *Bacillus subtilis*, *Variovorax paradoxus* and *Klebsiella oxytoca,* can be applied as biofertilizers to wheat crops to promote growth and yield under drought stress. This approach reduces the need for chemical fertilizers and also mitigates drought. By improving the drought tolerance of wheat, these PGPRs can help maintain crop yields in arid and semi-arid regions and ensure food security despite adverse climatic conditions. In addition, they contribute to soil health by improving soil fertility through mechanisms such as phosphate dissolution and nitrogen fixation, thereby increasing soil health and overall crop productivity. These findings can inform farmers' cropping strategies and enable the integration of microbial inoculants into integrated pest and nutrient management systems.

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References

AHEMAD, M. and KIBRET, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University. Science*, vol. 26, no. 1, pp. 1-20. http://doi.org/10.1016/j.jksus.2013.05.001.

AL-TURKI, A., MURALI, M., OMAR, A.F., REHAN, M. and SAYYED, R.Z., 2023. Recent advances in PGPR-mediated resilience toward interactive effects of drought and salt stress in plants. *Frontiers in Microbiology*, vol. 14, pp. 1214845. http://doi.org/10.3389/fmicb.2023.1214845. PMid:37829451.

BENEDUZI, A., AMBROSINI, A. and PASSAGLIA, L.M., 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology*, vol. 35, no. 4, suppl., pp. 1044-1051. http://doi.org/10.1590/S1415-47572012000600020. PMid:23411488.

CABALLERO-MELLADO, J., ONOFRE-LEMUS, J., ESTRADA-DE LOS SANTOS, P. and MARTINEZ-AGUILAR, L., 2007. The tomato rhizosphere, an environment rich in nitrogen-fixing Burkholderia species with capabilities of interest for agriculture and bioremediation. *Applied and Environmental Microbiology*, vol. 73, no. 16, pp. 5308-5319. http://doi.org/10.1128/AEM.00324-07. PMid:17601817.

CAREZZANO, M.E., ALVAREZ STRAZZI, F.B., PÉREZ, V., BOGINO, P. and GIORDANO, W., 2023. Exopolysaccharides Synthesized by Rhizospheric Bacteria: A Review Focused on Their Roles in Protecting Plants against Stress. *Applied Microbiology*, vol. 3, no. 4, pp. 1249-1261. http://doi.org/10.3390/applmicrobiol3040086.

CHEN, W.P. and KUO, T.T., 1993. A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic Acids Research*, vol. 21, no. 9, pp. 2260. http://doi.org/10.1093/nar/21.9.2260. PMid:8502576.

COMPANT, S., CLÉMENT, C. and SESSITSCH, A., 2010. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology & Biochemistry*, vol. 42, no. 5, pp. 669-678. http://doi.org/10.1016/j.soilbio.2009.11.024.

DANISH, S. and ZAFAR-UL-HYE, M., 2019. Co-application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. *Scientific Reports*, vol. 9, no. 1, pp. 5999. http://doi.org/10.1038/s41598-019-42374-9. PMid:30979925.

ETESAMI, H. and BEATTIE, G.A., 2018. Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Frontiers in Microbiology*, vol. 9, pp. 317452. http://doi.org/10.3389/fmicb.2018.00148. PMid:29472908.

GLICK, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research*, vol. 169, no. 1, pp. 30-39. http://doi.org/10.1016/j.micres.2013.09.009. PMid:24095256.

GORDON, S.A. and WEBER, R.P., 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiology*, vol. 26, no. 1, pp. 192-195. http://doi.org/10.1104/pp.26.1.192. PMid:16654351.

GUPTA, A., MISHRA, R., RAI, S., BANO, A., PATHAK, N., FUJITA, M., KUMAR, M. and HASANUZZAMAN, M., 2022. Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. *International Journal of Molecular Sciences*, vol. 23, no. 7, pp. 3741. http://doi.org/10.3390/ijms23073741. PMid:35409104.

HAFEZ, E., OMARA, A.E.D. and AHMED, A., 2019. The coupling effects of plant growth promoting rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions. *Agronomy (Basel)*, vol. 9, no. 9, pp. 524. http://doi.org/10.3390/agronomy9090524.

KANG, S.M., RADHAKRISHNAN, R., KHAN, A.L., KIM, M.J., PARK, J.M., KIM, B.R., SHIN, D.H. and LEE, I.J., 2014. Gibberellin secreting rhizobacterium, Pseudomonas putida H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiology and Biochemistry*, vol. 84, pp. 115-124. http://doi.org/10.1016/j.plaphy.2014.09.001. PMid:25270162.

KHOURY, C.K., HEIDER, B., CASTAÑEDA-ÁLVAREZ, N.P., ACHICANOY, H.A., SOSA, C.C., MILLER, R.E., SCOTLAND, R.W., WOOD, J.R., ROSSEL, G., ESERMAN, L.A., JARRET, R.L., YENCHO, G.C., BERNAU, V., JUAREZ, H., SOTELO, S., DE HAAN, S. and STRUIK, P.C., 2015. Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato [Ipomoea batatas (L.) Lam., I. series Batatas]. *Frontiers in Plant Science*, vol. 6, pp. 251. http://doi.org/10.3389/fpls.2015.00251. PMid:25954286.

KUMAR, D., SINGH, S.K., ARYA, S.K., SRIVASTAVA, D., RAJPUT, V.D. and HUSAIN, R., 2023. Multifunctional growth-promoting microbial consortium-based biofertilizers and their techno-commercial feasibility for sustainable agriculture. In: J.A. PARRAY, N. SHAMEEM, D. EGAMBERDIEVA and R.Z. SAYYED, eds. *Rhizobiome*. Cambridge: Academic Press, pp. 167-208. http://doi.org/10.1016/B978-0-443-16030-1.00010-9.

KUMAR, S., STECHER, G., LI, M., KNYAZ, C. and TAMURA, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, vol. 35, no. 6, pp. 1547-1549. http://doi.org/10.1093/molbev/msy096. PMid:29722887.

LANGRIDGE, P., ALAUX, M., ALMEIDA, N.F., AMMAR, K., BAUM, M., BEKKAOUI, F., BENTLEY, A.R., BERES, B.L., BERGER, B., BRAUN, H.-J., BROWN-GUEDIRA, G., BURT, C.J., CACCAMO, M.J., CATTIVELLI, L., CHARMET, G., CIVÁŇ, P., CLOUTIER, S., COHAN, J.-P., DEVAUX, P.J., DOOHAN, F.M., DRECCER, M.F., FERRAHI, M., GERMÁN, S.E., GOODWIN, S.B., GRIFFITHS, S., GUZMÁN, C., HANDA, H., HAWKESFORD, M.J., HE, Z., HUTTNER, E., IKEDA, T.M., KILIAN, B., KING, I.P., KING, J., KIRKEGAARD, J.A., LAGE, J., LE GOUIS, J., MONDAL, S., MULLINS, E., ORDON, F., ORTIZ-MONASTERIO, J.I., ÖZKAN, H., ÖZTÜRK, İ., PEREYRA, S.A., POZNIAK, C.J., QUESNEVILLE, H., QUINCKE, M.C., REBETZKE, G.J., CHRISTOPH REIF, J., SAAVEDRA-BRAVO, T., SCHURR, U., SHARMA, S., SINGH, S.K., SINGH, R.P., SNAPE, J.W., TADESSE, W., TSUJIMOTO, H., TUBEROSA, R., WILLIS, T.G. and ZHANG, X., 2022. Meeting the challenges facing wheat production: The strategic research agenda of the Global Wheat Initiative. *Agronomy (Basel)*, vol. 12, no. 11, pp. 2767. http://doi.org/10.3390/agronomy12112767.

LI, Z., CHANG, S., YE, S., CHEN, M., LIN, L., LI, Y., LI, S. and AN, Q., 2015. Differentiation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase from its homologs is the key for identifying bacteria containing ACC deaminase. *FEMS Microbiology Ecology*, vol. 91, no. 10, pp. fiv112. http://doi.org/10.1093/femsec/fiv112. PMid:26362924.

LIU, S., ZENG, J., YU, H., WANG, C., YANG, Y., WANG, J., HE, Z. and YAN, Q., 2023. Antimony efflux underpins phosphorus cycling and resistance of phosphate-solubilizing bacteria in mining soils. *The ISME Journal*, vol. 17, no. 8, pp. 1278-1289.

LIU, Y.Q., WANG, Y.H., KONG, W.L., LIU, W.H., XIE, X.L. and WU, X.Q., 2020. Identification, cloning and expression patterns of the genes related to phosphate solubilization in Burkholderia multivorans WS-FJ9 under different soluble phosphate levels. *AMB Express*, vol. 10, no. 1, pp. 108. http://doi.org/10.1186/s13568-020-01032-4. PMid:32504154.

LONG, H.H., SCHMIDT, D.D. and BALDWIN, I.T., 2008. Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. *PLoS One*, vol. 3, no. 7, pp. e2702. http://doi.org/10.1371/journal.pone.0002702. PMid:18628963.

MARRA, L.M., OLIVEIRA, S.M.D., SOARES, C.R.F.S. and MOREIRA, F.M.D.S., 2011. Solubilisation of inorganic phosphates by inoculant strains from tropical legumes. *Scientia Agrícola*, vol. 68, no. 5, pp. 603-609. http://doi.org/10.1590/S0103-90162011000500015.

MICKKY, B., ALDESUQUY, H. and ELNAJAR, M., 2020. Effect of drought on yield of ten wheat cultivars linked with their flag leaf water status, fatty acid profile and shoot vigor at heading. *Physiology and Molecular Biology of Plants*, vol. 26, no. 6, pp. 1111-1117. http://doi.org/10.1007/s12298-020-00807-0. PMid:32549676.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION – NCBI, 2023 [viewed 21 October 2023]. Available from: https://www.ncbi.nlm.nih.gov.

NITHYAPRIYA, S., LALITHA, S., SAYYED, R.Z., REDDY, M.S., DAILIN, D.J., EL ENSHASY, H.A., SURIANI, N.L. and HERLAMBANG, S., 2021. Production, purification, and characterization of bacillibactin siderophore of Bacillus subtilis and its application for improvement in plant growth and oil content in sesame. *Sustainability (Basel)*, vol. 13, no. 10, pp. 5394. http://doi.org/10.3390/su13105394.

O'TOOLE, G.A., 2011. Microtiter dish biofilm formation assay. *Journal of Visualized Experiments : JoVE*, no. 47, pp. e2437. PMid:21307833.

OMAR, S.A., FETYAN, N.A., ELDENARY, M.E., ABDELFATTAH, M.H., ABD-ELHALIM, H.M., WROBEL, J. and KALAJI, H.M., 2021. Alteration in expression level of some growth and stress-related genes after rhizobacteria inoculation to alleviate drought tolerance in sensitive rice genotype. *Chemical and Biological Technologies in Agriculture*, vol. 8, no. 1, pp. 1-19. http://doi.org/10.1186/s40538-021-00237-4.

PATTEN, C.L. and GLICK, B.R., 2002. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, vol. 68, no. 8, pp. 3795-3801. http://doi.org/10.1128/AEM.68.8.3795-3801.2002. PMid:12147474.

PEDRAZA, R.O., 2015. Siderophores production by Azospirillum: biological importance, assessing methods and biocontrol activity. In: F. CASSÁN, Y. OKON and C. CREUS, eds. *Handbook for Azospirillum: technical issues and protocols*. Cham: Springer International Publishing, pp. 251-262. http://doi.org/10.1007/978-3-319-06542-7_14.

PIGNATA, G., NIÑIROLA, D., CASALE, M., EGEA-GILABERT, C., HERNÁNDEZ, J.A.F. and SENESI, N., 2016. Inherent quality and safety of watercress grown in a floating system using Bacillus subtilis. *The Horticulture Journal*, vol. 85, no. 2, pp. 148-153. http://doi.org/10.2503/hortj.MI-091.

PIKOVSKAYA, R.I., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17: 362–370. *Plant and Soil*, vol. 287, pp. 77-84.

SAINI, H.S. and WESTGATE, M.E., 1999. Reproductive development in grain crops during drought. *Advances in Agronomy*, vol. 68, pp. 59-96. http://doi.org/10.1016/S0065-2113(08)60843-3.

SAITOU, N. and NEI, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, vol. 4, no. 4, pp. 406-425. PMid:3447015.

SALEEM, M., ARSHAD, M., HUSSAIN, S. and BHATTI, A.S., 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *Journal of Industrial Microbiology & Biotechnology*, vol. 34, no. 10, pp. 635-648. http://doi.org/10.1007/s10295-007-0240-6. PMid:17665234.

SALEM, M.A., ISMAIL, M.A., RADWAN, K.H. and ABD-ELHALIM, H.M., 2024. Unlocking the Potential of Plant Growth-Promoting Rhizobacteria to Enhance Drought Tolerance in Egyptian Wheat (Triticum aestivum). *Sustainability (Basel)*, vol. 16, no. 11, pp. 4605. http://doi.org/10.3390/su16114605.

SCHWYN, B. and NEILANDS, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, vol. 160, no. 1, pp. 47-56. http://doi.org/10.1016/0003-2697(87)90612-9. PMid:2952030.

SEDRI, M.H., NIEDBAŁA, G., ROOHI, E., NIAZIAN, M., SZULC, P., RAHMANI, H.A. and FEIZIASL, V., 2022. Comparative analysis of plant growth-promoting rhizobacteria (PGPR) and chemical fertilizers on quantitative and qualitative characteristics of rainfed wheat. *Agronomy (Basel)*, vol. 12, no. 7, pp. 1524. http://doi.org/10.3390/agronomy12071524.

SPAEPEN, S. and VANDERLEYDEN, J., 2011. Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology*, vol. 3, no. 4, pp. a001438. http://doi.org/10.1101/cshperspect.a001438. PMid:21084388.

UPADHYAY, S.K., SINGH, J.S., SAXENA, A.K. and SINGH, D.P., 2012. Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biology*, vol. 14, no. 4, pp. 605-611. http://doi.org/10.1111/j.1438-8677.2011.00533.x. PMid:22136617.

WALPOLA, B.C., KEUM, M.J. and YOON, M.H., 2012. Influence of different ph conditions and phosphate sources on phosphate solubilization by Pantoea agglomerans DSM3493. *Han'guk T'oyang Piryo Hakhoe Chi. Han'guk T'oyang Piryo Hakhoe*, vol. 45, no. 6, pp. 998-1003. http://doi.org/10.7745/KJSSF.2012.45.6.998.

YARZA, P., YILMAZ, P., PRUESSE, E., GLÖCKNER, F.O., LUDWIG, W., SCHLEIFER, K.H., WHITMAN, W.B., EUZÉBY, J., AMANN, R. and ROSSELLÓ-MÓRA, R., 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews. Microbiology*, vol. 12, no. 9, pp. 635-645. http://doi.org/10.1038/nrmicro3330. PMid:25118885.

ZAFAR-UL-HYE, M., DANISH, S., ABBAS, M., AHMAD, M. and MUNIR, T.M., 2019. ACC deaminase producing PGPR Bacillus amyloliquefaciens and Agrobacterium fabrum along with biochar improve wheat productivity under drought stress. *Agronomy (Basel)*, vol. 9, no. 7, pp. 343. http://doi.org/10.3390/agronomy9070343.

ZHANG, H., SUN, X. and DAI, M., 2021. Improving crop drought resistance with plant growth regulators and rhizobacteria: mechanisms, applications, and perspectives. *Plant Communications*, vol. 3, no. 1, pp. 100228. http://doi.org/10.1016/j.xplc.2021.100228. PMid:35059626.