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Enhancement of Foxtail millet Growth under Drought stress through Plant Growth-Promoting Rhizobacteria

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Abstract

Millets, known for their climate resilience, are predominantly grown in arid and semi-arid regions, with foxtail millet being one of the oldest cultivated crops and the second most produced globally. However, its productivity is significantly affected by drought, a major abiotic stress factor. Conventional breeding methods have been traditionally used to combat yield loss, but they are resource-intensive and time-consuming. In this context, through Plant Growth Promoting Rhizobacteria (PGPR) offer a promising alternative strategy to enhance crop resilience. This study focuses on the isolation and characterization of PGPR from the rhizosphere of millets, specifically assessing their drought tolerance and plant growth-promoting properties. A total of thirty-nine rhizobacterial isolates were obtained, among which two, SA6 and ATE5, demonstrated notable drought tolerance and exhibited key plant growth-promoting traits, including the production of Indole Acetic Acid (IAA), siderophores, ammonia, hydrogen cyanide, and phosphate solubilization. Molecular identification based on 16S rRNA sequencing revealed that these isolates belonged to *Pseudomonas* sp. To evaluate their potential in enhancing foxtail millet growth, plant experiments were conducted under both well-watered and drought conditions, using the seed bacterization technique. Results showed that the ATE5 strain significantly increased both root and shoot length of foxtail millet (Tenai ATL1) compared to the SA6 strain, under drought stress. This study underscores the potential of PGPR as a sustainable and cost-effective approach to improve drought tolerance in foxtail millet, offering a promising strategy for mitigating the impacts of climate change on millet production.

Key words : Millets, drought, PGPR, Tenai ATL 1, *Pseudomonas* sp.

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Plant response varies based on the different environmental conditions or factors to which they are exposed. These environmental factors include soil, temperature, pH, water, humidity, radiation etc. When the level of these abiotic factors exceeds or falls below the optimum range, they exhibit a severe constrain on the growth and development of the plants known as the abiotic stress. Drought stress is a critical abiotic stress, as water is essential for seed germination and plant growth[24]. Drought affects the morphology as well as the physiology of the plants leading to reduction in crop yield[23]. Conventional breeding methods and genetic engineering techniques have been adopted to develop plant varieties resistant to abiotic stress; however, these approaches are costly and labor-intensive. The use of chemical fertilizers not only diminishes crop quality but also depletes soil fertility. As a sustainable and eco-friendly alternative, Plant Growth Promoting Rhizobacteria (PGPR) offer a promising solution to address these issues. Therefore, Plant Growth Promoting Rhizobacteria (PGPR) can serve as an alternative and ecofriendly strategy to overcome this scenario. PGPR are a group of bacteria that are found in close vicinity of the rhizosphere of the plants[32,34]. Some of the examples of PGPR include *Rhizobium*, *Pseudomonas*, *Azospirillum*, *Azomonas*, *Bacillus* sp. etc.[20,15,29,10,11,35]. This rhizobacterial population vary according to the root exudates secreted by each plant. PGPR exhibits various mechanisms like production of phytohormone, siderophore, volatile organic compounds, 1-aminocyclopropane-1-carboxylic acid, exopolysaccharides, osmoregulators and phosphate solubilization for growth promotion and stress control in plants [9,31,33]. In the present study, an attempt was made to isolate potential rhizobacteria from the rhizosphere of millets and characterize them for drought tolerance and plant growth promoting traits. The present study aims to screen the effect of drought tolerant rhizobacteria for the growth of foxtail millet under drought stress.

Isolation of Rhizobacteria[3] *:*

Rhizobacteria were isolated from the foxtail and little millet rhizosphere samples collected from Centre for Excellence in Millets, Athiyandal, Thiruvannamalai by serial dilution method on nutrient agar medium. The colonies obtained were streaked on nutrient agar medium to get the pure culture of the isolate. These pure cultures were stored at 4°C for further study.

Screening of drought tolerance of Rhizobacteria[30] *:*

The isolated strains of rhizobacteria were screened for drought tolerance by growing them in the nutrient broth medium amended with two different concentrations (30% and 40%) of Polyethylene glycol 6000 (PEG 6000) for 24 hours at 120 rpm and 28 C. The optical density of the cultures was measured at a wavelength of 600 nm using a spectrophotometer. The drought tolerance level of the isolates was determined by using the optical density as follows:

OD < 0.3 - highly sensitive; OD = 0.3 - 0.4 - sensitive; OD = 0.4 - 0.5 - tolerant and OD > 0.5 - highly tolerant.

Screening of drought tolerant Rhizobacteria *for Plant growth promoting Traits :*

The following plant growth promoting

characteristics were screened by the following methods. Indole Acetic Acid Production[5], Phosphate solubilization[12], Siderophore production[25], Ammonia production[6] and Hydrogen Cyanide (HCN) production[16].

Molecular identification of the Rhizobacteria *using 16S rRNA* [27]*:*

The bacterial DNA was isolated and the PCR amplification was done using the universal primers 27F (5'AGAGTTTGATCCTGGCTCAG3') and 1492R (5' GGTTACCTTGTTACGACTT 3'). The sequencing was done by Sanger dideoxy method and the bacteria was identified using the NCBI Blast analysis. The sequences were submitted in the NCBI Gen Bank and accession numbers were obtained.

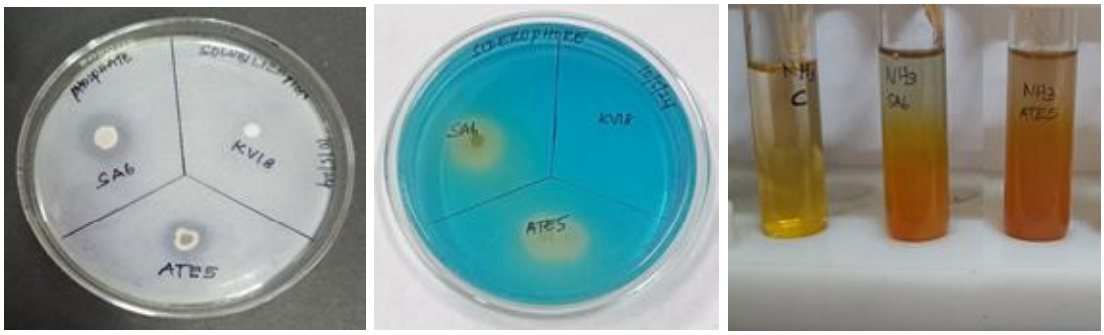
Assessment of growth parameters of Tenai ATL 1 using Rhizobacteria *under drought stress* [2] :

The experimental set up was designed using randomized block design and the seeds of Tenai ATL1 variety was procured from the Centre for Excellence in Millets, Athiyandal, Thiruvannamalai. The mixture of soil and sand was sterilized and used for the experiment. The millet seeds were bacterized with the 24 hours old cultures of drought tolerant rhizobacterial strains and 1% carboxymethyl cellulose (OD = 0.1 at 600 nm) for one hour. The bacterized seeds were then sowed in the soil and watering was done at different days interval (1, 2 and 3 days interval) to create drought stress while the control was watered daily. The root and the shoot length of the plants was measured after 21 days of sowing.

Statistical Analysis :

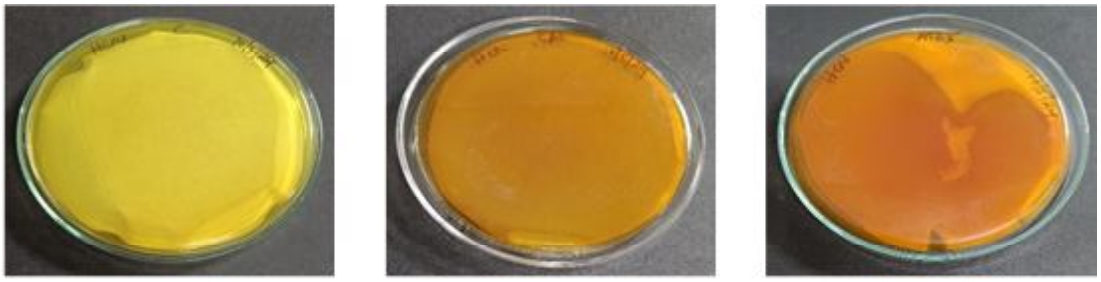
The data obtained from the plant experiment were statistically analysed using Analysis of Variance (Microsoft Excel, 2021).

Thirty-nine Rhizobacterial strains were isolated, of which, two strains were found to be drought tolerant since they exhibited the optical density in the range of 0.4-0.5 while screening it with Polyethylene Glycol (PEG



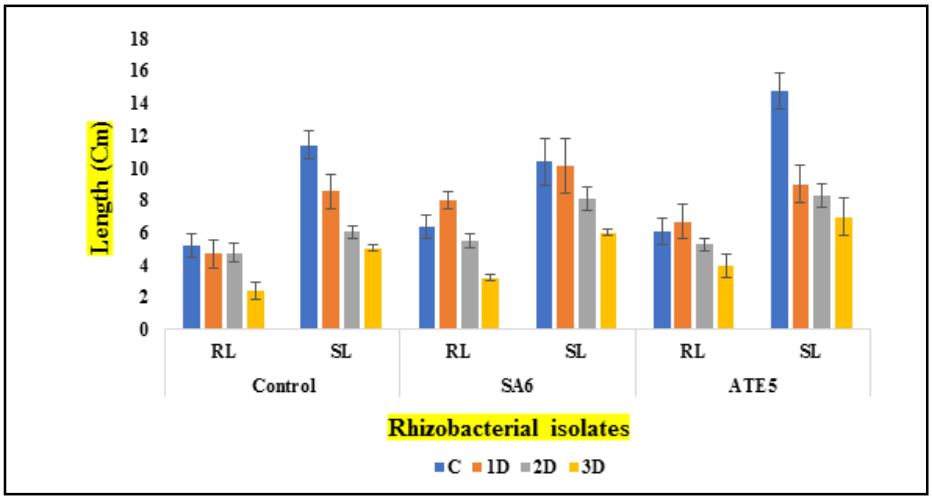
(a) **(b)** **(c)**

Fig. 1. (a) Phosphate solubilization (b) Siderophore production and (c) Ammonia production of SA6 and ATE5



(a) (b) (c)

Fig. 2. Hydrogen cyanide production (a) Control (b) SA6 (c) ATE5



C- Control (Watered daily); 1D- Watered at three days interval; 2D- Watered at two days interval; 3D- Watered at three days interval

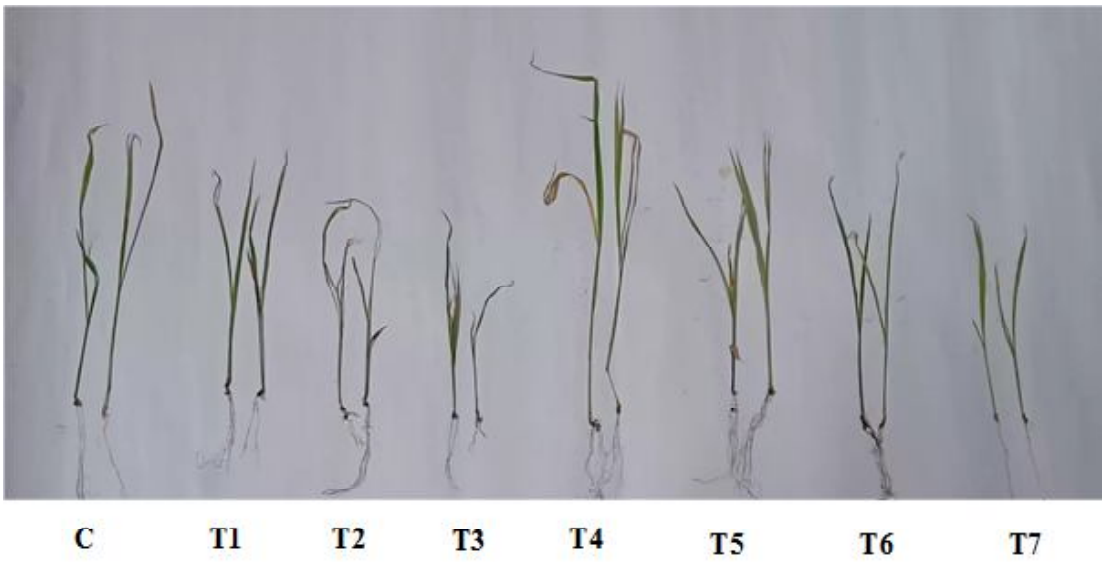
Fig. 3. RL- Root length and SL- Shoot length of Tenai ATL 1 under controlled and drought stressed conditions

6000) and found to possess the following growth promoting characteristics.

Production of Indole Acetic Acid (IAA) :

The results obtained from this study demonstrated that the rhizobacterial strains produced IAA, with strain SA6 showing the highest production of 2.89 µg/mL and strain ATE5 producing 2.46 µg/mL. IAA is known to promote the formation of lateral roots and root hairs, enhancing the plant's ability to absorb nutrients. Additionally, it stimulates cell elongation, contributing to overall plant growth[18]. These findings align with previous research where IAA production was observed in *Pseudomonas* strains isolated from the

(a)



(b)

Fig. 4. Effect of rhizobacterial strains (a) SA6 and (b) ATE5 on root length and shoot length of Tenai ATL1 under controlled and drought stressed conditions
C-Control (daily watering); T1, T2 and T3 indicates watering at 1, 2 and 3 days interval; T4-daily watering along with rhizobacterial treatment; T5, T6 and T7 indicates at 1, 2 and 3 days interval along with rhizobacterial treatment

rhizosphere of finger millet[26]. Similarly, *Pseudomonas migulae* DR35, isolated from foxtail millet, exhibited IAA production and was shown to improve seed germination and seedling growth, particularly under drought stress conditions. These results highlight the potential of rhizobacterial strains in enhancing plant growth and stress resilience[20].

Phosphate solubilization :

The phosphate solubilization index was found to be 3.26 and 2.78 for SA6 and ATE5 respectively (Fig 1a). This phosphate solubilizing ability of the bacterial isolate helps in promoting the plant growth. Plant Growth Promoting Rhizobacteria secretes organic acids which helps in solubilizing the insoluble phosphate complexes present in the soil to soluble form and makes it available to the plants[21]. *Pseudomonas fluorescens* DR7 and *Pseudomonas fluorescens* DR11 from the rhizosphere of foxtail millet were found to possess phosphate solubilization[20]. A phosphate solubilization index of 2.23 was observed in *Pseudomonas* sp. isolated from the tomato rhizosphere which is closer to the results of the present study[13].

Siderophore production :

These strains SA6 and ATE5 were found to be positive for siderophore production (Fig. 1b). Siderophores are low molecular weight compounds that are capable of chelating iron from the soil and makes it accessible to the plants under iron-deficient conditions. *Bacillus subtilis* and *Enterobacter* sp. from the rhizosphere of Sorghum[8] and eleven rhizobacterial isolates from maize namely *Bacillus licheniformis* A5-1, *Aeromonas caviae* A1-2, *A. veronii* C7-8, *B. cereus* B8-3, *Priestia endophytica* A10-11, *B. halotolerans* A9-10, *B. licheniformis* B9-5, *B. simplex* B15-6, *P. flexa* B12-4, *P. flexa* C6-7, and *P. aryabhattai* C1-9 have been reported to produce siderophores[1]. In a study[7], one strain of *Pseudomonas fluorescens* and two strains of *P. palleroniana* exhibited siderophore production.

Ammonia production :

The Rhizobacterial strains also exhibited ammonia production (Fig. 1c). This phenomenon facilitates the isolates to supply nitrogen to the plants and aids in their growth promotion[17]. Ammonia production was also observed in *Pseudomonas* sp. isolated from the rhizosphere of finger millet[26]. In a study[4], the rhizobacterial isolates of tea rhizosphere belonging to different genera namely *Bacillus*, *Staphylococcus*, *Ochrobactrum*, *Pseudomonas*, *Lysinibacillus*, *Micrococcus*, *Leifsonia*, *Exiguobacterium* and *Arthrobacter* were found to produce ammonia.

Hydrogen cyanide (HCN) production :

HCN production was found to be positive in both the strains (Fig. 2). Biological control of the pathogens has been attributed to HCN production. Two strains of *Pseudomonas fluorescens* and *Bacillus subtilis* from the rhizosphere of Sunflower and Sorghum plants respectively were found to produce hydrogen cyanide[8,22] as reported in the present study.

Molecular identification of the Rhizobacteria *using 16S rRNA :*

The two rhizobacterial isolates SA6

and ATE5 was identified as *Pseudomonas* sp. using 16S rRNA. The accession numbers obtained for the isolates were PP938950.1 (SA6) and PP938947.1 (ATE5).

Enhancement of root length and shoot length by Rhizobacterial *isolates :*

Root length and shoot length was found to be increased in control (without drought stress) and drought stressed conditions when treated with the rhizobacterial strains. The strain ATE5 performed better when compared to the strain SA6 (Fig. 3, Fig. 4). The increase in the root and shoot length was found to be statistically significant ($p < 0.05$). Foxtail millet treated with *Bacillus cereus* and *Pseudomonas putida* exhibited the maximum root length and shoot length, respectively[28]. *Pseudomonas fluorescens* DR7, *Pseudomonas fluorescens* DR11, *Enterobacter hormaechei* DR16, and *Pseudomonas migulae* DR35 were the drought tolerant PGPR associated with the rhizosphere of foxtail millet. When these strains were applied on the seeds of *Setaria italica* L. cv. Liaogu 2 (foxtail millet), they effectively enhanced seed germination and growth of the seedlings under drought stressed conditions[20]. *Bacillus amyloliquefaciens* was reported to induce drought tolerance and promote the growth of the pearl millet (*Pennisetum glaucum*) plants in drought conditions[19]. *Acinetobacter calcoaceticus*, a drought tolerant and phosphate solubilizing rhizobacteria was identified to mitigate the adverse effects of drought in foxtail millet plants[14]. Studies conducted by various authors demonstrated that PGPR are capable of alleviating the drought stress effects in millet plants and improve their growth.

Drought tolerant rhizobacterial isolates which were isolated from the rhizosphere of various millets were found to possess the plant growth promoting characters and also improved the growth of Tenai ATL 1 under water deficient conditions. Therefore, these strains can serve as effective bioinoculants under drought conditions, enhancing foxtail millet growth while simultaneously improving soil fertility.

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