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Effect of Plant Growth Promoting Rhizobacteria on the Growth and Yield of Foxtail Millet (*Setaria italica* L. Beauv)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of plant growth-promoting rhizobacteria (PGPR) is a sustainable way for efficient absorption and utilization of nutrients by crop species to enhance growth and yield and persistence against biotic and abiotic stresses. However, the use of PGPRs in drought tolerant crops such as foxtail millet is limited because of the low adaptability of PGPRs to the low moisture conditions. In this study, PGPRs acclimatised to low moisture conditions, were isolated from the rhizosphere of sorghum and their growth and yield promoting effect on foxtail millet was studied. Four isolates, *Pseudomonas putida, Bacillus subtilis*, *Bacillus cereus*, and *Pantoea Stewart* were isolated from the rhizosphere of sorghum cultivated on black soils. Further their identity was confirmed by sequencing 16S rDNA.

Foxtail millet seeds were inoculated with the newly isolated PGPR strains and evaluated for their effect on shoot and root growth under greenhouse conditions. Effect of PGPRs on yield related traits such as ear-head weight, grain weight, fodder weight, and ear-head length were assessed under field conditions. Newly isolated PGPRs boosted average root and shoot length when seeds were inoculated with *Bacillus cereus* (19.33 cm) and *Pseudomonas putida* (28.66 cm), respectively. On the other hand, among the four bacterial cultures, *Bacillus subtilis* enhanced grain weight (40.42%), ear-head weight (30.19%), fodder weight (49.72%) and ear-head length (42.56%)

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compared to the control group under field conditions. When considered collectively, the empirical evidence suggests that PGPR can improve crop growth and yield under greenhouse and field conditions.

Keywords: PGPR; foxtail millet; biofertilizers; plant growth; crop yield.

1. INTRODUCTION

Foxtail millet (*Setaria italica* L. Beauv), also known as Italian millet, is one of the oldest cultivated crops, originated in China and is now cultivated worldwide [1]. Foxtail millet is often grown in drought-prone areas and impoverished soils of semi-arid regions, where other crops fail to thrive [2]. It is primarily grown by small and marginal farmers in drought-prone arid and semi-arid zones [3]. Foxtail millet is also enriched with crude protein (12.3%), lipid, vitamins and minerals (3.3%) [4]. It is rich in dietary fibre and magnesium and possesses several nutritional and therapeutic benefits [5]. The high magnesium levels regulate glucose metabolism and help insulin secretion in type II diabetic patients [6].

Foxtail millet is often cultivated in marginal soils with poor fertility. However, soil erosion and intensive cultivation have reduced soil fertility drastically, requiring the application of minimal fertiliser doses for increasing crop productivity. However, indiscreet usage of agrochemicals further deteriorates soil fertility through nitrogen leaching, soil compaction, reduced soil organic matter and loss of soil carbon [7]. Also, agrochemicals often increase the cost of production.

Application of plant growth-promoting bacteria (PGPB) enhances plant growth by boosting nutrient use efficiency through phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering and other mechanisms [8]. The PGPBs are exogenous bacteria found in the rhizosphere in association with plant root systems, both at the root surface and in endophytic associations. When they are introduced into the agriculture ecosystem, the beneficial effects of PGPR include direct plant growth promotion, biological control of plant pathogens, inducing systemic resistance in host plants, nitrogen fixation for plant use, phytohormone production (including auxins, cytokinins and gibberellins), solubilization of mineral phosphates, and iron sequestration by bacterial siderophores [9-12]. Fortuitously, PGPB enhances plant nutrient absorption and efficient utilization, thus reducing fertilizer application [13]. The well-known PGPR includes organisms belonging to *Pseudomonas, Bacillus, Azotobacter, Azospirillum, Azoarcus, Klebsiella, Arthrobacter, Enterobacter, Burkholderia, Serratia*, and *Rhizobium*. These micro-organisms influence plant growth and development directly by producing indole acetic acid (IAA), siderophore and 1-aminocyclopropane1-carboxylate (ACC) deaminase enzyme, as well as by mobilizing nutrients like phosphorus (P) to the plants by solubilizing insoluble soil phosphates [14] and indirectly by exhibiting antagonistic effects towards many plant pathogenic fungi.

The use of PGPBs is considered an environment-friendly and cost-effective nutrient management practice for plant growth promotion and disease management. The rhizobacteria assemblages of many crops have been studied, and the use of PGPR holds promise for plant growth promotion and alleviation of drought stress also [15]. However, adapting PGPRs to drought-stressed soils is a serious issue, particularly for the microbes that had previously acclimatised to high water tension [16]. Conversely, foxtail is a hardy crop that thrives well in low moisture soils. Hence, it is necessary to test the efficiency of PGPRs for inclusion in the foxtail millet cultivation practices under the shared moisture ecosystem. Thus, the current study was framed to determine the effect of PGPRs on the growth and yield of foxtail millet.

2. MATERIALS AND METHODS

2.1 Isolation and Purification of Bacteria

The sorghum rhizospheric soil samples were collected from the fields of College of Horticulture, Kolar, India (altitude 533 m; 16. 18°N 75. 7° E) 10 g of soil was suspended in 90 ml sterile water blanks, vortexed three times for five seconds each, and serially diluted until 10^{-4}. 100 µL of each dilution was plated on tryptone glucose yeast extract agar media (TGY) by spread plate technique. Each dilution plate was incubated at 28 °C for 24 hours before being checked for colonies. The bacterial strains were

maintained in peptone glycerol for long-term storage at -80°C.

2.2 Molecular Identification of Bacteria

The CTAB method (cetyltrimethylammonium bromide) was used to isolate DNA from the bacterial biomass on the plates.16s rDNA was PCR amplified with primer pair 27F (5'AGAGTTTGATCCTGGCTCAG 3') 1492R (5'ACGGCTACCTTGTTACGACTT 3'). PCR was performed in a total volume of 40 µL, which contained 0.4 µL of Taq Phusion DNA Polymerase (New England Biolabs),08 µL of Buffer 5X (New England Biolabs), 0.8µL of dNTP (New England Biolabs), 2µL of 10 mM primer forward, two µL of 10 mM primer reverse, 100 ng of template DNA and 24.8 µL of nuclease-free water. 16S DNA was amplified with an initial denaturation at 95°C for 2 min 30 s, followed by 35 cycles of denaturation at 95°C for the 20s, annealing at 57°C for 30s and extension at 72 °C for 30s. A final extension was performed at 72°C for 5 min. Amplified 16S DNA sequence was purified using PCR purification kit (New England Biolabs) and sequenced on Sanger sequencing platform (ABI3730xl, Life Technologies) in both forward and reverse directions. Low quality bases with phred score <20 was trimmed, and a consensus sequence was obtained using BioEdit. Consensus 16S DNA was BLAST searched against NCBI-nr database and a neighbour-joining phylogenetic tree of top homologous sequences with identity >90% was constructed using MEGAX with 1000 bootstraps (Ref). The reference isolate of PGPR, D-NCIM was received from the National Collection of Industrial Micro-organism, Pune.

2.3 Seed Treatment

The seeds of the foxtail millet variety DHFT 109 were surface sterilized with 0.1% $HgCl_2$ for two minutes and rinsed six to seven times with sterile distilled water. The foxtail millet seeds were treated with three days old cultures (OD=0.6 at 600 nm) of newly isolated PGPR containing carboxy methyl cellulose (at 1%) as a sticking agent and allowed to dry overnight. Untreated foxtail millet seeds were used as control.

2.4 Evaluation of PGPB for Crop Growth and Yield

The culture-treated seeds were sown in portrays filled with pre-sterilized black soil (8 replications in each treatment). The soil was collected from the college field and was sieved through a mesh sieve and sterilized by autoclaving for 15 min. Approximately 200 grams of sterilized soil was filled into the trays. The uninoculated seeds were also sown in the trays as a control. Trays were kept under the greenhouse at a maximum temperature of 32 °C for 16 h with a relative humidity of 75%. Booster doses of the bacterial strains (1 ml per cup, 10^7 cfu ml^{-1}) were applied 15 days after sowing by the soil drench method. After 40 days of sowing, observations were recorded for shoot length and root length.

The field experiment was conducted in black soil following a completely randomized block design with four replications and sub-plot sizes of 9.45m² in black soil at College of Horticulture, Kolar Karnataka, India (altitude 533 m; 16. 18°N 75. 7° E) during the Kharif season of year. Seeds were treated with four potent bacterial cultures and sown at a depth of about 3 cm at a spacing of 45 x 15 cm by line sowing. Untreated seeds were sown in control plots. No chemical fertilizers or pesticides were applied to the crop. The crop was harvested on the 105th day after sowing, and the following observations were recorded: net plot grain weight, net plot ear-head weight, net plot fodder weight, and ear-head length.

2.5 Statistical Analysis

Effect of PGPR on the growth and yield of foxtail millet in both greenhouse and field conditions over the untreated control was statistically analysed by ANOVA using R programming.

3. RESULTS

The present investigation was undertaken to identify rhizobacterial isolates having plant growth promotion activity. The bacterial strains were initially isolated from the sorghum rhizosphere by serial dilution and plating on the TGY. Results need to be elaborated on length of the 16SrDNA sequenced, its homologous sequences and phylogeny. The isolates were identified as *Bacillus cereus* (DRS-3A), *Pantoea stewartii* (DRS-3B), *Pseudomonas putida* (DRS-3C), and *Bacillus subtilis* (LBPS) using 16s rDNA sequencing.

The foxtail millet variety DHFT 109 was inoculated with isolated bacterial strains to identify their effects on the shoot and root length (crop growth) under greenhouse conditions and net plot grain weight, net plot ear-head weight, and net plot grain weight, net plot ear-head plot

fodder weight and ear-head length under field condition.

Our analysis revealed that the bacterial isolates significantly improved the growth of foxtail millet seedlings in both shoot and root lengths over mock control (Fig. 1). The highest average shoot length of 28.66 cm was recorded in the treatment of *Pseudomonas putida* isolate, followed by isolates *Bacillus subtilis* (25.66 cm), *Bacillus cereus* (24.83 cm), and *Pantoea stewartii* (23.6 cm) over the control (15.66 cm). The isolate *Bacillus cereus* produced the highest average root length (19.33 cm) in comparison to *Pantoea stewartii* (16.66 cm), *Bacillus subtilis* (16.33 cm), *Pseudomonas putida* (15.33 cm) and the control group (13.33 cm).

Under field conditions, the bacterial strains significantly enhanced crop yield compared to the control treatment (Table 1). Bacillus subtilis showed the best performance among the four bacterial cultures regarding grain weight, ear-head weight, fodder weight, and ear-head length compared to the other three cultures. *Bacillus subtilis* enhanced grain weight, ear-head weight and fodder weight to 4.47kg, 4.8kg and 8.98 kg, respectively, over the control. Bacillus subtilis also increased ear-head length up to 6.92cm over uninoculated controls.

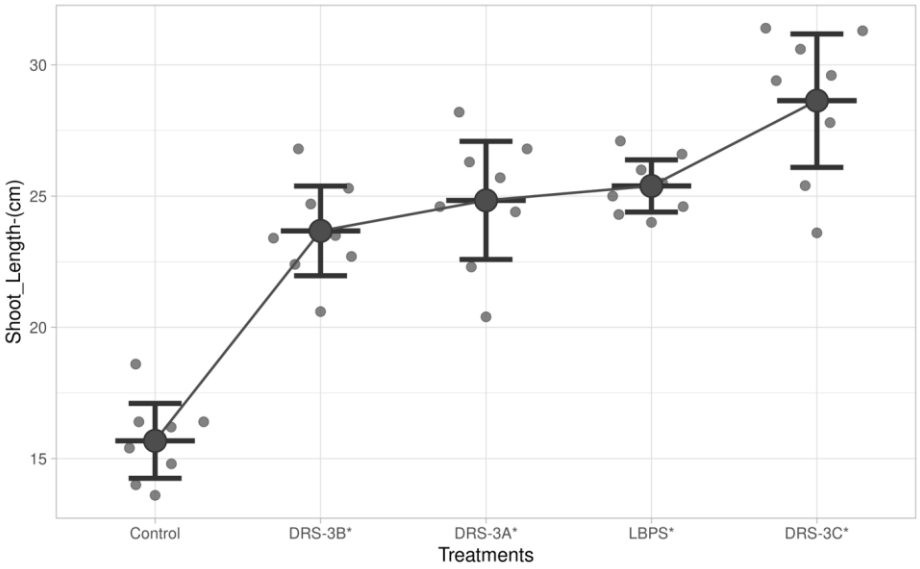

Fig. 1. Effect of seed treatment with PGPRs like DRS-3B, DRS-3A, LBPS and DRS-3C on shoot length of foxtail millet after 40days of sowing under greenhouse conditions

Table 1. Effect of seed treatment with PGPRs like DRS-3B, DRS-3A, LBPS and DRS-3C on net plot grain weight, net ear head weight, net fodder weight and net ear head weight of foxtail millet under field conditions

Treatment/ Parameter	Net Plot Grain Weight (q/ha)	Improvement (%)	Net Plot Ear Head Weight (q/ha)	Improvement (%)	Net Plot Fodder Weight (q/ha)	Improvement (%)	Net Plot Ear Head Length (cm)	Improvement (%)
Control	11.06	0.00	15.9	0.00	18.06	0.00	16.26	0.00
DRS-3A	12.52	13.20	16.9	6.29	21.95	21.54	21.98	35.18
DRS-3B	12.39	12.03	16.18	1.76	24.95	38.15	21.45	31.92
DRS-3C	14	26.58	16.81	5.72	23.52	30.23	20.65	27.00
LBPS	15.53	40.42	20.7	30.19	27.04	49.72	23.18	42.56
C.D.	1.9		1.95		4.57		3.98	
S.Em ±	0.63		0.65		1.52		1.32	

4. DISCUSSION

Plant growth-promoting rhizobacteria (PGPR are rhizosphere bacteria that can boost plant growth through various mechanisms. Studies on the effect of PGPR inoculation on plant growth of *Zea mays* revealed a significant increase in the shoot weight due to inoculation with *A. brasilense*. The increase in dry shoot weight was up to 57% with the inoculation of *A. brasilense* Cd strain alone, while in combination with *A. brasilense* strain AZ39, the increase rose to 91% [17]. When the Streptomyces strains were evaluated for their PGP activity and germination percentage on rice seedlings, the shoot and root length was significantly enhanced over the control [18,13]. In the field, the Streptomyces strains greatly improved the panicle length, filled grain numbers and weight, panicle weight, 1000 seed weight, tiller numbers, total dry matter, root length (39-65%), root volume (13-30%), root dry weight (16-24%), grain yield (9-11%) and stover (11-22%) over the control. According to Chandra et al. [19], the ability to produce IAA is widely linked with PGPR. The PGPR strains CA1001 and CA2004 can be employed as bioinoculants for various plants to increase root and shoot biomass. Chauhan et al. [20] identified the PGPR strain CKMV1, which significantly enhanced seed germination, shoot length, root length, shoot dry weight, and root dry weight of tomatoes under greenhouse conditions. Even though using beneficial plant micro-organisms such as PGPR in place of agrochemicals to improve plant growth is one of the most promising approaches. The utilization of plant growth promoting bacteria in modern agriculture is still limited. To increase the chances of PGPR strains becoming more widely accepted in the future, we investigated the influence of plant-associated rhizobacteria on crop growth and the yield of foxtail millet. A total of four strains were isolated from sorghum rhizosphere grown under semi-arid conditions. 16S rDNA sequencing revealed that they belonged to three different genera *Bacillus*, *Pantoea*, and *Pseudomonas*. The mechanisms of plant growth and nutrient uptake both consume more energy. However, plants primed by PGPR consume less energy to activate these processes [21] [20]. Hence plants will have more energy to devote to other crucial metabolic processes like reproduction and yield.

Our greenhouse and field experiment findings revealed that the crop growth and yield were increased in the PGPR-treated plants over the uninoculated control. The highest average shoot length was noticed in *Pseudomonas putida* treatment (28.66 cm), which is 83.7% higher than in untreated conditions. On the other hand, it also enhanced the net plot grain weight, net plot fodder weight, and ear head length up to 26.58%, 30.23% and 27.0%, respectively, over-controlled treatment. Under field conditions, Pseudomonas are active root colonizers capable of moving from seed to root and promoting plant yield. Initially, it was assumed that ability of the Pseudomonas to encourage growth was primarily due to their ability to exclude harmful microbes from the rhizosphere. However, [22] [18] discovered that in gnotobiotic circumstances, the growth enhancement of canola by a nitrogen fixer strain and a non-fixer mutant of *P. putida* GR12-2 was caused by phosphate solubilization instead of nitrogen fixation. The same strain of *P. putida* GR12-2 induced a two to three-fold increase in root length in canola seedlings due to IAA production [23]. *P. putida* does not fix nitrogen but generates IAA, solubilizes phosphate, and releases antifungal substances. Hence, more than one mechanism may be used by this organism to increase plant growth. The average root length of 19.33 cm was recorded in the treatment of *Bacillus cereus*, which is 45% higher than the control condition. This strain also improved the net plot fodder weight and net plot ear head length up to 21.54% and 35.18%, respectively, over uninoculated treatment. Bacillus *subtilis* showed the best performance among the isolated strains with 32.29% improvement in net-plot grain weight, 15.27% improvement in net plot ear head weight, 44.44% improvement in net-plot fodder weight and 35.85% improvement in net-plot ear-head length. *Bacillus* sp. are advantageous to plants through a wide range of mechanisms, including biofilm production, converting the complex form of essential nutrients (P and N), liberating ammonia from nitrogenous organic matter, fixing atmospheric N_2, siderophore production, phytohormone production and exudation of ACC deaminase. It also inhibits pathogenic microbial growth and strengthens pest defence mechanisms. In the present study, we found that *Bacillus subtilis* and *Bacillus cereus* improved crop growth and yield of foxtail millet. Hence, one or more of the above mechanisms may be accountable for growth improvement as exhibited by isolates [24-28]. *Pantoea stewartia* strain increased the average shoot length to 50.70% over the control group. It also enhanced net pot fodder weight and net pot ear head length up to 38.15% and 31.92%, respectively. It is suspected that nitrogen, phosphate dissolving, and indole-3-

acetic acid property of the *Pantoea sp*. are responsible for this outcome [29]. According to Monk et al. [30], 10% of bacteria in the roots of New Zealand fescue plants can generate auxin, which stimulates plant growth, and some PGPR can produce IAA and its analogues, which stimulates the growth of plant roots. The number of stem branches increased significantly after the inoculation of auxin-producing PGPR on Brassica napus, which grows in oily soil (Asghar et al. 2004). Gómez-Godnez et al. [31] investigated the effects of a set of PGPR on the growth of one-month-old maize seedlings. They discovered that these bacteria could activate nitrogen-fixing and encourage maize growth. Thus, it is reasonable to hypothesize that the nitrogen fixation, phosphorus solubilization and auxin secretion capacity of PGPR could be the probable general mechanism behind the ability of our isolates to boost foxtail millet plant growth.

Although roots were not inspected for colonization in our study, the data on net plot grain weight, ear head weight, fodder weight and ear head length strongly suggest that the rhizobacteria must have been established in the foxtail millet rhizosphere contributing to improved yield without external application of fertilizers.

5. CONCLUSION

The results of research of the greenhouse and field experiment findings revealed that the crop growth and yield were increased in the PGPR-treated plants over the uninoculated control. Thus, the present study confirmed that PGPRs exhibit high efficiency even under drought conditions by enhancing root volume in foxtail millet. PGPRs enabled foxtail millet to absorb more moisture and nutrients from the soil and increase shoot length, improving photosynthesis and overall grain yield. Isolated PGPRs can be used as effective bio-fertilizers in the cultivation of foxtail millet and other crops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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