Effect of plant growth promoting rhizobacteria on the crop growth and yield of foxtail millet (*Setaria italica* L. Beauv)

Abstract

The most productive use of land resources has emerged as a significant challenge for India's food production security in recent years. Soil micro-organisms have been generally assumed to have a beneficial impact on soil fertility and productivity. A sustainable approach to boost crop growth and yield is the use of plant growth promoting rhizobacteria (PGPR). This work primarily aimed to study the influence of isolated PGPR on promoting crop growth under greenhouse condition and the effects of PGPR on crop yield of foxtail millet under field condition. Initially, the PGPR were isolated from the sorghum rhizosphere using the serial dilution method. The molecular identity of the isolated bacteria was identified by 16S rDNA sequencing. The strains were identified as *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus cereus*, and *Pantoea Stewart*. Foxtail millet seeds were inoculated with bacterial strains, evaluated for shoot and root growth under greenhouse condition, whereas ear-head weight, grain weight, fodder weight, and ear-head length was evaluated under field condition. According to our findings, under greenhouse conditions foxtail millet produced the highest average root and shoot lengths 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* exhibited the best performance in terms of grain weight (40.42%), ear-head weight (30.19%), fodder weight (49.72%) and ear-head length (42.56%) compared to the control group under field conditions. When considered collectively, the empirical evidences suggest that PGPR can improve crop growth and yield under green house and field conditions respectively.

Keywords: PGPR, foxtail millet, biofertilizers, plant growth, crop yield

Introduction

Foxtail millet (*Setaria italica* L. Beauv), also known as Italian millet; it is one of the oldest cultivated crops, originated in China and is now cultivated worldwide (Yang *et al*., 2012) [31]. Foxtail millet is often cultivated in drought-prone areas and impoverished soils of semi-arid regions, where other crops fail to thrive (Lata *et al*., 2012) [12]. In India, it is primarily grown by the small and marginal farmers in the drought-prone arid and semi-arid zones (Sharma *et al*., 2014) [26]. The main components of foxtail millet include starch, crude protein (12.3%), lipid, vitamins and minerals (3.3%) (Rai, 2002) [22]. It is rich in fibre, magnesium and possesses several nutritional and therapeutic benefits (Choi *et al*., 2005) [6]. The high magnesium levels regulate the glucose metabolism in the body and helps in insulin secretion.

The use of agrochemicals is vital for increasing crop productivity. However, its indiscreet usage deteriorates soil fertility through nitrogen leaching, soil compaction, reduction in soil organic matter and loss of soil carbon. Also, agro-chemicals often increase the cost of production. The use of plant growth promoting bacteria is considered as an environment friendly, cost-effective and long-term substitute to dangerous chemical fertilizers (Egamberdieva *et al*., 2016) [9].

The PGPR's 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 includes direct plant growth promotion, biological control and inducing systemic resistance in host plants, nitrogen fixation for plant use, phytohormone production (including auxins, cytokinins and gibberellins), solubilisation of mineral phosphates, and iron sequestration by bacterial siderophores (Glick *et al*., 1999) [12]. Fortuitously, PGPR enhances plant nutrient absorption and efficient utilisation, thus reducing fertiliser application (Adesemoye *et al*. 2009) [1]. 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 mobilising nutrients like phosphorus (P) to the plants by solubilising insoluble soil phosphates (Glick *et al*., 1995; Glick *et al*., 1999) [10, 12] and indirectly by exhibiting antagonistic effects towards many plant pathogenic fungi. In recent years, the concept of PGPR-mediated plant growth promotion and disease management has been gaining importance and acceptance on a global scale. The rhizobacteria assemblages of many crops have been studied and the use of

PGPR holds promise for plant growth promotion and alleviation of plant drought stress (Mayak *et al*., 2004) [19].

However, adapting PGPR's to drought-stressed soils is a serious issue, particularly for the microbes that had previously acclimatized to high water tension (Van Meeteren *et al*., 2008) [28]. Conversely, foxtail is a hardy crop, that thrives well in low moisture soils. Hence, it is necessary to test the efficiency of PGPR's for inclusion in the foxtail millet cultivation practices under the low moisture ecosystem. Thus, the current study was framed to determine the effect of PGPR's on the growth and yield of foxtail millet.

Material and Methods

Isolation and purification of bacteria

The sorghum rhizospheric soil samples were collected from the University of Horticultural Sciences, Bagalkot, Karnataka. A total of 10 g of soil was suspended in 90 ml sterile water blanks and vortexed three times for five seconds each time and serially diluted until 10^{-4}. By using spread plate technique, 100 µL of each dilution were then plated on tryptone glucose yeast extract agar media (TGY). Each dilution plates were incubated at 28 °C for 24 hours before being checked for colonies. For long-term storage, the bacterial strains were maintained in peptone glycerol at -80 °C.

Molecular identification of bacteria

The CTAB method (cetyltrimethylammonium bromide) was used to isolate DNA from the bacterial biomass on the plates, and the DNA samples were quantified using the Eppendorf micro-cuvette in Eppendorf Bio-Spectrometer. The samples with an absorbance ratio of 1.8 to 2.0 at A260/280 were considered for further procedure. To identify the bacterial isolates, 16s rDNA PCR was done by using 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), 8 µL of Buffer 5X (New England Biolabs), 0.8µL of dNTP (New England Biolabs), 2µL of 10 mM primer forward, 2 µL of 10 mM primer reverse, 2 µL of template DNA and 24.8 µL of nuclease-free water. The reaction conditions were initial denaturation at 95 °C for 2 min 30 s, followed by 35 cycles of denaturation at 95 °C for 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. The obtained amplicon was prepared for

sequencing, using a Nextera XT library preparation kit in accordance with the manufacturer's recommended procedures. The libraries were sequenced by Sanger sequencing technology. The resulting sequences were edited, compared with others in the GenBank database, using BLAST to find for the most similar sequences. A neighbour-joining phylogenetic tree of the detected sequences was constructed using MEGAX with 1000 bootstraps. The reference isolate D-NCIM was received from National Collection of Industrial Micro-organism, Pune.

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) containing carboxy methyl cellulose (at 1%) as a sticking agent and allowed to dry overnight. Untreated foxtail millet seeds were used as control.

Evaluation of PGPB for crop growth and yield

The culture-treated seeds were sown in black trays filled with black soil (8 replications in each treatment). The soil was collected from the college field and was sieved through a mesh sieve and approximately 200 grams were filled into the trays. The uninoculated seeds were also sown in the trays as a control. Trays were kept under the greenhouse at maximum temperature 32 °C for 16 h light with relative humidity of 75%. Booster doses of the bacterial strains (1 ml per cup, 10^7 $cfuml^{-1}$) were applied 15 days after sowing by the soil drench method. Observations were recorded after 40 days of sowing 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 9.45m^2 in black soil at UHSB, Karnataka (altitude 533 m; 16. 18°N 75. 7° E) during the kharif season. 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 method. Untreated seeds were sown in control plots. No chemical fertilizers or pesticides were applied on the crop. The crop was harvested on the 105^{th} day after sowing and the following observations such as net plot grain weight, net plot ear-head weight, net plot fodder weight, and ear-head length were recorded.

Statistical analysis

The pot culture and field experiment were subjected to the analysis of variance (ANOVA) technique to evaluate the efficiency of PGP agents. The significance of differences between the treatment means was tested at $P = 0.01$ and 0.05.

Results

The present investigation was undertaken to identify rhizobacterial isolate having plant growth promotion activity. The bacterial strains were initially isolated from the sorghum rhizosphere by serial dilution method and plating on the TGY. 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 seeds of the foxtail millet variety DHFT 109 were inoculated with isolated bacterial strains in order to identify their effects, on shoot and root length (crop growth) under greenhouse condition and net plot grain weight, net plot ear-head weight, net 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 terms of both shoot length over mock control **(Figure 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 control group (13.33 cm).

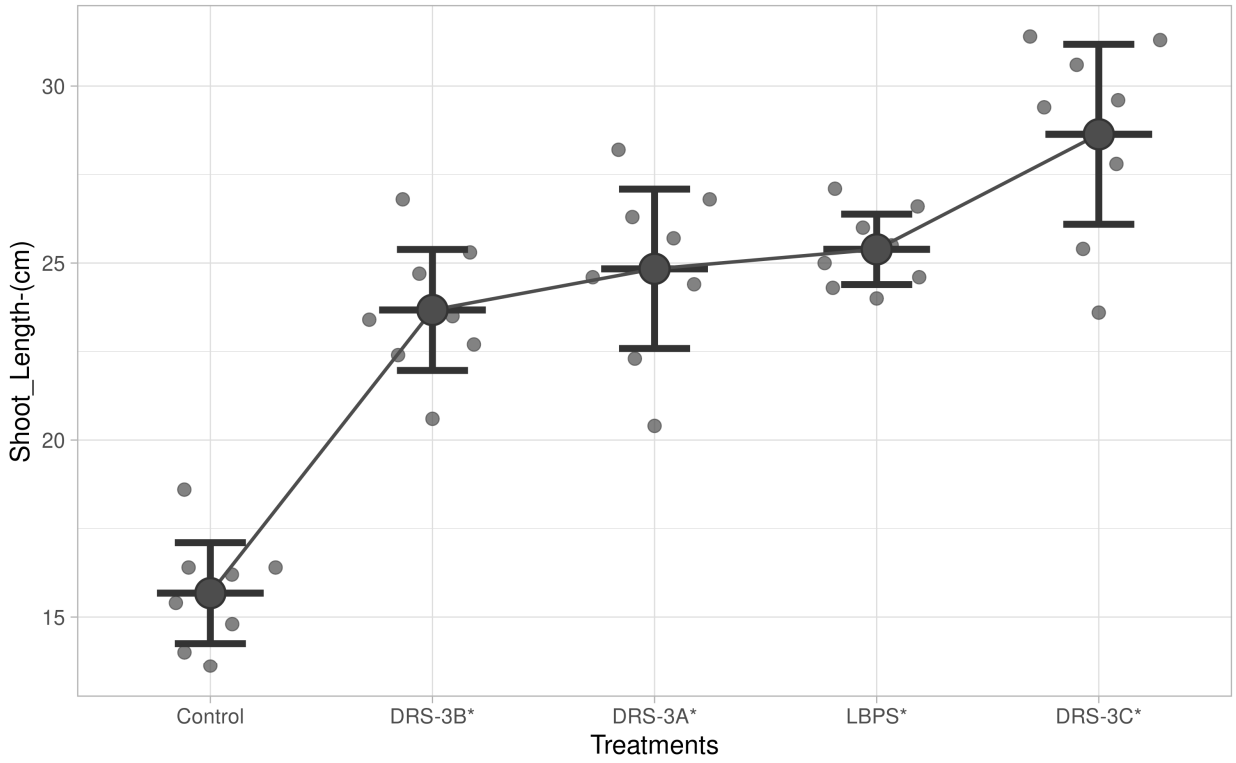

Fig.1. Effect of seed treatment with PGPR's like DRS-3B, DRS-3A, LBPS and DRS-3C on shoot length of foxtail millet after 40days of sowing under greenhouse condition.

Under field conditions, the bacterial strains significantly enhanced crop yield compared to control treatment **(Table 1)**. Among the four bacterial cultures, *Bacillus subtilis* showed the best performance in terms of 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 up to 4.47kg, 4.8kg and 8.98 kg, respectively, over the control. Ear-head length was also increased by *Bacillus subtilis* up to 6.92cm over uninoculated controls.

Table 1: Effect of seed treatment with PGPR's 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	

Discussion

Plant growth promoting rhizobacteria (PGPR) are rhizosphere bacteria that can boost plant growth through a range of mechanisms. Studies on the effect of PGPR inoculation on plant growth of *Zea mays* revealed a significant increase in shoot weight of *S. italica* 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%. 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 (Gopalakrishnan *et al*., 2012) [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. Despite the fact that using plant beneficial micro-organism 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 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 consumed less energy to activate these processes (Niu *et al*., 2011) [20], hence plants will have more energy to devote to other crucial metabolic processes like reproduction and yield.

The findings of our greenhouse and field experiment revealed that the crop growth and yield was 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, ear head length up to 26.58%, 30.23% and 27.0% respectively, over controlled treatment. Under field conditions, Pseudomonas are active root colonisers capable of moving from seed to root and promotes plant yield. Initially, it was assumed that ability of the pseudomonas to promote growth was mostly due to their ability to exclude harmful microbes from the rhizosphere. However, (Lifshitz *et al*., 1987) [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 as a result of IAA production (Xie *et al*., 1996) [29]. *P. putida* does not fix nitrogen, but it does generate 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 and also this strain improved the net plot fodder weight and net plot ear head length up to 21.54% and 35.18%, respectively over uninoculated treatment. *Bacilus 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 and 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 the pathogenic microbial growth and strengthens pest defense mechanisms. In the present study, we found that *Bacillus subtilis* and *Bacillus cereus* improved both 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 (Beauregard *et al*., 2013; Hayat *et al*., 2010; Ding *et al*., 2005; Arkhipova *et al*., 2005; Xu *et al*., 2013) [5, 14, 8, 2, 30]. *Pantoea stewartia* strain increased the average shoot length upto 50.70% over control group and it also enhanced net pot fodder weight and net pot ear head length upto 38.15% and 31.92%, respectively. It is suspected that nitrogen and phosphate dissolving and indole-3-acetic acid property of the *Pantoea* sp. is responsible for this outcome (Rungrueng *et*

al., 2021) [23].

Although roots were not inspected for colonisation 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.

Conclusion

The present study confirmed that PGPR's exhibit high efficiency even under drought conditions by enhancing root volume in foxtail millet. PGPR's enabled foxtail millet to absorb more moisture and nutrients from the soil and increasing shoot length, improving photosynthesis and overall grain yield. Isolated PGPR's may be used as effective bio-fertilisers in the cultivation of foxtail millet and other crops.

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