Influence of *Azospirillum* and PSB inoculation on growth and yield of Foxtail Millet

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**ABSTRACT**

Experiments were conducted to study the effect of inoculation of diazotrophic isolates of *Azospirillum lipoferum* and phosphate-solubilizing bacteria (PSB) individually and in combination on the yield of foxtail millet [*Setaria italic (L.) Beauv.*] (cv. Chitra) during Kharif and Rabi seasons, grown in Black soil of sandy loam type in earthen pots. Two selected isolates of *Azospirillum* A1 (45 C2) and A2 (45 L1) and one isolate of PSB (45 LR3) were chosen for inoculation studies. One set of the pots was inoculated separately with *Azospirillum* A1 alone, PSB alone, *Azospirillum* A1 and PSB in combination and another set of pots was inoculated with *Azospirillum* A2 alone, PSB alone, *Azospirillum* A1 and PSB in combination. Pots that received heat killed inoculum which served as control. Triplicates were maintained for each treatment. Inoculation of foxtail millet (cv. Chitra) with two strains of *Azospirillum* lipoferum and one strain of a Phosphate Solubilizing Bacterium (PSB) individually and in combination significantly increased plant height, shoot and root dry weight over control plants. Both the panicle and seed weight also increased due to inoculation with *Azospirillum* and PSB in combination. Among the individual inoculants *Azospirillum* A2 was superior to the other inocula.

**Key words**: *Azospirillum lipoferum*, PSB, Foxtail millet, growth, yield

**INTRODUCTION**

The existence of life on earth is favoured by the cycling of biological elements wherein the complex biological systems after their decay are converted into simpler forms. These cycles like carbon, sulphur, phosphorus and nitrogen are essential for biology and the role played by microorganisms in these cycles is important. Nitrogen is an essential and key element in improving crop productivity. Crop plants are able to use about 50% of the applied fertilizer N while 25% is lost from the soil-plant system through leaching, volatilization and denitrification [1]. The plants are unable to utilise molecular nitrogen unless it is converted into the suitable form (ammonia). This ability of reduction of atmospheric nitrogen to ammonia, known as biological nitrogen fixation, is confined to microorganisms. The well known asymbiotic diazotrophic bacteria belong to the genera like *Acetobacter, Azotobacter, Azospirillum, Azoarcus, Burkholderia, Enterobacter, Herbaspirillum, Pseudomonas, Klebsiella* etc., [2].

*Azospirillum* species belong to facultative endophytic diazotrophic group which colonize the surface and interior of roots [3]. The organisms are Gram negative curved rods of variable sizes which exhibit spirillar movement and polymorphism. The cells contain poly-β-hydroxy butyrate (PHB) granules and fat droplets. These are associative microaerophilic diazotrophs isolated from the roots and above ground parts of a variety of crop plants like forage grasses, cereals, legumes, millets and soils [4,5,3]. There are encouraging reports on the use of *Azospirillum* spp. as biofertilizers which contribute to the nitrogen economy of the plants and also promote the plant growth by producing growth hormones. [4,6,7,8,5].

Phosphorus is a vital nutrient for plants and microorganisms next only to nitrogen. Phosphorus is one of the major essential macronutrients for biological growth and for proper plant development. The deficiency of phosphorus may
occur in crop plants growing in soils containing adequate phosphates. This may be partly due to the fact that the plants are able to absorb phosphorus only in available form. Soil phosphates are rendered available either by plant roots or by soil microorganisms through their secretion of organic acids. Therefore, phosphate-solubilizing soil microorganisms play some role in correcting phosphorus deficiency of crop plants. Several soil bacteria particularly Bacillus, Pseudomonas and fungi possess the ability to solubilize insoluble phosphates into soluble forms. One way to correct the deficiency of phosphorus in plants is to inoculate seeds or soils with phosphate-solubilizing microorganisms (PSMO). There are number of reports on use of PSMO as biofertilizers in various crops. [9,10,11,12]. Small millets, produced mainly by small farmers as rainfed crops play an important role in the diet of people living in remote rural and tribal areas in semi-arid tropics. Foxtail millet (Setaria italica), a minor millet, is chosen for the present study because it is one of the important and staple food crops of semi-arid tropics which is nutritious (125 mg protein g$^{-1}$) and contains all essential amino acids. Further, it not only resists drought conditions but also withstands delayed monsoons and is suitable for light black and red soils of Anantapur district. Although growth responses of various crops to Azospirillum inoculation have been reviewed [13,5] information on the effects of Azospirillum inoculation on foxtail millet is very limited [14,15,16,17]. Further, there are no reports on inoculation effects of PSB and also co-inoculation studies with diazotrophic and PSB on foxtail millet.

Hence the present study focuses on the inoculation studies in pot cultures using selected and efficient strains of Azospirillum and PSB individually, which have been previously isolated from the rhizosphere samples of foxtail millet. Coinoculation studies in pot cultures with Azospirillum and PSB to investigate the effect of dual inoculation on growth, yield, and yield components of foxtail millet cultivar Chitra.

**MATERIALS AND METHODS**

Experiments were conducted during Rabi (Feb-May) and Kharif (July-September) seasons of 2005, considered as first and second experiments respectively.

**Soil Analysis:**  
Black soil was collected from the cultivated fields of Aanatapur District and analyzed in the laboratory for physico-chemical properties. The soil was analysed for sand, silt and clay employing Bouyoucos hydrometer [18]. Soil pH was measured by 1:1.25 soil to water ratio using Elico digital pH meter with calomel-glass electrode assembly. Organic carbon in the soil was determined by Walkley-Black method [19]. The total nitrogen (N) content of the soil was estimated by Kjeldahl method [19]. The cation exchange capacity (CEC) of soils was determined by ammonium acetate pH 7.0 method [19]. The total phosphorus (P) was estimated by Sodium bicarbonate method [20]. The details of soil properties were shown in the following table.

<table>
<thead>
<tr>
<th>Location</th>
<th>Type</th>
<th>Texture</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>pH</th>
<th>Organic matter (%)</th>
<th>Total nitrogen (%)</th>
<th>CEC</th>
<th>Total P mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jangalapalle</td>
<td>Black</td>
<td>67.7</td>
<td>19.9</td>
<td>12.5</td>
<td>7.69</td>
<td>1.24</td>
<td>0.21</td>
<td>14.2</td>
<td>92</td>
<td></td>
</tr>
</tbody>
</table>

**Seed Collection:** Seeds of foxtail millet [Setaria italica (L.) Beauv.] cultivar Chitra were collected from Agricultural Research Station, Rekulakunta, (Acharya N.G. Ranga Agricultural University), Anantapur.

**Experiments-1**  
Five kg black soil of sandy loam type was placed in earthen pots. Seeds of foxtail millet variety Chitra were surface sterilized by the method of Albrecht [21] and sown in the pots during Rabi (February-May) season of 2005. All the pots were watered once a week. One week after germination, thinning was made in such a way that only one plant remained per pot. Experiment was conducted to study the effect of inoculation (10 ml at 15-day intervals from 15 to 60 days) of diazotrophic isolates of Azospirillum and phosphate-solubilizing bacteria (PSB) individually and in combination. Two selected isolates of Azospirillum A1 (45 C2) and A2 (45 L1) and one isolate of PSB (45 LR3) were chosen for inoculation studies previously isolated from the rhizosphere of foxtail millet. One set of the pots was inoculated with Azospirillum A1 alone, PSB alone, Azospirillum A1 and PSB in combination and another set of pots was inoculated with Azospirillum A2 alone, PSB alone, Azospirillum A2 and PSB in combination. Some pots that received heat killed inoculum which served as controls. Triplicates were maintained for each treatment.

**Experiment-2**  
The experiment was repeated during Kharif (July-September) season of 2005.

**Preparation of bacterial inocula**
Azospirillum was isolated from rhizosphere of foxtail millet at different days (30, 45, 60 and 75 DAS on semi-solid nitrogen-free bromothymol blue malate (Nfb) medium [22]. Pure cultures of the two selected strains of Azospirillum were grown in malate broth with the following composition (g/l): DL-Malic acid - 5.0, K₂HPO₄ - 0.5, MgSO₄·7H₂O - 0.2, NaCl - 0.1, CaCl₂ - 0.02, Trace element solution-2 ml, Fe EDTA - 4 ml, Yeast extract - 0.5, KOH - 4.0, Distilled water - 1000 ml. (Trace element solution contained 200 mg of sodium molybdate, 235 mg of manganous sulphate, 280 mg of boric acid, 8 mg of copper sulphate and 24 mg of zinc sulphate in 200 ml distilled water). The medium was supplemented with NH₄Cl [23]. The pH of the medium was adjusted to 6.8 using potassium hydroxide. Similarly, one strain of PSB isolated from rhizosphere of foxtail millet was grown in Pikovskaya’s broth modified by Sundara Rao and Sinha, [24] with the following composition (g/l): Glucose - 10.00, Ca₃(PO₄)₂ - 5.0, (NH₄)₂SO₄ - 0.50, KCl - 0.20, MgSO₄·7H₂O - 0.10, MnSO₄·H₂O - Traces, FeSO₄·7H₂O - Traces, Yeast extract - 0.50, Agar agar - 20.00. The log phase cultures were used for inoculation. The cells were harvested by centrifugation at 5000 g at 4°C for 20 min. The supernatant was discarded and the pellet was washed two times with saline (5 g of NaCl and 0.12 g of MgSO₄·7H₂O in 1 l distilled water) and resuspended in saline at a concentration of 10⁸ colony forming units (CFU) per ml. Ten ml of the bacterial suspension was inoculated to each plant. Similarly the inoculum containing both Azospirillum and PSB was also inoculated to each plant. The plants were harvested 75 DAS.

Growth parameters
Growth parameters of the plants were recorded. Plant height (from base to tip of the plant) was measured on 30, 45, 60 and 75 DAS. The data recorded for three plants for each treatment were analyzed statistically. The dry weight of shoot and root was determined by drying the plant material at 80°C for 72 h in a hot air oven at 75 DAS.

Yield
The yield was estimated by taking into account the weight of the panicle and also weight of 1000 seeds [25].

Statistical analyses
In all the cases, analyses of significant differences of (P ≤ 0.05) between values of each sampling and treatment were performed using Duncan’s New Multiple Range (DMR) test [26].

RESULTS AND DISCUSSION

Shoot height
Data on the effect of inoculation of Azospirillum and PSB on shoot height in the first experiment are presented in the Fig. 1. All the inoculants enhanced plant height over control. Dual inoculation with A2 and PSB isolates resulted in maximum shoot height followed by the combination of A1 and PSB isolates. Among the individual inoculants of the diazotrophs and the PSB isolates, A2 isolate exerted maximum influence when compared with A1 or PSB isolate. Although the PSB isolate increased plant height, the effect is almost at par with A1 isolate. Results presented in Fig 2 reveal the effect of inoculants on shoot height in the second experiment. All the inoculants increased the shoot height significantly when inoculated either individually or in combination over control plants. Of the two diazotrophic isolates, A2 isolate exhibited more pronounced effect on shoot height when compared with A1 isolate. The PSB isolate also enhanced shoot height significantly which is almost at par with the effect produced by A1 isolate. Of the two combinations, co-inoculation with A2 and PSB isolates produced maximum effect followed by A1 and PSB isolates.

A perusal of the data indicates that the shoot height varied in the two experiments. Thus the plants in the first experiment exhibited higher shoot height when compared with the plants in the second experiment. Mixed inoculants stimulated shoot height significantly when compared with individual inoculant (A1 or A2 or PSB isolate). Maximum stimulation in shoot height was observed with A2 and PSB isolates followed by A1 and PSB isolates. Likewise, the isolate A2 was more effective compared with A1 isolate.
Significant increase in plant height of *Setaria italica* was observed due to inoculation with four isolates viz., Cd, Sp-7, Cd-1 and Cd-3 of *Azospirillum brasilense* over uninoculated control plants [14]. Results from the present study also showed the similar effect. Among the two isolates of *A. lipoferum* tested in the present study, A2 showed significant difference in plant height over A1. Similarly, plant height increased significantly when inoculated with two isolates (Cd and Sp-7) of *A. brasilense* on cultivars of *Setaria italica* [16]. In the present study, the two isolates of *Azospirillum* stimulated the plants differently. Significant increase in plant height in maize following inoculation with *Azospirillum* was also observed [27]. Similarly, increase in shoot length in rice and finger millet was also reported [10, 28]. Virtually no reports are available on the effect of inoculation of phosphate-solubilizing bacteria on *Setaria italica*. The effect of PSB inoculation on plant height was significant over control plants. A strain of *P. putida*, a PSB, stimulated the growth of shoot and roots and increased phosphate uptake in *Canola* [29] and in shola tree species [7]. Similar results were also reported in winter maize [12].

Coinoculation of *Azospirillum* and PSB in the present study significantly increased the shoot height of foxtail millet over the control and the individual inoculants. Virtually very little information is available on the effect of inoculation of *Azospirillum* and PSB on *Setaria italica*. However, a few reports are available with regard to other
crops. *Azospirillum* and phosphate-solubilizing microorganisms increased plant height of rice significantly at flowering stage over control [10], in shola tree species [7] and in pearl millet [30].

**Dry weight of shoot and root**

Dry weight of shoot and root was estimated 75 DAS in both the experiments and expressed in terms of mg g⁻¹ fresh weight of the shoot and root. All the inoculants increased the shoot and root dry weight in the first experiment. Among the individual inoculants, A2 isolate was more effective when compared with A1 isolate (Fig. 3 and 4). Likewise, coinoculation with A2 and PSB isolates resulted in maximum yield of shoot and root dry weight followed by A1 and PSB. The increase in shoot and root dry matter production was significant with all the inoculants over control.

Data on shoot dry weight indicate that dry matter yield increased significantly with all the inoculants either individually or in combination. Maximum increase in shoot and root dry matter was observed with the dual inoculants. Thus the combination of A2 and PSB was more effective followed by A1 and PSB isolates. The PSB isolate also increased the shoot dry weight significantly. The increase in yield of dry weight was significant and more pronounced with A2 isolate when compared with A1 isolate. A perusal of the data from the present study indicate that in general the response varied in the two experiments.

Reports available indicate an increase in shoot dry weight in *Setaria italica* upto 57% with the inoculation of *A. brasilense* Cd strain alone which increased upto 91% when it was combined with *A. brasilense* strain AZ-39 [17]. Similarly Mane et al. [8] observed increase in dry weight of shoot of pearl millet with *Azospirillum*. Das et al., [31] observed 4.2 to 6.1% increase in dry matter yield in three varieties of *Sorghum* over control plants inoculated with *Azospirillum*. Similarly, dry matter increase in rice due to *Azospirillum* inoculation was reported [10]. It was also reported the increase in dry weight of pearl millet inoculated with PSB [8]. Likewise, Sutaliya and Singh [12] also reported increased dry matter in maize due to PSB inoculation.

Results from the present study indicate that dual inoculation with *Azospirillum* and PSB increased dry weight of plants over other treatments which is in agreement with earlier reports [8] that dry matter of shoot and root in pearl millet increased with dual inoculants of *Azospirillum* and PSB.

![Shoot dry weight](image)

*Fig. 3. Effect of inoculation of Azospirillum spp. and PSB on shoot dry weight of foxtail millet in first and second experiments*

**Effect of inoculation on yield**

Yield attributes like panicle weight and weight of 1000 seeds were recorded at the time of harvest (75 DAS).

**Panicle weight**

At the time of harvest the panicle weight was determined in both the experiments. Data presented in Fig. 5 revealed that all the treatments significantly increased (33 to 100%) the panicle weight over control plants. Among the treatments, plants treated with A2+PSB exhibited maximum effect followed by A1+PSB, A2, A1 and PSB in that order. All the treatments in the second experiment also showed significant increase in panicle weight (35 to 104%)

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over control plants. Percent increase in panicle weight followed the order: A2+PSB > A1+PSB > A2 > A1 > PSB. The increase in yield percent was significantly higher (30 to 100%) in the second experiment when compared to the first experiment.

**Weight of 1000 seeds**

Data on the 1000 seed weight in the first experiment revealed that all the treatments significantly increased (41 to 68%) the panicle weight over control plants (Fig. 6). Among the individual treatments, plants responded significantly to A2 followed by A1 and PSB. Significant increase in weight of 1000 seeds was recorded by dual inoculation with A2+PSB followed by A1+PSB over other treatments.

All the treatments in the second experiment showed significant increase in seed weight (40 to 66%) over control plants. Percent increase in seed weight followed the order A2+PSB > A1+PSB > A2 > A1 > PSB. The increase in yield percent was slightly less (41 to 68%) than the first experiment. There was no significant difference in seed weight or in percent increase in seed weight in the two experiments. A comparison of the data on yield in terms of panicle weight in both the experiments revealed that percent increase in panicle weight was significant in plants treated with dual inoculum containing A2 and PSB.
A survey of literature reveals significant increase in grain yield and 1000 seed weight in wheat upon inoculation with *Azospirillum* [32]. Significant increase in grain yield with *Azospirillum* inoculants in rice plants over control was observed [33, 10]. Mishra *et al.* [27] reported significant increase in grain per cob and 100 seed weight in maize due to *Azospirillum* inoculation. Similarly, increased grain yield in pearl millet with *Azospirillum* inoculation over control was reported [8]. Further, Rao [34] observed increase (12.9 and 15%) in yield of rice variety ASD when inoculated with *Azospirillum*. Inoculation of foxtail millet with *Azospirillum* sp. alone increased the yield significantly [35]. Increased grain yield in pearl millet with PSB inoculation over control was observed [8]. Inoculation of PSB and along with FYM significantly increased the plant height, dry matter accumulation and grain yield per plant in maize over control [12].

Co-inoculation of *B. polymyxa* and *P. striata* strains showing phosphate-solubilizing ability along with a strain of *A. brasilense* resulted in significant improvement of grain and dry matter yield with a concomitant increase in N and P uptake, compared with individual inoculations in sorghum [36]. Improved grain yield and nutrient uptake in barley in pot and field experiments, when inoculated with *Agrobacterium radiobacter* (PSB) and *A. lipoferum* together when compared with single inoculant was reported [37]. Increase in grain yield in *Oryza sativa* upon dual inoculation with *Azospirillum* and PSB was also reported [38]. Higher seedling height, root dry matter in nursery and increase in grain yield of rice in the field due to combined inoculation of *Azospirillum* and PSB was reported [39]. Increased yield in rice inoculated with *Azospirillum* and *B. megaterium* (a PSB) was also reported [40]. Likewise, Guggari and Kalaghatagi [30] also observed higher grain yield (12.8%) over control plants in pearl millet inoculated with *Azospirillum* and PSB. The enhanced growth and yield of the plants in response to dual inoculations in the present study might be due to augmentation of nutrients (N and growth factors like IAA) by *Azospirillum* coupled with phosphorus made available by phosphate-solubilizing bacteria which are in agreement with those reported in pearl millet [8].

**REFERENCES**


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