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# RESPONSE OF PEARL MILLET TO *AZOSPIRILLUM* AS INFLUENCED BY N FERTILIZER UNDER FIELD CONDITIONS

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**ABSTRACT**

Response of pearl millet to inoculation with *Azospirillum brasilense*, in conjunction with nitrogen application, was studied under field conditions. There was an increase of 37.9% in tillers, 44.3% in heads and 31.2% in test weight upon inoculation. Inoculation effect was reduced with increase in N-application. *Azospirillum* inoculation resulted in enhanced $N_2$-ase activity and root dry weight. The population of *Azospirillum* in the roots of inoculated plants was higher as compared to uninoculated once. The inoculation response on grain yield was equivalent to about 13 kg N $na^{-1}$.

## INTRODUCTION

In areas where little nitrogen fertilizer is applied, the ability of various free living nitrogen fixing bacteria to enrich the soil environment with nitrogen offers considerable scope to supplement the N requirement of plants. Among these bacteria, *Azospirillum*, an associative symbiotic bacterium, was found to fix atmospheric nitrogen in association with many roots of tropical plants (Rao and Venkateswarlu, 1982). Inoculation with this organism increased the dry matter and grain yield of different crops (Cohen et al., 1980; Wani et al., 1985). In arid regions pearl millet is grown on nutritionally deficient soils and often without nitrogenous fertilizer resulting in low grain yields. Venkateswarlu and Rao (1983) reported the positive response of pearl millet to inoculation with *Azospirillum brasilense* under pot culture. The quantitative information on influence of *Azospirillum* and its contribution to nitrogen requirement of pearl millet under field conditions is inadequate, particularly for arid zone soils. In this paper, therefore, an attempt has been made to gauge and quantify the response of pearl millet to inoculation with *A. brasilense* in combination with nitrogenous fertilizer under field conditions.

## MATERIAL AND METHODS

Soils of the experimental site were coarse loamy, mixed hyperthermic camborthids with 11.2% coarse sand, 69.7% fine sand, 7.1% silt and 11.9% clay. The soil was low in organic carbon (0.162%), medium in available phosphorus (17 kg P $ha^{-1}$) and available potassium (190 kg K $ha^{-1}$). The treatments consisted of 4 nitrogen levels (0, 13, 20 and 40 kg N $ha^{-1}$) in combination with and without inoculation.

Each treatment was replicated 4 times in a randomised block design with a gross plot size of 5x4 $m^2$. Seeds of pearl millet cv BJ 104 were inoculated with the peat based culture of *A. brasilense* by slurry method. The seeds were sown with a seed drill at a row distance of 50 cm and 12.5 cm from plant to plant. Half of the nitrogen as urea, 20 kg $P_2O_5 ha^{-1}$ as super phosphate and 20 kg $K_2O$ $ha^{-1}$ as muriate of potash, were side dressed at the time of sowing. The remaining half of the nitrogen dose was top dressed at one month after sowing.

For assaying nitrogenase activity, two plants from every plot were dug carefully at 45 days after sowing and the roots after de-topping were put in 200 ml glass bottles. The bottles were closed with "Suba-seals" and tightened by metallic caps. Ten per cent of air from the bottles was replaced with acetylene and at the end of 24 h of incubation, $C_2H_4$ produced was estimated by gas chromatography (Venkateswarlu and Rao, 1983). Dry weight of the roots was determined after the assay. At maturity, yields of stover and grain were recorded alongwith 1000-grain weight. The data were analysed statistically for analysis of variance.

At different periods of growth, the population of *Azospirillum* in the crushed roots of inoculated and uninoculated plants were determined by MPN method employing N-free semi-solid malate (Rao and Venkateswarlu, 1985).

## RESULTS AND DISCUSSION

**Effect on tillers and yield attributes**

Inoculation of pearl millet with *A. brasilense* significantly enhanced the tillering compared with uninoculated in all the three years. On an average, plants in the uninoculated treatments produced 21.6 tillers $m^{-2}$ which increased to 29.8 tillers $m^{-2}$ (Table 1). Increasing the dose of nitrogen progressively increased the tillers significantly. Inoculation in combination with nitrogen application further increased the tiller number (Table 1). The response was, however, at a diminishing rate. The increase in tillers owing to inoculation at smaller dose (13 kg N) was 26.3 per cent which decreased to 20.9 per cent at 20 kg N. The inoculation effect was not exhibited with further increase in nitrogen dose (40 kg N). Reddy and Reddy (1981) and Kaushik and Mahendra Pal (1982) reported enhanced effective tillers in pearl millet upon inoculation with *Azotobacter*.

Inoculation favourably influenced the heads $m^{-2}$ in all the years (Table 1). The maximum mean increase (44.3%) with inoculation was recorded when no nitrogen was applied. Nitrogen in conjunction with inoculation though gave additive effects yet the influence was at decreasing rate. At 13 kg N, increase in heads due to inoculation was 23.3 per cent which decreased to 18.4 per cent with the application of 20 kg N. Inoculation effect was not expressed when nitrogen dose was raised to 40 kg.

Table 1. Tillers and yield contributes in pearl millet as influenced by inoculation

| Treatment | Tillers $m^{-2}$ | | | | Heads$^{-2}$ | | | | Test weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1979 | 1980 | 1982 | Pooled | 19 9 | 1980 | 1982 | Pooled | 1979 | 1980 | 1982 | Pooled |
| 0 N | 18.4 | 21.3 | 25.1 | 25.6 | 7.9 | 10 8 | 13.1 | 10.6 | 5.00 | 5.00 | 4.90 | 4.97 |
| 0 N+Inoculation | 29.2 | 27.7 | 32.6 | 29.8 (37.9) | 12.5 | 15.3 | 18 1 | 15.3 (44.3) | 6 95 | 6.15 | 6 45 | 6.52 (21 2) |
| 13 kg N | 26.5 | 30.5 | 36.6 | 31.2 | 11.8 | 17.6 | 19.5 | 16.3 | 6.81 | 6.35 | 6.50 | 6.55 |
| 13kg N+Inoculation | 40.7 | 36.1 | 41.3 | 39.4 (26.3) | 14.6 | 21.3 | 24.6 | 20 1 (23.3) | 7 55 | 7.45 | 6.95 | 7.32 (11.8) |
| 20 kg N | 32.0 | 33.3 | 44.2 | 36.8 | 12.8 | 18.5 | 22.3 | 17.9 | 7.25 | 6.80 | 6.85 | 6.97 |
| 20kg N+Inoculation | 47.9 | 38.6 | 47.1 | 44.5 (20.9) | 15.2 | 22.2 | 26.1 | 21.2 (18.4) | 4.95 | 7.65 | 7 35 | 7.65 (9.7) |
| 40 kg N | 45.6 | 40.3 | 51.7 | 45.9 | 14.7 | 24.0 | 25.3 | 21.3 | 7.85 | 7.90 | 7.65 | 7.80 |
| 40kg N+Inoculation | 44.1 | 43.8 | 48.3 | 45.4 (-1.1) | 14.4 | 24.7 | 24.7 | 21.3 (0.0) | 7.65 | 8.05 | 7.60 | 7.77 (-0.4) |
| LSD (P=0.05) | 5.7 | 4.0 | 4.2 | 4.9 | 3.3 | 4.0 | 4.2 | 2.7 | 0.82 | 1.38 | 1.00 | 0.37 |

Figures in parentheses indicate % increase over no inoculation

The test weight (1000-grain weight) increased significantly from 4.92 to 6.52 g due to inoculation in the absence of N. With every increase in N-dose there was a significant increase in test weight. Inoculation in combination with nitrogen applic-tion significantly increased the test weight upto 20 kg N. The complementarity of inoculation decreased with every increase in nitrogen dose and negated (-O.4%) at 40 kg N (Table 1).

**Effect on $N_2$-ase activity, root weight and *Azospirillum* population**

The root associated $N_2$-ase activity of pearl millet in general was low. Inoculation however, significantly enhanced the activity by about 3-folds (Table 2). In pot culture studies, increased $N_2$-ase activity upon inoculation with different strains of *A. brasilense* was observed (Bouton et al., 1979; Venkateswarlu and Rao, 1983). However, the increase was inconsistent. But Purushothaman and Oblisami (1986) while working with sorghum, reported enhanced $N_2$-ase activity upon inolculation under pot culture. Nitrogen application in smaller dose (13 kg), in combination with inoculation, significantly increased the $N_2$-ase activity as compared to inoculation without N. The difference between $N_2$-ase activity of inoculated plants with 13 kg N and 20 kg N was non-significant (Table 2). Further increase in nitrogen level to 40 kg significantly decreased the $N_2$-ase activity indicating inhibitory effects of higher nitrogen dose, which could be attributed to the end product inhibition. In sorghum, inhibition of $N_2$-ase activity of inoculated plants supplied with 15 ppm N under pot culture and 40 kg N $ha^{-1}$ under field conditions was observed (Wani, 1986).

Significant improvement in root dry weight upon inoculation and application of nitrogen was recorded (Table 2). The inoculation resulted in 43.5 per cent gain in root dry weight in the absence of nitrogen. The root dry weight significantly increased with each increase in nitrogen dose from 0 to 40 kg. With the application

Table 2. Nitrogenase activity and root dry weight of pearl millet as influenced by inoculation and nitrogen application

| Treatment | $N_2$-ase activity (n moles $C_2H_4$ $h^{-1}$ $g^{-1}$ dry root) | Root dry wt (g $plant^{-1}$) |
|---|---|---|
| 0 N | 1.03 | 2.00 |
| 0 N + Inoculation | 4.06 | 2.87 (43.5) |
| 13 N | 1.62 | 2.80 |
| 13 N + Inoculation | 4.85 | 3.37 (20.3) |
| 20 N | 1.65 | 3.93 |
| 20 N + Inoculation | 4.98 | 4.49 (14.2) |
| 40 N | 1.30 | 4.43 |
| 40 N + Inoculation | 4.26 | 4.68 (5.6) |
| LSD (P = 0.05) | 0.62 | 0.49 |

Figures in parentheses indicate % increase over no inoculation

of nitrogen the beneficial effect of *Azospirillum* on root weight decreased. The inoculation caused 20.3, 14.2, and 5.6 per cent gain in root dry weight at 13,20 and 40 kg N, respectively (Table 2). The enhanced root growth upon inoculation was due to the production of various growth regulating substances such as indoles, gibberellins and cytokinins by *Azospirillum* (Tein et al., 1979; Venkateswarlu and Rao, 1983).

The studies on *Azospirillum* population in washed and crushed roots of pearl millet indicated that the population was more in the inoculated plants as compared to the control plants (Table 3) indicating the establishment of the inoculated bacterium within the root. The population was maximum at 60 days of plant growth. This is in conformity with results of Rao and Venkateswarlu (1985) who reported higher number of bacteria both in histoplane and rhizoplane in the inoculated plants of maize and pearl millet.

Table 3. Effect of inoculation on the population of *Azospirillum* (x $10^4$ $g^{-1}$ dry root) in pearl millet

| Days after sowing | Uninoculated | Inoculated |
|---|---|---|
| 30 | 3.14±0.43* | 16.33±1.21 |
| 45 | 3.59±0.37 | 19.01±1.75 |
| 60 | 5.24±0.68 | 27.16±2.30 |
| 90 | 3.14±0.51 | 16.63±1.62 |

*Mean ± SD

**Grain and stover yield**

Inoculation favourably influenced the yield of pearl millet in all the years (Table 4). An increase of 39.4 per cent in the grain yield upon inoculation (718 kg $ha^{-1}$) was recorded as compared to uninoculated (515 kg $ha^{-1}$). The grain yield is a func-

Table 4. Grain and stover yield as influenced by inoculation

| Treatment | Grain (kg $ha^{-1}$) | | | | Stover (kg $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1979 | 1980 | 1982 | Pooled | 1979 | 1980 | 1982 | Polled |
| O N | 357 | 440 | 748 | 515 | 650 | 781 | 781 | 737 |
| O N + Inoculation | 556 | 764 | 834 | 718 | 1083 | 1516 | 1563 | 1387 |
| | | | | (39.4) | | | | (88.2) |
| 13 kg N | 497 | 814 | 837 | 716 | 1417 | 1750 | 1797 | 1654 |
| 13 kg N + Inoculation | 601 | 968 | 986 | 851 | 1833 | 2125 | 1875 | 1944 |
| | | | | (18.8) | | | | (17.5) |
| 20 kg N | 581 | 968 | 1007 | 852 | 1667 | 2141 | 2109 | 1972 |
| 20 kg N + Inoculation | 789 | 1197 | 1028 | 1004 | 2167 | 2270 | 2403 | 2280 |
| | | | | (17.8) | | | | (15.6) |
| 40 kg N | 751 | 1374 | 1129 | 1084 | 2000 | 2844 | 2500 | 2448 |
| 40 kg N + Inoculation | 681 | 1339 | 1114 | 1044 | 1917 | 2953 | 2422 | 2430 |
| | | | | (-3.7) | | | | (-0.7) |
| LSD (P = 0.05) | 162 | 211 | 107 | 204 | 652 | 114 | 337 | 304 |

tion of number of heads, grains per head and 1000-grain weight. In inoculation treatments, resultant 44.3 per cent more heads and 31.2 per cent higher test weight (Table 1) were instrumental in increasing the grain yield. Better root growth, as evidenced by higher root weight under inoculation, might have helped the plants to explore more moisture and nutrients eventually resulting in enhanced yield.

The yield obtained with the application of 13 kg N (716 kg $ha^{-1}$) was similar to that of inoculation treatment (718 kg) indicating that *Azospirillum* inoculation could contribute an amount equivalent to 13 kg N $ha^{-1}$ in crop nitrogen requirement. Application of 13, 20 and 40 kg N increased the grain yield by 39.0.65.4 and 110.7 per cent over no nitrogen application (515 kg grain $ha^{-1}$). The increase in yield due to nitrogen application is of universal occurrence, particularly, in nutritionally deficient arid soils (Gautam et al., 1981; Joshi and Panjab Singh, 1985). Use of *Azospirillum* inoculation in combination with nitrogen gave additional benefits of 18.8 and 17.8 per cent at 13 to 20 kg N $ha^{-1}$, respectively (Table 4). Subba Rao (1986) reported that the yield response of pearl millet to inoculation varied under different locations and additional benefit in conjunction with the *Azospirillum* inoculation at different nitrogen levels are also different from one location to the other. At 40 kg N the inoculation did not increase the grain yield. The lack of inoculation effect at 40 kg N is due to the fact that nitrogenous fertilizers, after a threshold concentration, inhibit the activity. This is in conformity with result of Rao and Venkateswarlu (1982) who observed the inhibition of $N_2$-ase activity of *A. brasilense* by the addition of both organic and inorganic nitrogen compounds under in vitro conditions.

Inoculation in the absence of external N application resulted in stover production of 1387 kg $ha^{-1}$ (pooled) as against 737 kg $ha^{-1}$ under no inoculation (Table 4). The pooled analysis revealed a significant increase in stover production with every incremental dose of nitrogen. Inoculation coupled with application of 13 and 20 kg N $ha^{-1}$ resulted in further increase in stover yield by 17.5 and 15.6 per cent, respectively (Table 4). Similar to grain yield, the response of stover yield to inoculation at 40 kg N $ha^{-1}$ was non-additive, confirming the inhibitory effects of nitrogenous fertilizers.

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