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Full Length Research Paper

Evaluation of effect of inoculation of *Azospirillum* on the yield of *Setaria italica* (L.)

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Inoculation of foxtail millet (variety Lepakshi) with three strains of *Azospirillum lipoferum* either alone or in combination with nitrogen fertilizer (40 kg N ha^{-1}) increased the plant height, dry weight of shoot and root, and total N content of shoot, root and grain. The grain yield of the inoculated plants was significantly higher compared to the control plants. Both the panicle and the seed weight increased due to inoculation. Among the inocula of *A. lipoferum* tested, Inoculum-2 (In-2) was found superior to the other two inocula. Further, seasonal (Kharif and Rabi) variations in growth and yield of the plants were also observed.

Key words: *Azospirillum*, effect, foxtail millet, fertilization, growth, inoculation, yield.

INTRODUCTION

Small millets, which are produced mainly by subsistence farmers as rainfed crops, continue to play an important role in the diets of people living in interior rural and tribal areas in semi-arid tropics. Foxtail millet [*Setaria italica* (L.) Beauv.], a minor millet, is one of the important staple food crops in the semi-arid tropics like Anantapur district in Andhra Pradesh, India. It is nutritious (125 mg protein g^{-1}) and contains all essential amino acids. Further, it not only resists drought conditions but also withstands delayed monsoon. Hence it is cultivated as one of the minor millets in this drought prone semi-arid region. Despite many reports on inoculation of cereals and millets (Rao et al., 1983; Venkateswarlu and Rao, 1983; Gunarto et al., 1999), information on the effects of inoculation with diazotrophs and N fertilization on foxtail millet is rather limited (Nur et al., 1980; Kapulnik et al., 1981a; Yahalom et al., 1984; Di Ciocco and Rodriguez Caceres, 1994). Therefore, the present study deals with the effect of inoculation with *Azospirillum lipoferum* and nitrogen (N) fertilization on foxtail millet during Rabi and Kharif seasons in 2000.

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Abbreviations: F = Fertilizer; In-1 = Culture No. 60 NR-2; In-2 = Culture No. 45 L-1; In-3 = Culture No. 45 C-1; FIn = Culture in combination with fertilizer.

MATERIALS AND METHODS

Pot experiments

Black soil (5 kg) of sandy loam type (pH 7.8; 1.18% organic matter; 0.19% total N), placed in earthen pots (in triplicate) was sterilized at 121°C for 3 h three times on alternate days. Seeds of foxtail millet variety Lepakshi were surface sterilized following the method of Albrecht et al. (1977) and sown in the experimental pots during Rabi (February-April) and Kharif (July-September) seasons. After emergence, the plants were thinned down to one plant per pot. One set of pots was inoculated separately with three strains of *A. lipoferum* 60 NR-2 (Inoculum-1), 45 L-1 (Inoculum-2) and 45 C-1 (Inoculum-3) from 15th day onwards at 15 day intervals until harvesting period. The second set of pots received N fertilizer at the recommended level (40 kg N ha^{-1}) along with the bacterial inoculum. The third set of pots was treated with N fertilizer alone without inoculum. Control pots received only heat killed inoculum.

Table 1. Effect of inoculation of *A. lipoferum* and N fertilizer on shoot height of foxtail millet variety Lepakshi in Rabi season.

Days after sowing	Shoot height (cm)							
	Control	Fertilizer (F)	In-1	In-2	In-3	FIn-1	FIn-2	FIn-3
15	3.3a	3.2a	3.3a	3.2a	3.3a	3.3a	3.3a	3.2a
30	9.5a	14.2c	12.8b	14.3c	13.1b	15.9d	16.8c	15.2d
45	44.0a	58.0c	56.5b	58.9c	57.2b	76.0d	79.4f	77.2e
60	51.0a	67.2c	65.8b	68.2d	66.4b	84.1e	88.6g	85.3f
75	52.5a	68.0c	66.1b	68.9d	67.0b	85.0e	89.2f	86.0f

F = Fertilizer; In-1 = Culture No. 60 NR-2; In-2 = Culture No. 45 L-1; In-3 = Culture No. 45 C-1; Fln = Culture in combination with fertilizer. Means (n=3) for each treatment followed by the same letter in the columns are not significantly different ($P \leq 0.05$) according to DMR test.

Preparation of bacterial inocula

A. lipoferum strains were isolated from the rhizosphere of foxtail millet varieties Lepakshi, Chitra and non-rhizosphere at different days of plant growth. Pure cultures of three selected strains of *A. lipoferum* were grown in malate broth (Dobereiner and Day, 1976) supplemented with NH_4Cl (Okon et al., 1977). The log phase cultures were used for inoculation. The cells were harvested by centrifugation at 5,000 g at 4°C for 20 min. The supernatant was discarded and the pellet was washed two times with saline (5 g NaCl and 0.12 g $MgSO_4.7H_20$ in 1 l distilled water) and resuspended in saline at a concentration of 10^8 colony forming units (CFU) per ml. 10 ml of the bacterial culture was inoculated to each plant in a pot. The plants were grown under glasshouse conditions (31 ± 9°C day / 26 ± 2°C night) and were harvested at the end of 75 days.

Growth parameters and yield

The plant height of all the plants was measured from basal part to the tip of the plant after 15, 30, 45, 60 and 75 days of sowing. Three plants were recorded for each treatment and the data were statistically analysed. The dry weights of shoot and root were estimated at the end of 75 days by drying the plant material at 60°C for 72 h in a hot air oven. The total N content in powdered samples of the shoot, root and grain was estimated by Kjeldahl method (Jackson, 1973). The yield was determined by taking into account weight of the panicles as well as 1000 seeds.

Statistical analysis

Data were subjected to analysis of variance and means of samples were compared by Duncan's Multiple Range (DMR) test (Duncan, 1955).

RESULTS AND DISCUSSION

Data on the effects of fertilizer (F) and inoculum (In) either alone or in combination with fertilizer (FIn) on shoot height of variety Lepakshi during the Rabi season are presented in Table 1. All the treatments stimulated the plant growth over the control at different stages. Fertilizer (F) treatment led to significant increase in shoot height. Inoculum-2 (In-2) treatment also showed significant increase in shoot height which is almost on par with the increase obtained by F treatment. Although treatment with Inoculum-1 (In-1) and Inoculum-3 (In-3) also resulted in increased shoot height, the increase was not as pronounced as in the case of either F or In-2 alone. There was no significant difference in shoot height in plants treated with In-1 or In-3 alone. Application of F in combination with In-2 resulted in maximum stimulation in shoot height compared to FIn-1 or FIn-3. FIn-3 treatment in general led to significant increased shoot height compared to FIn-1. All the treatments resulted in increased shoot height over the controls irrespective of the sampling day (15 to 75 days).

In Kharif, the treatment with In-2 led to significant stimulation in shoot height compared to F treatment. No significant difference in shoot height was observed in the plants treated with In-1 or In-3 alone and it was always less when compared to F treatment. Maximum stimulation was noticed in FIn-2 treated plants (Table 2). Stimulation in shoot height was similar in FIn-1 and FIn-3 treated plants and the difference between the two treatments was not significant. However, inoculum treatment in combination with F was superior in all the cases (FIn-1, FIn-2 and FIn-3). A comparison of the data obtained during the two seasons revealed that in general the plant height was more in Kharif season compared to Rabi season.

Studies on the effect of inoculation on plant growth of *S. italica* revealed significant increase in plant height following inoculation with four isolates (Cd, Sp7, Cd-1 and Cd-3) of *A. brasilense* over the uninoculated plants (Nur et al., 1980). However, the difference in plant height among the four isolates was not significant. Results from the present study involving the same plant are also in agreement with the above observation. Thus, among the three isolates of *A. lipoferum* tested in the present study, there was no significant difference in plant height

Table 2. Effect of inoculation of *Azospirillum lipoferum* and N fertilizer on shoot height of foxtail millet variety Lepakshi in Kharif season

Days after sowing	Shoot height (cm)							
	Control	Fertilizer (F)	In-1	In-2	In-3	FIn-1	FIn-2	FIn-3
15	3.9a	4.1a	4.0a	3.9a	3.9a	4.0a	4.0a	3.9a
30	11.8a	16.0c	14.2b	16.6c	14.8b	17.0d	17.2e	17.9d
45	48.0a	62.0c	61.1b	65.0d	60.8b	83.0e	86.0f	82.5e
60	55.0a	70.0c	69.0b	72.0d	68.5b	91.0e	95.0f	90.5e
75	56.0a	72.5c	71.0b	74.0d	71.8b	92.1e	96.2f	91.8e

F = Fertilizer; In-1 = Culture No. 60 NR-2; In-2 = Culture No. 45 L-1; In-3 = Culture No. 45 C-1; Fln = Culture in combination with fertilizer.
Means (n=3) for each treatment followed by the same letter in the columns are not significantly different ($P \leq 0.05$) according to DMR test.

Table 3. Effect of inoculation of *Azospirillum lipoferum* and N fertilizer on growth and yield parameters of foxtail millet variety Lepakshi in Rabi season

Treatment	Growth parameters					Yield parameters	
	Dry weight (mg/g fresh weight)		Total N (mg N/g dry weight)				
	Shoot	Root	Shoot	Root	Grain	Panicle weight (g)	1000 seed weight (g)
Control	333a	715a	2.98a	0.76a	0.719a	1.61a	1.72a
Fertilizer	384c	722b	3.39b	1.63b	0.987bc	2.30c	2.75c
In-1	385c	726c	3.40b	1.67b	0.960b	2.08b	2.56b
In-2	395d	783d	3.50c	1.77c	1.910cde	2.45d	2.85cde
In-3	360b	728c	3.41b	1.69b	1.020bcd	2.38c	2.78cd
FIn-1	399e	793e	3.63d	1.92d	1.230de	2.47d	2.83de
FIn-2	412g	821g	4.12f	2.12e	1.540f	3.08f	3.03f
FIn-3	405f	804f	3.94e	1.96d	1.370e	2.61e	2.89e

F = Fertilizer; In-1 = Culture No. 60 NR-2; In-2 = Culture No. 45 L-1; In-3 = Culture No. 45 C-1; Fln = Culture in combination with fertilizer.
Means (n=3) for each treatment followed by the same letter in the columns are not significantly different ($P \leq 0.05$) according to DMR test.

inoculated with In-1 and In-3 whereas treatment with In-2 alone resulted in significant increase in shoot height. Likewise, plant height increased significantly when inoculated with two isolates (Cd and Sp7) of A. *brasilense* on cultivars of wheat, *Sorghum, Panicum* (Kapulnik et al., 1981b) and *S. italica* (Kapulnik et al., 1981a). Similar results were also obtained with other crops like pearl millet (Venkateswarlu and Rao, 1983) and cotton (Fayez and Daw, 1987). Similarly, inoculation of indigenous strains of *Azospirillum* to rice led to increased plant height at some growth stages (40 and 75 days after transplantation) and the increase was significant over the control although the difference in increase among the strains was not significant (Gunarto et al., 1999). Sunflower when inoculated with Cd strain of *A. brasilense* and a strain of *A. lipoferum* positively affected plant growth especially under irrigated conditions (Itzigsohn et al., 1995). Although inoculation of wheat with strains of *A. brasilense* resulted in increased plant height, the difference was not significant (Kapulnik et al., 1985; 1987).

Results on the effect of inoculation on dry weight of the shoot in variety Lepakshi in Rabi season showed that all the treatments resulted in significant increase (8 to 24%) over the control plants (Table 3). Among the inocula, In-2 (19%) followed by In-1 (16%) gave higher dry matter production over the plants treated with F alone (15%). However, the increase in dry weight in In-1 treatment was not significant when compared to In-2 treated plants. The plants treated with In-3 exhibited the least increase (8%) in dry matter production among all the treatments. Combination of inocula and F also resulted in significant increase in dry weight ranging from 20 to 24% over the control plants. Fin-2 treated plants recorded the maximum dry weight followed by FIn-3 and FIn-1 and the difference in dry matter production among them was

Table 4. Effect of inoculation of *Azospirillum lipoferum* and N fertilizer on growth and yield parameters of foxtail millet variety Lepakshi in Kharif season

Treatment	Growth parameters					Yield parameters	
	Dry weight (mg/g fresh weight)		Total N (mg N/g dry weight)				
	Shoot	Root	Shoot	Root	Grain	Panicle weight (g)	1000 seed weight (g)
Control	316a	728a	3.65a	0.725a	0.881a	0.882a	1.75a
Fertilizer	396c	815c	3.98b	1.920d	2.210c	1.350c	2.91cd
In-1	362b	804b	4.21c	1.370b	1.640b	1.080b	2.74bc
In-2	415d	821c	4.45d	1.450c	1.740b	1.740d	2.95d
In-3	358b	798b	4.16c	1.390b	1.690b	1.060b	2.57b
FIn-1	470e	835d	4.79e	1.950d	2.580d	2.620e	3.24e
FIn-2	512g	865e	5.32f	2.230e	2.920e	2.910f	3.50f
FIn-3	482f	839d	4.82e	1.970d	2.620d	2.580e	3.15e

F = Fertilizer; In-1 = Culture No. 60 NR-2; In-2 = Culture No. 45 L-1; In-3 = Culture No. 45 C-1; Fln = Culture in combination with fertilizer. Means (n=3) for each treatment followed by the same letter in the columns are not significantly different ($P \leq 0.05$) according to DMR test.

significant.

Dry matter production in Kharif was significantly higher (13 to 62%) when compared to Rabi. In-2 treated plants recorded the maximum (31%) dry matter production followed by F (25%), In-1 (15%) and In-3 (13%). Among the inocula in combination with F, FIn-2 treated plants exhibited the maximum stimulation (62%) in dry matter production followed by FIn-3 (53%) and FIn-1 (49%). The increase in dry matter production among them was significant (Table 4).

A perusal of the data from the present study indicates that, in general, In-2 treatment either alone or in combination with F resulted in the maximum increase in shoot dry weight of foxtail millet. Similar results were also reported by Nur et al. (1980) who studied the effect of inoculation of Israeli isolates of *A. brasilense* on *S. italica* and *Zea mays*. The organism tested significantly increased the dry weight of shoot of both the plants. Cohen et al. (1980) also observed that the inoculation of *S. italica* and *Z. mays* with strains of *A. brasilense* increased plant dry weight from 50 to 100% and the increase varied with the soil type. Okon et al. (1983) reported significant increase in shoot dry weight of *S. italica* as a result of inoculation with *A. brasilense*. Investigations in Argentina, where *Azospirillum* strains were inoculated in combinations, also revealed the increase in shoot dry weight up to 57%, while in combination with *A. brasilense* strain AZ-39, the increase rose to 91%. However, the combination of Cd and AZ-39 strains with AZ-30 strain of *A. lipoferum* resulted in only 30% increase (Di Ciocco and Rodriguez Caceres, 1994). Such studies of comparison on the response of *S. italica* with *A. brasilense* and *Azotobacter chroococcum* were also conducted (Yahalom et al., 1984).

Dry weight of roots significantly increased in all the treated plants over the control plants in Rabi (Table 3). In-2 treated plants alone showed the maximum (10%) dry matter production among the inocula and F treatment. Further, the difference in dry weight between In-3 and In-1 treated plants was not significant. The plants treated with F exhibited the least increase in dry matter production when compared to that of inocula alone. Significant stimulation in root dry weight was observed in FIn-2 (15%) followed by FIn-3 (12%) and FIn-1 (11%) treated plants.

Root dry matter production was considerably higher in Kharif season. All the treatments resulted in higher root dry weight over the control plants (Table 4). Although In-2 treated plants exhibited higher root dry weight among the inocula, the increase was almost on par with F treated plants but the difference was not significant. Similar trend was also noticed between In-3 and In-1 treated plants. Even among the FIn treated plants, the increase in root dry weight between FIn-3 and Fin-1 treated plants was not significant. However, FIn-2 treated plants exhibited the maximum and significant stimulation.

The root dry matter production was more in Kharif season compared to Rabi season. Similar trends due to inoculation on root dry matter production were also reported by others. Inoculation with two strains of *A. brasilense* (Cd and Cd-1) alone resulted in significant increase in root dry weight over the control plants in *S. italica* (Nur et al., 1980). Kapulnik et al. (1981) also observed that the root system of *S. italica* inoculated with the strain Cd of *A. brasilense* increased in size and branching compared to the control plants. Likewise, significant increase in the root dry weight of the same plant as a result of inoculation with *A. brasilense* has been reported (Okon et al., 1983).

All treatments showed increase (14 to 30%) in shoot total N over the control plants in Rabi season (Table 3). In-2 treated plants exhibited higher and significant increase in total N compared to In-3, In-1 and F treated plants. Although In-3 and In-1 treated plants contained more total N than F treated plants, the increase was not significant. Fin treated plants recorded higher total N compared to In or F treated plants. Among the Fin treatments, FIn-2 treated plants contained the maximum (38%) N content. The total N in shoot was higher in Kharif (9 to 46%) than in Rabi (14 to 38%). All the inoculated plants contained higher total N compared to the F treated plants or the control plants (Table 4). But there was no significant increase in total N content of In-1 and In-3 treated plants. In-2 treatment resulted in higher total N among the inocula. Fin treated plants recorded enhanced N content over all other treatments with FIn-2 being the maximum. No significant difference in increase in N was noticed between FIn-1 and FIn-3 treated plants.

All the treatments resulted in higher total N content of root compared to the control plants in Rabi (Table 3). In-3, In-1 and F treated plants contained almost the same amount of N with no significant difference among them. In-2 treated plants had higher N content among inoculated and F treated plants. Combination of inoculation and fertilization resulted in higher N content compared to the other treatment with FIn-2 exhibiting the maximum N content. Further, there was no significant increase in total N between FIn-3 and FIn-1 treated plants. In Kharif season also all the treatments led to increased root total N compared to the control plants (Table 4). F treated plants contained more total N in their roots compared to inoculated plants including In-2 treated plants. But In-2 treated plants had significantly higher N content compared to In-3 and In-1 treated plants while no significant difference was noticed between In-3 and In-1 treated plants. Similarly, significant differences existed in total N content of FIn-3, FIn-1 and F treated plants. FIn-2 treated plants exhibited the maximum total N over all other treatments.

In Rabi season the total N content in grains of all the treated plants was significantly higher over the control plants (Table 3). In spite of a marginal increase in total N content among In-1, F and In-3 treated plants, no significant difference in total N was observed. Similarly, there was no significant difference in N content among FIn-3, FIn-1 and In-2 treated plants. In general, there was no significant difference in grain N content among most of the treated plants except FIn-2.

The N content in grain was significantly higher in Kharif season (86 to 231%) compared to Rabi season (34 to 114%). All the treatments resulted in higher N content compared to the control plants (Table 4). F treated plants had significantly higher N content over the inoculated plants which exhibited no significant difference among them. Likewise, the total N content in FIn-3 and FIn-1 treatments did not differ significantly while FIn-2 treatment resulted in significant difference in N content of grain.

Results from the present study revealed that inoculation either alone or in combination with F resulted in significant increase in total N content of shoot, root and grain over the control plants as illustrated by In-2. There are very few reports available in literature on the effect of inoculation on N content of shoot, root and grain in *S. italica*. Inoculation of *S. italica* with three strains of *A. brasilense* resulted in 50 to 200% increase in total N content of the plant while another strain Cd of *A. brasilense* recorded 128% increase (Nur et al., 1980). The total N content in inoculated plants either alone or in combination with F increased during Kharif season compared to the N content in Rabi season in the present study. This is in agreement with the reports by others. Cohen et al. (1980) reported that the N content increased significantly (115 to 118%) in *S. italica* plants grown during May-June compared to the plants grown during February-March (12 to 51%) following inoculation with different strains of *A. brasilense*. Further, increase in N content in the inoculated plants varied with the soil type which ranged between 25 and 150%. Kapulnik et al. (1981a) also reported a significant increase (72.8%) in the total n content of *S. italica* plants inoculated with strain Cd of *A. brasilense*. Although Okon et al. (1983) observed increase in total plant N in *S. italica* plants, the increase was not significant.

Yield parameters like panicle and seed weight (1000) were recorded at the time of harvest. In Rabi season all the treatments increased the panicle weight over the control plants (Table 3). Between In and F treatments, In-2 treated plants exhibited significant increase over the others while In-3 and F treatments were on par but with significant increase over In-1 treatment. FIn treatments recorded significant increase in panicle weight and followed the order FIn-2>FIn-3>FIn-1. The yield percentage increased significantly in Kharif (20 to 230%) compared to Rabi (29 to 91%) season. Inoculation and fertilization led to significant increase in panicle weight when compared to In-1 and In-3 treatments. However, there was no significant difference between In-1 and In-3 treatments while In-2 showed significant increase over F treatment. Among FIn treated plants, FIn-2 recorded significant increase over FIn-1 and FIn-3 whereas there was no significant difference between FIn-1 and FIn-3 treated plants.

Irrespective of the treatment, seed weight increased in all the treated plants over the control plants in Rabi season (Table 3). Although seed weight in In-2 and In-3 treated plants increased over F treated plants, the increase was not significant. However, the increase in seed weight in In-2, In-3 and F treated plants was significant compared to In-1 treated plants. Among the FIn treated plants, FIn-2 alone exhibited significant increase in seed weight while FIn-3, FIn-1 and FIn-2 treatments were almost on par with F treated plants. The seed weight as well as the percent increase in seed weight were higher in Kharif season compared to Rabi season. All the treated plants recorded significantly higher seed weight over the control plants (Table 4). There was no significant increase in seed weight between In-2 and F treated plants and between In-1 and In-3 treated plants. Among FIn treated plants, FIn-2 treatment exhibited the maximum and significant increase in seed weight while it was not significant between FIn-1 and FIn-3 treated plants.

Inoculation with In-2 either alone or in combination with F gave the maximum yield of grain and panicle weight in both the seasons. Among the other inocula, In-3 was superior to In-1 in Rabi season while In-1 was more efficient than In-3 in Kharif season. In general, yield in In-1 and In-3 treated plants was almost on par with F treated plants. The combination of inocula and F gave significantly higher yields compared to In or F treatment alone. In Rabi season, FIn-3 gave higher yields compared to FIn-1 while in Kharif FIn-1 exhibited better yield over FIn-3.

The effects of inoculation of *Azospirillum* on yield of several crop plants have been reviewed (Sumner, 1990; Wani, 1990; Kennedy and Tchan, 1992; Okon and Labandera Gonzalez, 1994; Kannaiyan, 2003). However, very few reports are available with regard to *S. italica*. Inoculation of *S. italica* with *A. brasilense* (Cd) increased plant growth and yield significantly at low or medium levels of combined N whereas the effect was less marked at zero or at high levels of N fertilization (Kapulnik et al., 1981a). Further, significant increases in shoot dry weight, panicle weight and length were obtained in inoculated plants fertilized with suboptimal levels of N fertilizer. Similarly, Okon et al. (1983) also studied the effect of inoculation on nitrogen fixation and yield of *S. italica* using the same bacterium. The results obtained corroborated those reported by Kapulnik et al. (1981a). In another experiment conducted at semi-arid Pampas of Argentina, with the combined inoculation of *A. brasilense* strains AZ-39 and Cd and AZ-30 of *A. lipoferum* to *S. italica* cv. Yaguana, the yield increased significantly by 30% in the first season. In the second season, AZ-39 and Cd, when inoculated individually increased yields significantly by about 21% (Di Ciocco and Rodriguez Caceres, 1994). Results from the present study revealed that in general, In-2 either alone or in combination with F exhibited significant increase in growth parameters and yield irrespective of the season.

Azospirillum strains are known to produce phytohormones like indole acetic acid, gibberellins and cytokinins under *in vitro* conditions (Venkateswarlu and Rao, 1983; Tien et al., 1979; Hartmann et al., 1994; Rademacher, 1994). These hormones in turn have been reported to affect nitrogen-fixing capability of the diazotrophs (Christiansen Weniger, 1988). Okon and Labandera-Gonzalez (1994) concluded that various strains of *A. brasilense* and *A. lipoferum* are capable of promoting the yield of agriculturally important crops in different soils and climatic regions. A critical review of the data revealed that success with statistically significant increases in yield ranging from 5 to 30% was achieved in 60 to 70% of the inoculation experiments which is in agreement with the reports of Okon et al. (1988) and Wani (1990). In spite of the negative results reported mainly on wheat, Sumner (1990) while reviewing the crop responses to *Azospirillum* inoculation concluded that the responses have been quite substantial and well in excess of the likely costs of inoculation thus making this technique highly attractive to the farmer.

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REFERENCES

Albrecht SL, Okon Y, Burris RH (1977). Effect of light and temperature on the association between *Zea mays* and *Spirillum lipoferum*. Plant Physiol. 60: 528-531.

Christiansen-Weniger C (1988). An influence of plant growth substances on growth and nitrogenase activity from *Azospirillum brasilense*. In: Klingmuller W (Ed.), *Azospirillum* IV. Genetics, Physiology, Ecology. Springer Verlag, Berlin. pp. 141-149.

Cohen E, Okon Y, Kigel J, Nur I, Henis Y (1980). Increase in dry weight and total nitrogen content of *Zea mays* and *Setaria italica* associated with nitrogen fixing *Azospirillum* spp. Plant Physiol. 66: 746-749.

DiCiocco AC, Rodriguez-Caceres EA (1994). Field inoculation of *Setaria italica* with *Azospirillum* spp. in Argentine humid pampas. Field Crop Res. 37: 253-257.

Dobereiner J, Day JM (1976). Associative symbiosis in tropical grasses: Characterisation of microorganisms and dinitrogen fixing sites. In: Proc. First Int. Symp. on N_2 fixation. Washington University Press, Pullman. pp. 518-538.

Duncan GB (1955). Multiple range and multiple tests. Biometrics 42: 1-42.

Fayez M, Daw ZY (1987). Effect of inoculation with different strains of *Azospirillum brasilense* on cotton (*Gossypium barabadense*). Biol. Fertil. Soils 4: 91-95.

Gunarto I, Adachi K, Senboku T (1999). Isolation and selection of indigenous *Azospirillum* spp. from a subtropical island and effect of inoculation on growth of lowland rice under several levels of N application. Biol. Fertil. Soils 28: 129-135.

Hartmann A, Singh M, Klingmuller W (1994). Isolation and characterization of *Azospirillum* mutants excreting high amounts of indole acetic acid. Can. J. Microbiol. 29: 303-314.

Itzigsohn R, Abbass Z, Sarig S, Okon Y (1995). Inoculation effects of *Azospirillum* on sunflower (*Helianthus annus*) under different fertilization and irrigation regimes. NATO ASI Ser. G 37: 503-513.

Jackson ML (1973). Nitrogen determinations for soils and plant tissues. In: Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd, New Delhi. pp. 183-204.

Kannaiyan S (2003). Inoculant production in developing countries – problems, potentials and success. In: Hardarson G, Broughton WJ (Eds.), Maximising the use of biological nitrogen fixation in agriculture. Kluwer Academic Publishers. The Netherlands. pp. 187-198.

Kapulnik Y, Okon Y, Kigel J, Nur I, Henis Y (1981a). Effect of temperature, nitrogen fertilization and plant age on nitrogen fixation by *Setaria italica* inoculated with *Azospirillum brasilense* (strain Cd). Plant Physiol. 68: 340-343.

Kapulnik Y, Kigel J, Okon Y, Nur I, Henis Y (1981b). Effect of *Azospirillum* inoculation on some growth parameters and N content of wheat, sorghum and *Panicum*, Plant and Soil. 63: 627-631.

Kapulnik Y, Feldman M, Okon Y, Henis Y (1985). Contribution of nitrogen fixed by *Azospirillum* to the N nutrition of spring wheat in Israel. Soil Biol. Biochem. 17: 509-515.

Kapulnik Y, Okon Y, Henis Y (1987). Yield response of spring wheat cultivars (*Triticum aestivum* and *T. turgidum*) to inoculation with *Azospirillum brasilense* under field conditions. Biol. Fertil. Soils 4: 27-35.

Kennedy IR, Tchan YT (1992). Biological nitrogen fixation in non-leguminous field crops: Recent advances. Plant and Soil 141: 93-118.

Nur I, Okon Y, Henis Y (1980). An increase in nitrogen content of *Setaria italica* and *Zea mays* inoculated with *Azospirillum*, Can. J. Microbiol. 26: 482-485.

Okon Y, Albrecht SL, Burris RH (1977). Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. Appl. Environ. Microbiol. 33: 85-88.

Okon Y, Heytler PG, Hardy RWF (1983). N_2 fixation by *Azospirillum brasilense* and its incorporation into host *Setaria italica*. Appl. Environ. Microbiol. 46: 694-697.

Okon Y, Kapulnik Y, Sarig S (1988). Field inoculation studies with *Azospirillum* in Israel. In: Subba Rao NS (Ed.), Biological nitrogen fixation–Recent developments. Oxford & IBH Publishing Co, New Delhi. pp. 175-195.

Okon Y, Labandera-Gonzalez CA (1994). Agronomic application of *Azospirillum*. An evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem. 26: 1591-1601.

Rademacher W (1994). Gibberellin formation in microorganisms. Plant Growth Regul. 15: 303-314.

Rao VR, Nayak DN, Charyulu PBBN, Adhya TK (1983). Yield response of rice to root inoculation with *Azospirillum*. J. Agric. Sci. 100: 689-691.

Sumner ME (1990). Crop responses to *Azospirillum* inoculation. Adv. Soil Sci. 12: 53-123.

Tien TM, Gaskins MH, Hubbel DH (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37: 1016-1024.

Venkateswarlu B, Rao AV (1983). Response of pearl millet to inoculation with different strains of *Azospirillum brasilense*. Plant and Soil 74: 379-396.

Wani SP (1990). Inoculation with associative nitrogen fixing bacteria: Role in cereal grain production improvement. Indian J. Microbiol. 30: 363-393.

Yahalom E, Kapulnik Y, Okon Y (1984). Response of *Setaria italica* to inoculation with *Azospirillum brasilense* as compared to *Azotobacter chroococcum*. Plant and Soil 82: 77-85.