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REVIEW

Inoculation with Associative Nitrogen-Fixing Bacteria : Role in Cereal Grain Production Improvement

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Introduction

Since Winogradsky (1) established in 1893 that *Clostridium pasteurianum* could fix atmospheric N_2 and Beijerinck (2) described in 1901 the first *Azotobacter*, the list of N_2-fixing bacteria associated with cereals and grasses has increased. The list includes species of *Achromobacter, Acetobacter, Alcaligenes, Arthrobater, Azotobacter, Azomonas, Bacillus, Beijerinckia Clostridium, Campylobacter, Corynebacterium, Derxia, Desulfovibrio, Enterobacter, Erwinia, Herbaspirillum, Klebsiella, Lignobacter, Mycobacterium, Methylosinus, Pseudomonas, Rhodospirillum, Rhodopseudomonas,* and *Xanthobacter*. Although many genera and species of N_2-fixing bacteria can be isolated from the rhizosphere soil of various cereals, mainly members of *Azotobacter* and *Azospirillum* genera have been widely tested to increase yields of cereals under field conditions.

The occurrence of the association of N_2-fixing bacteria with roots of cereals and grasses is well documented (3-8) and these bacteria are stimulated in the rhizosphere of these crops (9-10). Azospirilla and azotobacters are active N_2 fixers under laboratory conditions, generally found wherever these are sought and can use a variety of carbon and energy sources for their growth on combined N or N_2. It was thought that these bacteria could be exploited to increase crop yields through increased biological nitrogen fixation (BNF). To increase crop yields, the route of artificial inoculation of plants with N_2-fixing bacteria has been tried. Many experiments have been performed in several countries to investigate the effects of inoculation of various strains of *Azotobacter chroococcum* and *Azospirillum* spp. on cereals and grasses. Several field experiments in Belgium, Brazil, Czechoslovakia, Egypt, Israel, India, Germany, Poland, USA, and USSR with different crops inoculated with different N_2-fixing bacteria showed increased yields and/or increased N accumulation by plants, and sometimes resulted in decreased yields because of inoculation. This review summarises the findings of several field experiments conducted in various countries on cereals inoculated with azotobacters and azospirilla and to ascertain the benefits from inoculation experiments. The literature on mechanisms of increasing crop yields is also reviewed to find the extent of BNF contributing towards increasing cereal yields because of inoculation with N_2-fixing bacteria.

2. Inoculation Responses

It is not possible to cover all the reports published on field inoculation responses and an attempt has been made to cover maximum number of reports from various countries covering cereals and different N_2-fixing bacteria. More emphasis is given to reports from USSR, Israel, and India, as these are the countries where maximum number of inoculation trials were conducted.

2.1. Grain and plant biomass yield: The first attempt in 1902 to study the effect of *Azotobacter* on the growth of oats in a pot experiment showed no increase in plant dry weight and nitrogen content because of inoculation; thus the first attempt of using *Azotobacter* to increase crop yields was unsuccessful (11). However, subsequent experiments showed positive benefits of *Azotobacter* inoculation in pots (12, 13) that rasulted in conducting the first field experiment in the USSR in 1933 on the effect of *Azotobacter* on the yield of plants. Since then, many field experiments were conducted in the USSR to study the benefits from *Azotobacter* inoculation. The first comprehensive survey of the data obtained from such field experiments revealed that out of 1095 experiments, 890 (81%) experiments showed increases in yields of cereals and vegetables, and the increases amounted to >10% in only 514 (47%) experiments (14). Further, the 1949 report of the Agriculture Ministry, USSR, showed that in 14 out of 17 (82%) experiments on wheat, oats, barley, and rye, the increases in crop yields exceeded 10% because of *Azotobacter* inoculation. The results of 105 experiments with wheat and oats performed at research institutes during 1949-55 revealed that in 83% of the experiments use of *Azotobacter* increased crop yields (15). Similarly, several field experiments conducted in different parts of the USSR showed increased crop yields because of *Azotobacter* use (Table 1). It was observed that in the Volga region positive results with *Azotobacter* were obtained only in irrigated soils (16). In poorly cultivated soils of the Gorki and Arzomas regions good results were obtained with standard doses of *Azotobacter*, whereas, higher doses were needed in well-cultivated Chernozem and Forest-steppe soils (17). Of the 241 experiments with cereal crops, in >71% of the experiments the use of *Azotobacter* increased yields by >10%. In flood plains of the Northern Reaches of the Yenisei, use of *Azotobacter* proved effective in the presence of sufficient amounts of organo-mineral fertilizers. Use of *Azotobater* caused a 12.5% increase in yield of rye also in acidic soils. In many regions, strains of *Azotobacter* isolated from the local soils were found to be effective (16, 18-20). *Azotobacter* inoculation increased crop yields effectively; in irrigated calcareous soil wheat yield increased by 20-25% and in Chestnut soils, poor in humus, wheat yield increased by 10-14%. *Azotobater* inoculation had no effect in slightly calcareous chestnut soils rich in humus and in acidic Alpine-meadow Chernozems rich in nitrogenous organic compounds (21). In the Estonian S. S. R. region, results of 117

Table 1. Summary of cereal crop responses to *Azotobacter* inoculation in different regions of the USSR.

Crop	No of expt.	Av. % increase in yield	Crop	No. of expt.	Av. % increase in yield
Spring wheat	6[1]	13	Oats	8	16
	2	6		2	24
	7	28		1	9
	38	17		1	15
	1	15		26	15
	6	13		2	24
	6	4		1	9
Winter wheat	4	18		32	9
	5	17	Rye	2	24
	25	11		4	18
Barley	2	12		1	14
	1	22	Millet	3	17
	1	7		1	49
	15	9		5	1
	7	15	Corn	8	18
	30	7		1	18
Foxtail millet	2	39		18	6

[1] Each number indicate the number of experiments conducted in a region.

[2] Locally isolated strain of *Azotobacter* was used for inoculation.

Source : Extracted from Rubenchik (16).

field experiments on the use of *Azotobacter* concluded that *Azotobacter* is effective only in soils with a native *Azotobacter* population. This observation looks strange since it is generally thought that inoculation is successful in soils that have very low or no population of the inoculant bacteria. Further, it was suggested that instead of *Azotobacter* inoculation it would be more convenient to enhance the growth of the native *Azotobater* population in the soil by treating seeds with trace elements and other growth factors (22, 23). In Poland, pure *Azotobacter* cultures proved to be ineffective and the introduction of soil containing large numbers of *Azotobater* cells led, in some experiments, to an increased crop capacity of the plants tested (16).

In Australia, out of 71 field trials with *Azotobacter* inoculation of wheat, in 28 trials grain yields increased by >5%, in 4 trials negative results were observed and in 39 trials no effect on grain yields were observed (24).

While most workers in the USSR found positive benefits from *Azotobacter* inoculation on the yield of various crops, some workers from other countries drew insufficiently grounded conclusions after obtaining negative results in the few experiments (3, 25-32). While comparing results obtained by Soviet and non-Soviet workers, one must remember that the scope of investigations on *Azotobacter* in the USSR was much wider and more comprehensive than those of the non-Soviets.

Schmidt (29) concluded that *Azotobacter* was not effective in German soil. However, the use of an *Azotobacter* strain from the USSR increased the potato yields in a field trial by 15% (33).

Of late, attention has been shifted from *Azotobacter* to *Azospirillum* as an inoculant as it has widespread distribution in soil, is easy to culture and identify because of its curved form and type of motility, and is relatively efficient in utilization of carbon to support N_2-fixation.

A comprehensive list of field experiments with *Azotobacter* and *Azospirillum* inoculation is given in Table 2. The results (Tables 1-3) indicate that in many cases inoculations increased plant yields and such increases are statistically significant or otherwise and also sometimes negative. In many cases, experiments with negative responses are not reported and it becomes difficult to assess the agronomic significance of the positive responses that have been obtained for many parameters-grain yield, plant biomass yield, nutrient uptake, N uptake, grain N content, nitrogenase activity, time to 50% flowering, tiller number and 1000 grain weight. Responses usually do not occur in all these parameters in a given experiment. Various cereal crops have responded positively to inoculation with *Azotobacter* and *Azospirillum* with a wide range in yield increases. The responses vary with crops, locations, seasons, agronomic practices, bacterial strains, and some of these factors are discussed later in this review.

In India, multilocational trials with pearl millet conducted under different agroclimatic conditions for 3 years showed that seed inoculation with *A. brasilense* increased the mean grain yields significantly at six out of nine locations tested. Increase in mean yield because of inoculation over noninoculated control with no nitrogen addition, was equivalent to that of 10-15 kg N ha^{-1} application (34). The results of 5 years of testing at four locations revealed that seed inoculation brought an increase in grain yields over noninoculated control (no N). The increase in all-India mean grain yield because of seed inoculation over noninoculated

Table 2. Crop responses to inoculation with N_2-fixing bacteria in field trials.

Crop	Inoculation treatment	Percentage increase in yield over noninoculated control		Remarks and references
		Range	Average	
Rice	*Aztb. chroococcum*	-6 to 17^{NS}	8^{NS}	Inoculation increased yield only with applied N treatments (137)
Rice	*Beijerinckia indica*	-12 to 24^{*}	2^{NS}	Significant increase was observed with 40 kg N ha^{-1} inoculated treatment (139)
Forage grass	*Azosp. brasilense*	NS	NS	Out of 40 genotypes only in *Digitaria decumbens* and *panicum maximum* inoculation increased biomass significantly by 50 and 63% over respective controls (140)
Pearl millet	*Azosp. brasilense*	0 to 20^{*}	—	Increases with only 20 and 60 kg N ha^{-1} treatments (141)
Maize (17 cvs) & sorghum (2 cvs)	*Azosp. brasilense*	NS	–	Positive and negative effects (142)
P maximum	*Azosp. brasilense*	NS	–	
Pearl millet	*Azosp. brasilense*	–	29^{*}	With 90 kg N ha^{-1} applied (143)
Pearl millet	*Azosp. brasilense*	-10 to 15^{NS}	—	Five hybrids and 15 inbreds were tested. Pooled analysis of 2 yrs. data showed significant increase by 19 and 14% in Gahi 3 hybrid and Bil 38 inbred (144)
Pearl millet	*Azotobabter*		11.2^{*}	Average of 3 trials (145)
Pearl millet	*Aztb. chroococcum*	No effect		(146)
Pearl millet	*Azosp. brasilense*	-10 to 17^{NS}	–	Trials were conducted for 2 yrs. at 9 locations. Significant increases were observed at 6 locations only (52)
Pearl millet	*Azosp. brasilense*	4 to 19	10^{*}	Average of 4 locations over 5 yrs trials. Mean inoculation effects were significant only with 10 kg N ha^{-1} treatment (35)
Pearl millet	*Aztb. chroococcum*		8^{NS}	Mean across the N levels (35)
Pearl millet	*Azosp. brasilense*		8^{NS}	
Rice	*Aztb. chroococcum*		13^{NS}	Two isolates were tested with diff. N levels (148)
Rice	*Aztb. chroococcum*		12^{NS}	
Rice	*Aztb. chroococcum*+ Glyricidia or sunnhemp		14^{*}	
	Aztb. chroococcum+ paddy straw or sesbania		7^{NS}	(65)
Rice	*Azospirillum*		11^{*}	(149)
Rice	*Azospirillum*		33^{NS}	(160)

Table 2. Contd.

Crop	Inoculation treatment	Percentage increase in yield over noninoculated control		Remarks and references
		Range	Average	
Rice	*Azospirillum* Irrigated		28*	Mean across the 4 N levels (147)
	Rainfed		16*	
Sorghum	*Aztb. chroococcum*	-1 to 10	3	(150)
Sorghum	*Azosp. lipoferum*		0	
Sorghum	*Azosp. brasilense*		18*	Alongwith organic matter inoculation increased yield by 31% over control (40)
Sorghum	*Azosp. brasilense*		10*	Average of 2 trials. Inoculation effects were more pronounced (127)
Sorghum	*Aztb. chroococcum*	-22 to 38	13	Out of 5 locations the results were significant at 1 location only (151)
Sorghum	*Aztb. chroococcum*		25*	Differcnt N levels were used (152)
Sorghum	*Azosp. brosilense*		28*	
Sorghum	*Azospirillum*		13*	Mean across N treatments (153)
Sorghum	*Azosp. brasilense*	7 to 31*	19*	Average of 9 locations over 4 yrs. Significant increases were observed at 3 locations only (36)
Sorghum	*Azosp. lipoferum*	—	6.5^{NS}	Mean across 3 cvs. Total dry matter was significantly increased (P=>0.1) by 11% in case of *Azosp. lipoferum* treatments (37)
Sorghum	*Aztb. chroococcum*	—	6^{NS}	
Sorghum	*Azospirillum* spp.	2 to 10	5^{NS}	Mean across 3 cvs. Similar results were observed for total plant biomass also (37)
Sorghum	*Aztb. chroococcum*	2 to 40	30*	In the presence of wheat straw and sugarcane bagasse innoculation increased yield over organic matter amendments alone (67)
Sorghum	*Aztb. chroococcum*	2 to 20	8^{NS}	Average of 2 trials–120 kg N ha^{-1} was applied (154)
Maize	*Aztb. chroococcum*	4 to 19	11	Significance not mentioned (140)
Maize	*Azosp. lipoferum*		36*	Increased plant N uptake by 40 kg ha^{-1}
Maize	*Azosp. brosilense*		19^{NS}	(33)
Maize	*Aztb. chroococcum*	27 to 72	44*	Significant at all the 4 locations (151)
Maize	*Aztb. chroococcum*		50*	Mean across the N levels (152)
Maize	*Azosp. brasilense*		53*	
Wheat	*Azosp. lipoferum*		28^{NS}	Reduced plant biomass
	Azosp. brasilense		34^{NS}	Plant biomass was increased significantly by 16% (85)

Table 2. Contd.

Crop	Inoculation treatment	Percentage increase in yield over noninoculated control		Remarks and references
		Range	Average	
Wheat	*Aztb chroococcum*	10 to 13	11*	Significant increases were observed only with inoculated N treatments (151)
Wheat	*Aztb. chroococcum* *Azosp. brasilense*		16* 22*	Mean across diff. N levels (152)
Wheat	*Azosp. brasilense*		39*	Diff. strains of *A. brosilense* were tested and SP 245 showed significant difference over other strains. Total N uptake and concentration was also significantly increased (77)
Wheat	*Azosp amazonense*		37*	
	Azotobactor	-5 to 19^{NS}	7	Average of three trials, 2 strains were used (155)
Wheat	*Aztb. chroococcum*	0.4 to 11	5.2^{NS}	Average of 3 years trial. Treatments included 5 to 10 t ha^{-1} FYM and 60 & 120 kg N ha^{-1} combinations with *Aztb. chroococcum* (68)
Rice, wheat, oat, barley and sorghum	*Azosp. brasilense*	0 to 81*	17	At 14 locations out of 56 inoculation treatments combinations only 14 combinations showed significant increases. Significant increases were observed with 0 to 120 kg N ha^{-1} treatments combination (156)
Pearl millet, grain sorghum, *p americanum* × *p purpureum* & forage sorghum	*Azosp. brasilense*	-1 to 24*		Out of 3 locations and 5 crops only grain sorghum and *P. purpureum* × *P. americonum* showed significant increase at 2 loccations (51)
Maize, wheat and sorghum	*Aztb chroococcum*	No effect		(157)
Finger millet	*Azosp brasilense*		22*	(158)
Finger millet	*Azosp. lipoferum*			(159)
Corn, wheat, sorghum, *Seteria italica* & *P. miliaceum*	*Azospirillum*	0 to 47		Out of 31 trials only 18 trials showed significant increases All the trials with sorghum and grain corn showed significant increases (38)
Barley	*Azosp brasilense*	0 to 6	4	Mean of two trials conducted for two years with 0, 50 & 60 kg N ha^{-1} (76)
	Aztb. chroococcum	0 to 15	9*	
	Azosp + *Aztb.*	14 to 26	19*	

* P = <0 05

NS = Nonsignificant.

Table 3. Summary of the *Azospirillum* inoculation trials conducted on large plots in Israel

Crop	No. of experiments	Increase in yield (%)		No. of experiments showing	
		Range	Average	Significant increases	Increase above 5%
Corn (forage and sheet corn and grain corn)	13	0–38	16	7	11
Wheat (forage and grain)	10	3–19	9	5	8
Sorghum (forage and grain)	5	12–20	17	5	5
Setaria italica	2	38–47	43	2	2
Panicum milliaceum	1	13	13	1	1

Source : Exracted from Okon (38).

control was 15% which was on par with the yield obtained with 10 kg N ha^{-1} (urea) application alone. Further, inoculation proved beneficial alongwith upto 40 kg N ha^{-1} as a basal dose increasing grain yield over that of their corresponding controls. However, it was recommended that for maximum benefit of bacterial inoculation in pearl millet, application of fertilizer N @ 10–20 kg ha^{-1} is suitable (35). Similary, in multilocation trials with sorghum under different agroclimatic conditions of India, the grain yields were increased significantly at four out of nine locations because of inoculation with *A. brasilense*. The mean increase in grain yield because of seed inoculation over the control, averaged for all trials over 4 years, amounted to 19% which was on par with the yield obtained with 15–20 kg N ha^{-1} application (36).

Our experience with field experiments conducted at the ICRISAT Centre and other locations in India, using different millet cultivars, N doses, and FYM additions to study the responses to inoculation with N_2-fixing bacteria revealed that responses varied with locations, cultivars (cvs), and agronomic practices.

Mean grain yields increased significantly (up to 33%) because of inoculation with N_2-fixing bacteria over the respective noninoculated controls in 14 out of the 25 experiments. Of the 24 experiments with *Azospirillum lipoferum* (ICM 1001), in 11 experiments increases in grain yields (average 18.7%) were significantly ($P = <0.05$) high; in 10 experiments the increases in grain yields (9.3%) were not statistically significant; in one experiment no response was observed and in 2 of them, grain yields decreased (2.7%) after inoculation. Similarly, of the 24 experiments with *Azotobacter chroococcum* (ICM 2001), in eight trials, mean grain yields across the cultivars/treatments increased significantly ($P = <0.05$) (average increases 13.6%); in 12 experiments grain yield increases (with an average increase of 8.3%) were not statistically significant; in 2 experiments no response was observed, and in 2 other experiments grain yields decreased (by 4.5%) after inoculation. *Azospirillum brasilence* (SP 7) caused a reduction in grain yield in the two experiments where this strain was used. In a few other experiments, inoculation with other strains of *Azospirillum brasilense* resulted in higher grain yields by an average of 8% over the noninoculated control (37).

Experiments with sorghum showed that inoculation with *Azospirillum* and *Azotobacter* increased the grain yields marginally over the uninoculated control. In a field trial on an Alfisol with three sorghum hybrids CSH 1, CSH 5, and CSH 9, inoculated with *Azospirillum lipoferum* and *Aztb. chroococcum* grain yield was marginally increased by 6% over the control because of inoculation (8). Another trial with three sorghum cvs CSH 5, CSH 9, and SPV 351 and 10 inoculation treatments showed only marginal increase (2-10%) in grain and plant dry matter yields across the cvs because of inoculation with N_2-fixing bacteria over the uninoculated control (8).

In Israel, field inoculation experiments with *Azospirillum* were carried out using different cereal crops, varieties, and different fertilization levels. These experiments were conducted on large plots (20-100 m^2) with 4-6 replications and the agronomic practices used were identical to those used for commercial production. Thirty-one such field experiments were conducted and in most cases, the effect of *Azospirillum* varied with the season, years, and the crop. In general, inoculation of the C_4 plants corn, sorghum, *Panicum*, and *Setaria* showed greater yield increases than the inoculated spring wheat, a C_3 plant. With the summer crops, 75% of the experiments showed significant increases and 90% of the experiments showed increases >5%. The optimum temperature for *Azospirillum* growth is 32-35°C and it is possible that bacterial activity, including BNF was greater in the summer, particularly in irrigated crops. During vegetative phase of wheat growth, the soil temperatures in Israel are 10-15°C; nevertheless, inoculation of wheat with *Azospirillum* also showed significant increases in foliage and grain yield with lower increases than the summer crops (Table 3) (38).

2.2. Nutrient Uptake : It has been observed from several field experiments that total N, P and K assimilation by the inoculated plants was higher than the uninoculated plants. Inoculation often causes increases in grain and plant dry matter yields with decreases or no increases in N concentration (37, 39–42) and these responses have, therefore, been attributed to effects of plant-growth substances. In other experiments, increased grain and plant dry matter yields are accompanied by increased N concentration because of inoculation with N_2-fixing bacteria indicated increased BNF or increased N assimilation by plants (42—47).

The results in Table 4 show that in pearl millet inoculated with *Azospirillum* or *Azotobacter* total plant N assimilation increased generally, and such increases were higher with zero or 20 kg N ha^{-1} inoculated treatments. The average increase in N assimilation by pearl millet inoculated with *Azospirillum* works out to be 5 kg ha^{-1}. The results of millet inoculation experiments conducted for 3 years in the same plot showed that the mean total N uptake of cultivars varied significantly from year to year. The mean nitrogen uptake increased ($P = <0.05$) following inoculation and addition of N (Table 5). There was no interaction between N levels and bacterial cultures for plant N uptake, although there was a significant variety × bacterial culture interaction particularly for total plant N uptake (Table 5) of BJ 104 with *Aztb. chroococcum* and *Azosp. lipoferum.*

Previous inoculations in above-mentioned experiment with N_2-fixing bacteria resulted in increased ($P = <0.05$) N uptake by cv ICMV 1 (Table 6), which was grown as a cover crop to measure the residual benefits of continued inoculations. In another experiment conducted for 2 years in the same plot using FYM, N levels and bacterial strains, the mean

Table 4. Total N uptake by Pearl millet plants inoculated with N_2-fixing bacteria from field trials.

Experiment	Treatment	Total N uptake (kg ha^{-1})		Increased N uptake (kg ha^{-1})
		Inoculated	Control	
1.	*Azosp. lipoferum* + 0 N	26	21	5
	+ 20 N	32	25	7*
	+ 40 N	38	31	7*
	Aztb. chroococcum + 0 N	25	21	4
	+ 20 N	32	25	7*
	+ 40 N	37	31	6*
2[1].	*Azosp. lipoferum*	71	57	11*
	Azosp. brasilense (1)	62	57	5
	Azosp. brasilense (2)	65	57	8*
	Azasp. brasilense (1+2)	65	57	8*
	Aztb. chroococcum	63	57	6*
3.	*Azosp. lipoferum*	40	33	7
	Azosp. brasilense	40	33	7
	Aztb. chroococcum	35	33	2
4.	*Azosp. brasilense* (7 strains)	26	33	3[2]
	Azosp. brasilense	22	33	-1
	Aztb. chroococcum	27	23	4*
5.	*Azosp. lipoferum*	31	22	9*
	Azosp. lipoferum + 80 N	50	50	0
	Aztb. chroococcum	31	22	9*
	Aztb. chroococcum + 80 N	51	50	1
6.	*Azosp. lipoferum* + 0 N	41	29	12^{NS}
	+ 20 N	48	38	10^{NS}
	+ 40 N	47	48	0^{NS}
	+ 80 N	57	53	4^{NS}
	Aztb. chroococcum + 0 N	36	29	7^{NS}
	+ 20 N	51	38	13^{NS}
	+ 40 N	44	47	-3^{NS}
	+ 80 N	52	53	-1^{NS}
7.	*Azosp. lipoferum* 0 N	29	26	5
	20 N	29	24	-5
	40 N	32	31	1
	Aztb. chroococcum 0 N	28	26	2
	20 N	26	34	-8
	40 N	30	31	-1

[1] Average of 2 locations and 3 cvs were grown at each location.

[2] In 2 strains increases were significant (5 kg N).

* P = <0.05.

NS = Nonsignificant.

Source : Derived from Wani et al. (9, 37, 42).

Table 5. Mean grain and total plant biomass yield (t ha^{-1}), mean total plant N uptake (kg ha^{-1}) and plant dry matter nitrogen percentage of pearl millet cultivars inoculated with N_2-fixing bacteria at three N levels across three years[1].

	Culture					
N applied (kg ha^{-1})	*A. lipoferum* (ICM 1001)	*A brasilense* (SL 33)	*A. chroococcum* (ICM 2001)	Noninoculated control	Mean	SE±
		Grain yield				
0	1.97	1.91	1.92	1.79	1.90	
20	2.50	2.48	2.58	2.43	2.50	0.057*
100	2.66	2.79	2.84	2.62	2.73	
Mean	2.38	2.40	2.45	2.48		0.033*
CV (%)		13.2				
		Total plant biomass				
0	5.68	5.56	5.51	5.42	5.54	
20	6.82	6.81	6.96	6.51	6.78	0.092*
100	7.62	7.75	7.83	7.44	7.66	
Mean	6.71	6.71	6.77	6.46		0.077*
CV (%)		11.4				
		Total plant N uptake				
0	37.3	36.4	36.5	32.8	35.8	
20	56.3	54.9	59.1	52.9	55.8	3.05*
100	92.1	90.3	89.7	83.5	88.9	
Mean	62.0	60.6	61.1	56.3		1.18*
CV (%)		19.9				
		Plant dry matter nitrogen (%)				
0	0.31	0.33	0.30	0.26	0.30	
20	0.39	0.36	0.42	0.37	0.39	0.031*
100	0.70	0.63	0.65	0.62	0.65	
Mean	0.47	0.44	0.45	0.42		0.009*
CV (%)		27.2				

[1] Average of 48 replications.

* $P = <0.05$

Source : Wani et al. (37).

Table 6. Mean grain and total plant biomass yield (t ha^{-1}) and plant nitrogen uptake (kg ha^{-1}) of millet cv ICMV 1 grown in plots inoculated earlier with N_2-fixing bacteria.

N applied (kg ha^{-1})	*A. lipoferum* (ICM 1001)	*A. brasilense* (LS 33)	*A. chroococcum* (ICM 2001)	Noninoculated control	Mean	SE ±
		Grain yield (t ha^{-1})				
0	2.13	2.01	1.98	1.67	1.95	
20	2.33	1.99	2.55	2.01	2.22	0.076**
100	2.60	2.82	2.83	2.74	2.75	
Mean	2.35	2.27	2.45	2.14		0.070**
CV (%)		15.1				
		Total plant biomass yield (t ha^{-1})				
0	6.01	5.78	6.01	2.42	5.81	
20	7.00	6.24	7.20	6.31	6.69	0.193**
100	7.28	7.56	7.60	7.48	7.48	
Mean	6.77	6.53	6.04	6.40		0.115**
CV (%)		9.2				
		Total plant nitrogen uptake (kg ha^{-1})				
0	41.0	44.1	41.5	34.1	39.9	
20	51.4	45.2	56.7	43.0	49.0	5.43**
100	86.3	87.5	86.0	81.9	85.4	
Mean	59.6	58.6	61.4	53.0		1.68**
CV (%)		13.6				

** $P = <0.01$

Source : Wani et al. (37).

plant N uptake varied with seasons. Increased plant N uptake (30 kg ha^{-1}) was observed with FYM addition (5 t ha^{-1}), compared to the zero FYM treatment (27 kg ha^{-1}). Nitrogen uptake also increased after application of N and inoculations with N_2-fixing bacteria. Similarly, enhanced plant N assimilation without and also with N upto 40 kg ha^{-1} has been observed in pearl millet inoculated with *Azosp. brasilense* (35). Maximum increase in N assimilation (21 kg N ha^{-1}) because of inoculation was observed in 20 kg N ha^{-1} treatment over the 20 kg N ha^{-1} alone treatment (35). These results showed that inoculation increased total plant N assimilation and by application of low levels of N (10-20 kg N ha^{-1}), increases in assimilated N were higher than in the presence of high or no applied N.

2.3. N fertilizer and inoculation : Soil nitrogen levels (soil N + fertilizer N) affect the response to inoculation. Generally, good responses to inoculation have been obtained at intermediate levels of intial N-fertilizer in the range of 10 to 80 kg N ha^{-1} enhancing the responses of sorghum, maize, millet, and wheat (35, 37, 45, 48-51). "Intermediate levels" is an arbitrary term because it depends on the level of available combined N present in the soil before fertilization and on rates of mineralization in particular soil. Therefore, the largest differences in yield were obtained when the soil was adequately but not excessively fertilized. Higher doses of mineral N application drastically reduced or abolished the responses.

The experiments conducted with pearl millet at different locations showed that higher increases in grain and total plant biomass yield and also total plant N uptake (at three locations) were observed with zero N + inoculation treatments and the extent of response declined with the increasing levels of applied N (Table 7).

Table 7. Mean grain, total plant biomass yield and total plant N uptake by Pearl millet inoculated with N_2-fixing bacteria with different N levels.

N levels (kg ha^{-1})	Bacterial culture		Noninoculated control	Mean	SE
	Azosp. lipoferum	*Aztb. chroococcum*			
	Grain yield (t ha^{-1})[1]				
0	1.8 (16)[2]	1.8 (16)	1.5	1.7	
20	2.0 (10)	1.9 (4)	1.8	1.9	0.059^{NS}
40	2.0 (6)	2.0 (3)	1.9	2.0	
Mean	1.93	1.88	1.76		0.033**
CV (%)		20	0.036**		
	Total plant dry matter (t ha^{-1})[2]				
0	5.4 (13)	5.2 (9)	4.8	5.1	
20	5.7 (4)	5.6 (4)	5.4	5.6	0.141^{NS}
40	6.1 (5)	5.8 (0.2)	5.7	5.9	
Mean	5.7	5.5	5.3		0.082**
CV (%)		16	0.079**		
	Total plant N uptake (kg ha^{-1})[3]				
0	32.2 (27)	29.9 (18)	25.3	29.1	
20	37.0 (13)	36.6 (12)	32.6	35.4	
40	39.2 (8)	37.3 (3)	36.2	37.6	
Mean	36.1	34.6	31.4		

[1] Mean across 7 locations, at each location four replications were grown.

[2] Figures in parentheses indicate percentage increase over respective control.

[3] Mean across three locations.

** $P = < 0.01$.

NS = Nonsignificant.

Source : Based on ICRISAT trials data.

In India, experiments conducted at four locations with pearl millet over 5 years revealed that the highest increases in grain yield were observed because of inoculation alongwith zero or 10 kg N ha^{-1} application than with 20 or 40 kg N ha^{-1} application (Table 8). Similar results were observed at six other locations over 2 years (52).

Table 8. Response of Pearl millet variety BJ 104 to inoculation with *Azospirillum brasilense* on grain yield (kg ha^{-1})[1].

	Soil pH				
	Kanpur	Hyderabad	Parbhani	Delhi	
Treatment	7.5	6.8	7.2	7.8	Mean
Control (noninoculated and no nitrogen)	1275	1250	1450	975	1238
A. brasilense	1550	1275	1765	1125	1429
10 kg N ha^{-1}	1625	1415	1950	1175	1544
10 kg N ha^{-1} + *A. brasilense*	1910	1450	2275	1480	1770
20 kg N ha^{-1}	1750	1575	2250	1250	1706
20 kg N ha^{-1} + *A. brasilense*	1875	1695	2315	1575	1845
40 kg N ha^{-1}	2012	1850	2425	18 0	2084
40 kg N ha^{-1} + *A. brasilense*	2050	1925	2610	2375	2165
LSD P = 0.05%	2 0	150	285	302	212

[1] Mean of 5-year field trials.

Source : Extracted from Tilak and Subba Rao (35).

2.4. Organic Manures and Inoculations : The soils in the tropics are generally poor in their organic matter contents and such soils are deficient in organic substances that serve as energy source to N_2-fixing bacteria. In such instances, the addition of organic substances introduced into the soil not only serve as nutrients (53,54) for N_2-fixing bacteria but also help the bacteria to overcome the antagonistic effect of soil fungi and bacteria (55). Increased nitrogenase activity was observed in the soil when straw was incorporated and the activity was enhanced further with warm moist conditions (54,56) Similarly, addition of 3% W/W farmyard manure to sand considerably enhanced nitrogenase activity associated with sorghum and millet (57). In nonplanted lysimeters containing sandy, ferruginous dior soil, a net gain of 2 g N (60 kg of soil)$^{-1}$ was observed when millet residues were added to the soil at 15 to 30 t ha^{-1} (58). These levels of residue addition are larger than would be normally used by farmers, but the experiment illustrates that high levels of non-symbiotic N_2-fixation can be associated with the return of plant residues to the soil, stimulating N_2-fixation by the supply of carbohydrates. Increased efficacy of inoculated *Azotobacter* in soils spread with manure was noted in different regions of the USSR (22, 54, 60). In loamy soils, application of 30 t ha^{-1} manure stimulated *Azotobacter* growth and enhanced its effect on winter rye yields (61). The addition of *Azotobacter* increased the number of microorganisms and nitrifying bacteria in the compost. Rye grown in soils fertilized with bacterized composts increased yields by 10% over the yields obtained from soils fertilized with uninoculated compost (62, 63).

Increased corn yield by 23% was obtained because of the addition of organomineral mixture plus *Azotobacter* over the organo mixture alone (64). Incorporation of straw (5% w/w) into Nile Delta soil together with *Azospirillum* inoculation increased the dry matter, nitrogen content of 12-week old maize plants and plant height. Nitrogenase activity associated with corn roots was also increased (44). The inoculation experiment conducted for 2 years in the same plot with pearl millet showed that addition of FYM at 5 t ha^{-1} increased the yield over no FYM plot and further inoculation with *Azosp. lipoferum* or *Aztb. chroococcum* alongwith FYM increased the yields by 9% and 12% over the FYM alone treatment (Table 9) (42).

Table 9. Mean grain and total plant biomass yield of pearl millet inoculated with N_2-fixing bacteria alongwith farmyard manure[1].

Farmyard manure (t ha^{-1})	Inoculation		Noninoculated control	Mean	SE
	Azosp. lipoferum	*Aztb. chroococcum*			
	Grain yield (t ha^{-1})				
0	1.71	1.75	1.59	1.68	±0 019
5	1 82	1.89	1.68	1.80	
Mean	1.76	1.82	1.64		±0 034
CV (%)		14			
	Total plant biomass (t ha^{-1})				
0	4 48	4.39	4.09	4 32	
5	4 73	4 83	4.32	4 63	0 070
Mean	4 60	4 61	4 20		0.075

[1] Mean of 2 years and 2 N levels. Each treatment was replicated four times.

Source : Wani et al. (42).

In field studies, inoculation of rice with *Azotobacter* alongwith green manures such as *Sesbania*, *Glyricidia*, sunnhemp, and paddy straw, increased grain yield by 9-19% and straw yield by 7-21% over noninoculated controls (Table 10) (65). Further, while studying three levels of glyricidia (2.5, 5, and 7.5 t ha^{-1}) and application with 60 and 90 kg N ha^{-1}, it was observed that 7.5 t ha^{-1} glyricidia applied with 60 kg N ha^{-1} and inoculated with *Aztb. chroococcum* gave increased grain yield over the treatments of only 90 kg N ha^{-1}. With increasing levels of glyricidia application rice grain yield kept increasing. Similarly, neem cake application (6-25 t ha^{-1}) in combination with *Aztb. chroococcum* inoculation and 90 kg N ha^{-1} increased rice grain yield by 12-15% and straw yield by 16-19% over the treatment having an application of 120 kg N ha^{-1} alone (66). However, in such trials comparisons should be made with organic amendments alone, without inoculating with N_2-fixing bacteria and comparisons with higher N doses are not valid as the exact amount of N added through amendments is not known.

Table 10. Effect of organic amendments and *Aztb. chroococcum* inoculation on grain and straw yield of rice.

Treatment	Grain yield (t ha^{-1})	Percentage increase over respective control	Straw yield (t ha^{-1})	Percentage increase over control
Noninoculated control	3.14	—	10.12	—
Aztb. chroococcum	5.53	12	11.75	16
Sesbania	3.83	—	12.84	—
Sesbania + *Aztb. chroococcum*	4.12	9	14.19	13
Glyricidia	3.53	—	12.91	—
Glyricidia + *Aztb. chroococcum*	4.11	19	14.84	21
Sunhemp	3.47	—	11.72	—
Sunhemp + *Aztb. chroococcum*	3.86	13	12.70	10
Paddy straw	3.09	—	11.07	—
Paddy straw + *Aztb. chroococcum*	3.61	17	11.80	7
CD ($P = <0.05$)	0.40		1.20	

Source : Prasad (66).

Sorghum was grown with application of wheat straw or sugarcane bagasse @ 25 t ha^{-1} with C : N ratio adjusted to 36 : 1 and 50 : 22 : 25 (N : P : K ha^{-1}) and inoculated with *Aztb. chroococcum*. Sorghum grain yield increased by 20% because of wheat straw and 6% in sugarcane bagasse application over control. Further, inoculation with *Aztb. chroococcum* increased grain yield by 4% over the treatment of wheat straw alone (67). In a trial with wheat conducted on medium-black soil for 3 years, grain yield was significantly increased with *Aztb. chroococcum* inoculation alone without farm-yard manure. The grain yield obtained with 60 kg N ha^{-1}+ 10 t FYM ha^{-1} alongwith *Azotobacter* inoculation was equal to the yield obtained with 120 kg N ha^{-1} alone (68).

2.5. Interaction between N$_2$-fixing bacteria and other microorganisms : Interactions between N$_2$-fixing bacteria and other beneficial microorganisms like cellulose decomposers, phosphate solubilizers and mycorrhiza have been studied to attempt simultaneous application of two or more biofertilizers to promote plant nutrition. Increased efficacy of *Bacillus megaterium* with barley, oat, and corn was observed when simultaneously *Aztb. chroococcum* was also inoculated resulting in increased grain yields by 2-8% over *B. megaterium* inoculation alone (69). Similar results were observed with wheat grown in soils, containing low humus, whereas in light-chestnut soils, separate application of *Aztb. chroococcum* or *B. megaterium* was found more effective than their simultaneous application (70). Similar results were observed in some other experiments also (71, 72). In Turf-podsol soils of the Byelorussian S. S. R., *Azotobacter* inoculation increased barley grain yield by 19% and a simultaneous application of *Azotobacter* and *Trichoderma* increased the barley yield by 53% over noninoculated control (73). In brown-chestnut soils *Azotobacter* inoculation increased wheat grain yields by 12% and simultaneous application of

Azotobacter, *Pseudomonas radiobacter* and *Bacillus mycoides* increased grain yield by 30% (74). In a field trial with rice, effects of inoculation with *Aztb chroococcum*, *Aztb. chroococcum* + *B. polymyxa*, *Aztb. chroococcum* + *B. megaterium* and mixture of three bacterial cultures with 80 to 160 kg N ha^{-1} application were studied. Simultaneous application of *Aztb. chroococcum* and *B. polymyxa* performed better with 80 and 100 kg N ha^{-1} than with 120 and 160 kg N ha^{-1}. *Aztb. chroococcum* and *B. megaterium* application with 80 kg N ha^{-1} increased grain yield by 9% over 80 kg N ha^{-1} alone and with increased N levels above 80 kg N ha^{-1}, responses were reduced reaching marginal decrease in yield with 160 kg N ha^{-1} treatment. With simultaneous application of all the three inoculants, a maximum increase in yield of 12% over noninoculated control was observed for the 100 kg N ha^{-1} treatment (75).

In a trial with 3 N levels (0, 30 and 60 kg ha^{-1}) conducted at two locations for 2 years on loamy soils of low fertility, dual inoculation showed higher benefits over single inoculation in barley grain yield. Simultaneous inoculation or barley with *Aztb. chroococcum* and *Azosp. brasilense* increased grain yield by 19% over noninoculated control as compared to increases of 9% by *Aztb. chroococcum* and 4% by *Azosp. brasilense* inoculation (76).

In a field experiment, simultaneous inoculation of sorghum with *Azosp. brasilense* and *Glomus fasiculatum* (Vesicular-arbuscular mycorrhizal fungus) showed significant ($P = > 0.05$) increase in grain and fodder yield over noninoculated control and single inoculation with either *Azosp. brasilense* or *G. fasiculatum* (Table 11) (35).

Table 11. Grain and plant dry matter yield (t ha^{-1}) of sorghum inoculated with *Azosp brasilense* and *Glomus fosiculatum* (VAM) fungus.

Treatment	Grain yield	Plant biomass yield
Noninoculated control	1 99	4.28
Azosp. brasilense	2.15	4.61
G fasiculatum	2.10	4.48
Azosp. brasilense + *G fasiculatum*	2 66	5 68
CD ($P = <0$ 05)	0 39	0 63

Source : Tilak and Subha Rao (35).

2.6. *Effect of continued inoculation* : There are several reports on effects of inoculation with N_2-fixing bacteria on crop yields, but information has been scanty on the benefits of continued inoculation on the yields of the main and the succeeding crops. An inoculation experiment with pearl millet cultivars and N levels was conducted for 3 years in the same plot. A pooled analysis of 3 years data revealed that the mean grain yield of pearl millet cultivars across the years increased significantly in inoculated treatments over the noninoculated treatment (Table 5). The three inoculants were equally effective in increasing grain yield. The interaction between N levels and inocula was not significant. Similarly, mean total plant biomass increased significantly with addition of N and also from inoculation with N_2-fixing bacteria (Table 5). The interaction between millet cultivars and inoculations with N_2-fixing bacteria for plant biomass was significant. No significant interactions were observed between N level and inoculations, cultivars and N levels, and years and inoculations.

Data on cumulative nitrogen uptake in the above-ground plant biomass during the three seasons showed significant increases ($P = >0.001$) after the addition of 20 and 100 kg N ha^{-1}. In the zero applied N treatments a mean cumulative N uptake of 107 kg ha^{-1} was recorded; with 20 kg N ha^{-1} it increased to 167 kg N ha^{-1}. A maximum N uptake of 262 kg ha^{-1} was recorded in the 100 kg N ha^{-1} treatment. Similarly, inoculation with N_2-fixing bacteria increased ($P = >0.05$) mean cumulative N uptake. A maximum cumulative plant N uptake of 185 kg ha^{-1} (19 kg N ha^{-1} more) was observed in cultivars inoculated with *Azosp. lipoferum* (ICM 1001), followed by 182 kg N ha^{-1} with *Azosp. brasilense* (SL 33) and *Aztb. chroococcum* (ICM 2001) inoculated treatments, compared to 166 kg N ha^{-1} in the noninoculated millet cultivars (37).

In all, 3 years of continued inoculation enabled the crops (three main crops and one succeeding crop) to assimilate 25.6 kg extra N ha^{-1} over the noninoculated control plots. The lack of significant interaction between the cultures and seasons in the experiment suggests that continued inoculation may be necessary to obtain increased yields (37).

The grain yield of millet cv. ICMV 1 from the plots inoculated previously increased in comparison with the respective control plots (Table 6). A maximum mean grain yield of 2.45 t ha^{-1} (14.4% increase) was observed from the plots inoculated previously with *Aztb. chroococcum*. Earlier inoculations with *Azosp. lipoferum* and *Aztb. chroococcum* increased plant biomass. Previous inoculations for 3 years showed increased N uptake by a cover crop (Table 6).

In another inoculation experiment with millet conducted for 2 years in the same plot also, there was no interaction between years and inoculations and the benefits observed from inoculations were similar in terms of increased grain and plant biomass yields (37). While studying residual benefits from inoculation, it was observed that inoculation of sorghum with *Azotobacter* alone or with wheat straw resulted in significant ($P = >0.05$) increase in grain yield of succeeding wheat crop by 28 and 13% over the respective noninoculated controls. However, the main sorghum crop grain yield was increased by 4% only over the noninoculated control (67). In another experiment, grain and straw yield of wheat cover crop was increased in treatments inoculated previously with *Azotobacter* alone or with 30 kg N ha^{-1}. With increasing N doses to 45 and 90 kg N ha^{-1} plus inoculation of main crop, reduced the yield of a cover crop; however, the increases or decreases were not significiant (78).

Strains isolated from the roots of the same crop into which they were subsequently inoculated have been termed 'homologous' (6). The strains used in the experiments conducted at the ICRISAT Center were not homologous and except for *Azosp. brasilense* (SP 7); in general, inoculation with all the strains increased the yields. The most probable number (MPN) count of N_2-fixers in the pre-sowing soil samples were 10^2 (g of dry soil)$^{-1}$. Boddey *et al.* (77) suggested that when azospirilla populations are low, *Azospirilium* strains of diverse origin may cause significant response, but in the areas where these bacteria are abundant, 'homologous' strains are more likely to stimulate yield increases. However, the evidence accumulated so far suggests that there is no definite pattern observed for the benefits from homologous or heterologous strains of azospirilla.

2.7. *Establishment of inoculated Bacteria* : The success of inoculation experiments depend on the ability of the inoculated bacteria to establish in the rhizosphere. Few experiments have followed the fate of the inoculated bacteria during the crop season. The reason for few studies on establishment of the inoculated bacteria is the difficulty in tracing the strains after inoculation. There has been little use of genetic markers to identify inoculant strains probably because many strains have a high level of intrinsic resistanse to antibiotics. Serological techniques have been used to identify strains (29, 79, 80, 83); however, only one report gives quantitative data on the number of *Azospirillum* and *Azotobacter* populations in the soil (83). In Israel, pink strains of *Azosp. brasilense* seem to be absent from soils (84) and this enables the identification of the pink inoculum strain in these soils.

Inoculated *Azospirillum* successfully established (65 fold increase over noninoculated control) in soils under Wisconsin (USA) conditions, where no *Azospirillum* was present. However, under Brazilian conditions increases in *Azospirillum* numbers by inoculation were up to four fold only with different crops over noninoculated controls. This was mainly because of the high numbers of native azospirilla in the soil (85). Similar results were observed with many *Azospirillum* associated with roots of inoculated sorghum plants (86). Six fold increase in the number of azospirilla in the roots sterilized in 1% chloramine T for 10 min was observed for inoculated wheat over the noninoculated control. However, a good positive correlation was observed between the *Azospirillum* numbers in chloramine T treated roots and total N accumulation in plant tops ($r=0\ 92$) (43). A continued decline in the population of *Azosp. brasilense* strains (CD and CDSR) to less than 10^2 bacteria g^{-1} of soil by the 6th week after inoculation was observed (51). Exceptional results are of Hegazi et al. (44) from Egypt where maize grown with 100 kg N ha^{-1} inoculated with *Azospirillum* showed continued increase in azospirilla numbers up to the 12th week and the increase was 156 fold over the number from noninoculated plots. Such increases are unusual particularly where the *Azospirillum* population was quite high 10^4 (g of $soil^{-1}$)

We used the enzyme linked immunosorbent assay (ELISA) and MPN techniques to study the establishment of inoculated *Azosp. lipoferum* and *Aztb. chroococcum* in the millet rhizosphere under field and greenhouse conditions. Continued inoculation of the same plot for three consecutive seasons showed that during the 4th year, earlier inoculations with *Azosp. lipoferum* and *Aztb. chroococcum* resulted in increased MPN counts in the rhizosphere soil over the noninoculated control by 1.4 to 2 fold; however, increases were statistically not significant. The host cvs. used in the earlier inoculation experiments had no effect on the population of N_2-fixing bacteria during the 4th year. The mean MPN count of N_2-fiixers from macerated roots increased significantly ($P= <0.05$) in plots fertilized with 100 kg N ha^{-1} [9.8×10^5 g^{-1} of dry roots] compared to MPN counts from 20 kg N ha^{-1} treatments (4.0×10^5 g^{-1} of dry roots) and zero N treatment (3.8×10^5 g^{-1} of dry roots). Previous inoculations with *Azosp. lipoferum* increased the MPN counts from the roots of cv ICMV 1 upto 6.7×10^7 g^{-1} of dry roots, and *Aztb. chroococcum* to 6.0×10^7 g^{-1} of dry roots, as against 5×10^7 g^{-1} of dry roots in the noninoculated treatment. Using ELISA it was observed that the counts of *Azops. lipoferum* in the rhizosphere soil and macerated roots of cv ICMV 1 grown in the plots inoculated earlier for 3 years increased significantly (Table 12). Similarly, with the addition of 20 kg N ha^{-1}, *Azosp. lipoferum* counts increased to 2.9 × 10^{6} plant^{-1} compared to 1.8 × 10^{6} plant^{-1} and with 100 kg N ha^{-1} to 3.4 × 10^{6} plant^{-1} with zero N treatment (37). Similar results were observed with *Aztb. chroococcum* counts also using ELISA (Table 12).

Table 12. Number of *A. lipoferum* and *A chroococcum* using ELISA associated with millet cv. ICMV 1 grown in the plots which were inoculated earlier for 3 consecutive years.

Nitrogen applied (kg ha^{-1})	Rhizosphere soil*			Root macerate*		
	A lipoferum	Contral	Mean	*A. lipoferum*	Control	Mean
0	7.3	5.1	6.2	31.6	20.5	26.1
20	9.0	7.4	8.2	36.2	59.2	32.7
100	8.3	6.5	7.4	49.6	40.1	44.8
Mean	8.2^{a}	6.3^{b}		39.2^{a}	29.9^{b}	
CV (%)	2			3		

Nitrogen applied (kg ha^{-1})	Rhizosphere soil			Root macerate		
	Aztb. chroococcum	Control	Mean	*Azth chroococcum*	Control	Mean
0	2.9	0.6	1.8	712	452	582
20	4.5	1.5	3.0	1050	416	733
100	4.4	4.0	4.2	1202	622	912
Mean	3.9^{a}	2.0^{b}		988^{a}	361^{b}	
CV (%)	10			12		

1. Average of eight replications, mean across the cultivars. Log transformations of data used for analysis and figures with different letters vary significantly ($P = <0.05$) from each other.

* P = Number expressed as × 10^{3} g^{-1} of dry rhizospheric soil/dry root

Source : Wani et al. (37).

In another experiment, the mean ELISA counts of *Azosp. lipoferum* in the rhizosphere soil of Pearlmillet cv BJ 104 increased significantly ($P = < 0.01$) with inoculation [9.6 × 10^{8} g^{-1} of dry soil), compared to 5.8 × 10^{8} (g^{-1} of dry soil) with the noninoculated control plants. Similarly, ELISA and MPN counts of *Azosp. lipoferum* with roots increased two fold over the noninoculated control after inoculation (37). Similar results were observed in case of *Aztb. chroococcum* also. There was no change in the population of the bacteria from the macerated root samples because of plant age or inoculation suggesting that the inoculated bacteria were closely associated with the roots and rhizosphere but did not enter the roots internal surfaces.

3. Mechanisms of Response

Azospirilla, azotobacters and other bacteria were initially selected for inoculation experiments because of their N$_2$-fixing ability and because they are closely associated with ots of cereals and grasses. The mechanism by which the cereals inoculated with N_2-fixing cteria derive benefit is not clearly understood. However, knowledge has accumulated to dicate the possible mechanisms involved in deriving the benefits from the N_2-fixing bacteria.

1. Colonization of Roots and Rhizosphere : The first step in colonization of roots by bacteria volves their ability to reach colonization sites by chemotaxis and/or by aerotaxis. Azospirilla re very motile bacteria. Several strains of azospirilla showed aerotaxis and responded to xygen gradients in capillary tubes and actively moved towards a specific zone with low dissolved xygen (87). *Azospirillum* responded chemotactically to root exudates (88), amino acids, sugars nd organic acids (89). Plant roots release water-soluble sugars, amino acids, organic acids nd peptides into the soil making the rhizosphere a natural place for microbial colonization 5, 90, 91). The soluble exudates of millet cv Gahi 3 contained substances that bind to *zospirillum* and promoted adsorption of the bacteria to root hairs (92). During the first 3 ays after inoculation, colonization took place mainly on the root elongation zone, on the ase of root hairs and to a lesser extent, on the surface of young root hairs. Inoculation of everal cultivars of wheat, corn, sorghum, and *Setaria* with several strains of *Azospirillum* aused morphological changes in roots starting immediately after germination (93). With earl millet and guinea-grass seedlings grown in axenic conditions inoculated *Azosp. brasilense* ells adsorbed in few seconds, to root hairs and old epidermal cells (92).

It was shown that in liquid medium, *Azospirillum* attaches in a polar fashion to root airs, epidermal cells and mucigel but may also occur in clumps (94). *Azotobacter* tends to form ggregates on the root cell (95). The adsorption studies of *Azosp. brasilense* to corn roots using ^{2}P-labelled cells showed that adherence of bacteria to roots increased during the first 90 min and attained a maximum level within 4.5 h of incubation. Bacterial adherence to corn roots varied with the strains and the adherence increased linearly following a Langmuir isotherm, with increasing *Azospirillum* concentration up to 10^{9} cells ml^{-1} of binding mixture (96).

3.2 Root and Root Hair Development : Inoculated seedlings of *Pennisetum* developed more extensive root systems than the noninoculated control seedlings (97). Our experiment with pearl millet and sorghum grown in tubes containing either agar medium or sand : FYM or an Alfisol and inoculated with *Azosp. lipoferum* and *Aztb. chroococcum* showed increased root development, more lateral roots and also more root hairs. Similarly, increased root development and branching was observed in inoculated *Setaria*, wheat, sorghum, and pearl millet (34, 46, 98, 99). Root elongation and total surface area of wheat roots was increased by inouclation of seedlings with 10^{5}–10^{6} cells of *Azospirillum* while 10^{8}–10^{10} cells inhibited root development.in Petri dishes and pots. Higher inoculum concentrations were necessary to produce effects when *Azospirillum* was applied in combination with other saprophytic rhizosphere bacteria (100). Inoculation with *Aztb. chroococcum* enhanced root elongation (101). Under field conditions, wheat seedlings inoculated with strains of *Azospirillum* caused two types of branching of root hairs, turning forked deformation and branching of unequal length. There was a good correlation between the number of turning fork deformations in seedlings inoculated with different strains and total N gain by inoculated wheat by these strains. Root hair deformations were also observed in roots of field-grown maize (94). Inoculation of pearl causing cell collapse thus enhancing the mineral absorption surface of cortex cells in a kind of "sponge" effect.

Table 14. Correlation matrix of nitrate reductase activity in leaves with different growth parameters of pearl millet inoculated with N_2-fixing bacteria, ICRISAT Centre, rainy season 1984.

	NRA cm^2 leaf		NRA (g of leaf tissue)[1]	
	43 DAS	58 DAS	43 DAS	50 DAS
NRA cm^2 43 DAS	1.00			
NRA cm^2 58 DAS	0.54	1.00		
NRA g^{-1} 43 DAS	0.97	0.45	1.00	
NRA g^{-1} 58 DAS	0.56	0.99	0.48	1.00
Grain yield	0.46	0.46	0.41	0.50
Total plant biomass	0.33	0.54	0.21	0.52
N uptake thru grain	0.53	0.74	0.44	0.75
Grain N content	0.46	0.75	0.35	0.73
N uptake thru TDM	0.54	0.77	0.44	0.77

[1] At each assay 96 observations were used for computing the correlations.

3.6. Biological N_2-fixation : In several experiments inoculation with N_2-fixing bacteria caused increases in plant dry matter along with increased N percentage in plant tissue of sorghum (45, 47, 112, 117), millet (37, 42), *Setaria italica* (45), maize (44, 45, 84, 106), and wheat (39, 43, 105, 111, 118), indicating effects of inoculation on N_2-fixation or N assimilation. Measurements of N_2-fixation by isotopic (77, 108, 119) or N balance (121) methods suggested significant amounts of N_2-fixation associated with grasses in some experiments.

In certain experiments, high N_2ase activities [(1000, 3000 n mol C_2H_4 h^{-1} g^{-1} of dry roots)] have been observed in case of inoculated plants (44, 45, 108, 122) which could account for total N gains by inoculated plants. However, most of these experiments have measured activity at one time generally after flowering and peak activity reaches during flowering to grain-filling stage (9, 45, 123, 124). In several experiments even at flowering the activity recorded is low for inoculated plants which could not explain the N gains (37, 84, 125). In our experiments, nitrogenase (C_2H_2 reduction) activity associated with millet plants inoculated with N_2-fixing bacteria increased in field but such increased activity was observed only during later stages of plant growth for a shorter period. As most of the N required for plant growth in millet and sorghum is taken up before flowering (Wani et al, unpublished data) and increased nitrogenase activity was observed after flowering for a short period, the nitrogenase activity may not account solely for the increased N uptake observed in the experiments (37). The $^{15}N_2$ incorporation studies suggested that only approximately 5% of the fixed nitrogen incorporated into plant tissue (124). Increased plant dry matter and total nitrogen content was observed in 8 week old maize plants inoculated with *Azosp. brasilense* and 12.6% of the plant

N was derived from nitrogen fixation, although these ylants were less than 0.5 g dry weight and were deficient in N (0.55%) (126). In greenhouse studies, wheat cultivars grown in soil and inoculated with *Bacillus polymyxa* and *Azosp. brasilense* derived 0-32.3% and 0-28.9% of total plant N through BNF. However, in such experiments unless the soil is labelled uniformly with ^{15}N in time and depth (127), the ^{15}N isotope dilution results could not be interpreted conclusively. Moreover, in Renni's experiment (126) there was no correlation between total N yield and isotope dilution and it is not certain that the lower ^{15}N enrichments observed in the inoculated plants were because of nitrogen derived from N_2-fixation. Even in the presence of high levels of fertilizer N which are inhibitory to N_2-fixation, inoculation responses have been observed (45, 99, 105, 106, 128).

3.7 Other Mechanisms : Some of the other mechanisms which may be involved in obtaining positive inoculation effects could be, improved water status of the plant because of inoculation with *Azospirillum* (38), and antagonistic effects on plant pathogens (129,-132). There are indications that the use of *Azotobacter* increased microorganisms in the rhizosphere or under certain conditions, *Azotobacter* might enhance the activity of bacteria antagonists to pathogenic bacteria (59). Introduction of *Azotobacter* into soil also brought shifts in species composition of the bacterial flora of plant rhizospheres (33). Inoculation of plants with *Azotobacter* and *Azospirillum* may improve the iron nutrition of the plants by making the nonavailable form of iron to available form through production of siderophores. *In vitro*, *Aztb. chrococcum* and *Azosp. lipoferum* (ICM) produced siderophores when grown in Fe-deficient medium. The production of siderophores by these bacteria may also be beneficial to the plants by way of offering protection from minor pathogens (29, 135). The siderophores are high affinity Fe^{+++} chelators that specifically enhance the acquisition of iron. This highly efficient iron scavenging mechanism is thought to compete with that of fungal pathogens, thereby creating an iron-deficient eevironment deleterious to fungal growth (136). Inoculation of sorghum with *Azotobacter* resulted in marked decline of shoot fly (*Atherigona soccata* Rond.) as compared to noninoculated control, although it was not as effective as carbofuran. It was found to be more economical and safer than carbofuran (137). The possible reason for low incidence could be the faster growth of the inoculated seedlings that results in escape from the shootfly attack as by the time shootfly population builds up, the susceptible plant stage is over.

4. Conclusions

The main purpose of studying *Azotobacter* and *Azospirillum* was to exploit the potential BNF properties of these bacteria to economize the use of valuable nitrogen fertilizer while ensuring good cereal crops. The inoculation studies in field using azotobacters and azospirilla have shown increased cereal crop yields in several countries. The extent of the response obtained varies with crop, variety, location, season, agronomic practices, bacterial strains, level of soil N, organic matter and interaction with native soil microflora. Statistically significant yieid increases have been observed in upto 60% of the trials in USSR, Israel and India. Increased yields because of inoculation would contribute significantly to the economy of the subsistance farming. In the field even with legumes, significant increases are

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