http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (2): 544-552, 1999 Research Article

Comparative Response of C_3 and C_4 Cereal Plants and Soil *Azospirillum* Spp. Distribution to Bacterization and N-fertilization

F.M. Hammouda* and Aida H. Afify**
*Bacteriological Laboratory, Sakha Agricultural Research Station, A.R.C.
** Department of Microbiology, Faculty of Agriculture, Mansoura University, Mansoura Egypt.

Abstract
Four greenhouse experiments to assess the response of two physiologically different cereal crops, *i.e.* sorghum and rice crops representing C_4 and C_3 plants as well as *Azospirillum* spp. populations in their rhizosphere and root surface soil to inoculation with *A. lipoferum* in combination with five levels of N fertilizer were conducted. The results indicated that sorghum was more responsive to inoculation than rice plant. Maximum benefits were obtained when the N fertilizer level was reduced to 30 kg/fed. in combination with *A. lipoferum* inoculant, giving the superiority in yields and yields components of both plants. There was a moderate difference of the rhizosphere and root surface soil effect on *Azospirillum* spp. population density between the two different groups of plants. However, MPN of *Azospirillum* spp. surrounded the root system of C_4 plant recorded greater value than that of C_3 one.

Introduction
The association of efficient nitrogen fixing bacteria with plant roots in appreciable number in the rhizosphere region of cultivated cereal plants is often attributed to the presence of a variety of soluble promoting substances exuded by the plant roots (Martinez *et al.*,1988). Plant physiologists admit that plants using the C_4 type of CO_2 assimilation obviously possess a rapid rate of photosynthesis, more dry matter production, and highest growth rate in the world (Black, 1973). The C_4 plants species are thought to translocate more of their carbohydrates to the roots and to exude more in their rhizosphere (Dart, 1976).

In as much as the vary physiology of C_4 plant species is distinctly different from that of C_3 plants in respect of CO_2 fixation, it is reasonable to expect subtile qualitative and quantitative differences in the root surface microflora and nitrogen fixing bacteria in particular. A few investigators (Paul *et al.*,1971; Raju *et al.*, 1972; Dobereiner and Day, 1974; Kipe-Nolt *et al.,* 1985; Wani *et al.,* 1985 and Wani, 1986), have already come out with valuable information about the expected varied effects of nitrogen fixed in rhizosphere and root surface soil of C_3 and C_4 plant species on growth and yield of these quite distinct plants.

That the nitrogenous fertilization level could be reduced, if supplemented with *Azospirillum* inoculation has been reported by Sanathanakrishna and Oblisami (1980); ICRISAT annual report (1984). Hence, the studying of the responses of each sorghum and rice plant representing the C_4 and C_3 group plants to inoculation with *A. lipoferum* in combination with graded levels of N fertilizer is reasonable. The establishment and competitive survival of such nitrogen-fixing bacteria in the rhizosphere and root surface soils of each sorghum and rice plant needs to be understood by this study.

Materials and Methods
Four pot experiments were carried out in a wireproof greenhouse during the two successive summer seasons, 1997 and 1998 at the Agricultural Research Station, Sakha, two with sorghum var. Giza 15 and two with rice var. Giza 176. Each experiment contained 10 treatments which were, with and without inoculation and five N fertilizer levels, i.e., 0, 10, 20, 30 and 40 kg/fed as urea (46% N) for each of both the crops. The phosphatic fertilizer was applied at the recommended levels, i.e., 250 and 100 kg/fed. as calcium super phosphate (15% P_2O_5) for sorghum and rice plant, respectively.

The soil used in the study having 44.7 per cent clay, pH 8.2, E.C. 2.7 mmhos/cm; Na^+, K^+, Ca^{++} and Mg^{++} 20.7, 0.1, 5.0 and 3.7 meq/l, respectively. Available nutrients: N and P were 22.11 and 6.81 ppm (Chapman and Parker, 1981). It was mixed and distributed uniformly into all the pots of two experiments in each season. Sorghum and rice grains were treated separately with peat based inoculant of *A. lipoferum* (Ca. 10^8 cells/gram) and the grains were air dried and then planted. Grains soaked in water served as control. The 60 pots of each experiment were distributed in randomized complete blocks. For sorghum, the height of the plant, total plant dry matter yield (g/plant), plant crude protein yield (%) and the comulative N uptakes in the above-ground plant parts at three cutting periods were also detected. Regarding rice, the plant height at 60 and 120 day after sowing (DAS), number of total and productive tillers per hill, panicle length, grain and straw-yields, grain crude protein percent and N uptake by plant and grains were recorded (Chapman and Parker, 1981).

The most probable number (MPN) of *Azospirillum* spp. in the rhizosphere and roots surface soil samples was estimated at harvest time following the standard dilution technique according to Vincent (1970) using the N-free semi-solid malate medium of Dobereiner (1980). The medium used was 5 g malic acid, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 20 mg $CaCl_2$, 2 ml trace elements solution, 2 ml alcoholic solution of Bromothymol Blue (5%), 4 ml Fe EDTA; 1 ml vitamin solution, 4 g KOH, .75 g agar, 1000 ml H_2O, NaOH to adjust pH to 6.8. The trace element solution was 200 mg $Na_2MoO_4.2H_2O$, 235 mg $MnSO_4.H_2O$, 280 mg H_3BO_3, 8 mg $CuSO_45H_2O$, 24 mg $ZnSO_4.7H_2O$, 200 ml H_2O. The vitamin solution was: 10 mg biotin, 20 mg pyridoxine, 100 ml H_2O. Data were subjected to statistical analysis according to Snedecor and Cochran (1967), using L.S.D. test for comparison between means.

Results and Discussion

The results of the effect of *Azospirillum lipoferum* inoculant along with varied levels of N fertilization and their interaction on both physiologically different cereal crops, sorghum and rice are presented in Tables 1-8.

Plant growth and yield components: Regarding sorghum, plants from *Azospirillum lipoferum* treated pots were significantly taller than the uninoculated plants along the three cutting stages except the first cut in the first season, 1997 (Tables 1 and 3). *Azospirillum lipoferum* inoculated sorghum pots gave significantly increased mean of plant dry matter yield over that of uninoculated treatment with all the five levels of N fertilizer along with both seasons, the increases amounted 15.0, 10.0, 14.2, 18.1 and 14.2 percent with 0, 10, 20, 30 and 40 kg N/fed., respectively (Tables 1 and 3).

Regarding the influence of nitrogen fertilization and its different levels on plant dry matter yield, data presented in Tables 1, 3, 6 and 8 revealed that nitrogen fertilization significantly increased the dry matter produced. The average increase due to nitrogen fertilization was 39.9 per cent with sorghum and 47.? per cent with rice compared with the control treatment (0 kg N/fed.). The percentage increase means of both seasons for N level treatments (10, 20, 30 and 40 kg/fed.) were 16.2, 30.6, 49.7 and 63 per cent, respectively with sorghum plant compared to 10.7, 20.7, 76.9 and 82.9 per cent, respectively with rice plant. With both the plants, the highest level of N added (40 kg/fed. resulted in a significant increment in the amount of the dry matter produced. Thus, it is clear that fertilization with 40 kg N/fed. alone is significantly the proper level either with sorghum or rice plant under the conditions of this study.

Considering the results of both seasons, it may be pointed that the treatments receiving the *Azospirillum lipoferum* along with the reduced level (30 kg N/fed.) recorded significantly largest means in plant height (148.9 and 102.9 cm) of sorghum and rice plants, accumulated dry matter yield (3.34 g/plant and 58.9 g/hill) in sorghum and rice as well as number of total and productive tillers (40 and 30.5/hill) in rice plant over those of the treatments receiving 40 kg N/fed. either applied alone or in combination with bacterial inoculation. The panicle length in rice plant showed the same influence of the aforenamed treatment (Tables 5 and 7).

In the inoculated rice, the treatment received 30 kg N/fed. gave a significant increase mean of grain yields of 10.13 and 12.86 percent over those obtained from either inoculated or uninoculated treatments combined each with 40 kg N/fed., respectively. However, the beneficial effect of *Azospirillum* inoculant along with the 40 kg N/fed. application was relatively poor than that with 30 kg/fed., which might be probably, because of the deleterious effect of readily available N in repressing not inhibiting the activities of *Azospirillum* (Hardy *et al.,* 1973; Rangaswami, 1975 and Hammouda and Zidan, 1991).

Whatever, the magnitude mean of this increase due to the same treatment was 129.1 percent over that obtained from the uninoculated control treatment did not receive N fertilizer (Tables 6 and 8). The corresponding increase mean values for straw yield amounted 1.4, 14.9 and 110.3 percent, respectively on the same order. These results are in confirmity with the earlier reports (Subba Rao, 1981; Hammouda and Zidan, 1991).

Amounts of N uptake and crude protein content: Data in Tables 2, 4, 6 and 8 show the effect of *A. lipoferum* inoculant and levels of N fertilizer application on the amounts of N uptake by each sorghum and rice plant (mg/plant) and by rice grain (mg/hill) as well as the crude protein content of both aforenamed plants organs.

Concerning the effect of bacterization in this respect, it is clear that there is a significant increase in the amounts of N uptake by sorghum and rice plants and/or rice grain. The present results are similar to the earlier reports by various workers with other cereal crops that, the C_4 plants like *Zea mays* and sorghum are more responsive than those which possess the C_3-Calvin cycle of photosynthesis, like *Oryza* and *Triticum* to the free N_2-fixing bacteria treatments (Dobereiner and Day, 1974; Purushothaman *et al.,* 1976). With that respects, in the present study, sorghum exhibited better response to *Azospirillum lipoferum* than the rice crop, Knowles (1974) has also reported that sorghum could be better responsive to *Azospirillum* and *Azotobacter* treatments and the present results confirm the above view. Considering the results of both seasons the mean N uptake by the uninoculated sorghum was 1.51 times as much as that by the uninoculated rice plant. The *Azospirillum lipoferum* inoculant changed this ratio to become 1.78 times (Tables 2, 4, 6 and 8). The data also show that N uptake by the inoculated control (N, O) treatment in case of sorghum plants was 1.42 times as much as that by the uninoculated control treatment.

Higher N application changed this ratio to become 1.35, 1.41, 1.75 and 1.16 for 10, 20, 30 and 40 kg N/fed., respectively. The corresponding values by rice plant are 1.17 times changed to 1.10, 1.72, 1.27 and 1.01 times with higher N application on the same order (Tables 6 & 8).

Table 1: Effect of *Azospirillum* inoculant in combination with N fertilizer on plant height and dry matter in Giza 15 variety of sorghum, 1997 season.

Treatment	Plant height (cm/plant)				Plant dry matter yield (g/plant)			
	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean
No Azospirillum treatment								
0 kg N fed (zero % N)	153	105	77.5	111.8	5.12	1.78	1.40	2.77
5 + 5 kg N/fed. (25% N)	158	112	79.3	116.4	5.20	2.34	1.44	2.99
10 + 10 kg N/fed. (50 % N)	159	124.5	89.8	124.4	5.52	2.64	1.63	3.26
20 + 10 kg N/fed. (75 % N)	168	125.5	92.5	128.7	6.02	3.21	1.67	3.63
30 + 10 kg N/fed. (100 % N)	169	128	103.0	133.3	6.66	3.23	1.69	3.86
Mean	161.4	119.0	88.4		5.70	2.64	1.57	
Azospirillum treatment								
0 kg N fed (zero % N)	154	112.5	83.0	116.5	5.54	2.16	1.51	3.07
5 + 5 kg N/fed. (25 % N)	160	132	85.5	125.8	5.75	2.43	1.53	3.24
10 + 10 kg N/fed. (50 % N)	165	132.5	93.0	130.2	6.43	3.06	1.64	3.71
20 + 10 kg N/fed. (75 % N)	170	143.5	109.0	140.8	7.55	3.44	2.01	4.33
30 + 10 kg N/fed. (100 % N)	168	137.8	97.3	134.4	6.95	3.29	1.99	4.08
Mean	163.4	131.7	93.6		6.44	2.88	1.74	

Nitrogen (N) L.S.D.	(0.05)	2.35	3.49	1.43	0.52	0.18	0.10
	(0.01)	3.19	4.74	1.94	0.70	0.25	0.14
Inoculation (I) L.S.D.	(0.05)	N.S	1.86	2.03	0.28	0.149	0.078
	(0.01)	-	3.42	3.73	0.52	-	0.143
(N) x (I) L.S.D.	(0.05)	3.33	4.94	N.S	N.S	N.S	0.143
	(0.01)	-	6.70	-	-	-	0.193

Table 2: Effect of *Azospirillum* inoculant in combination with N fertilizer on crude protein and N uptake in Giza 15 variety of sorghum, 1997 season.

Treatment	Plant crude protein yield (%)				N uptake by plant, mg/plant			
	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean
No Azospirillum treatment								
0 kg N fed (zero % N)	6.09	6.06	6.04	6.06	50	17	14	27.0
5 + 5 kg N/fed. (25 % N)	7.17	6.65	6.37	6.73	60	25	15	33.3
10 + 10 kg N/fed. (50 % N)	7.16	7.05	6.45	6.89	63	30	17	36.7
20 + 10 kg N/fed. (75 % N)	752	7.23	6.76	7.17	72	37	18	42.3
30 + 10 kg N/fed. (100 % N)	8.07	7.77	7.62	7.82	86	40	21	49.0
Mean	7.20	6.95	6.65		66.2	29.8	17	17.0
Azospirillum treatment								
0 kg N fed (zero % N)	6.97	6.92	6.45	6.78	62	24	16	34
5 + 5 kg N/fed. (25 % N)	8.35	8.33	6.90	7.86	77	32	17	42
10 + 10 kg N/fed. (50 % N)	8.56	8.34	6.90	7.93	88	41	18	49
20 + 10 kg N/fed. (75 % N)	8.24	7.87	8.60	8.24	100	43	28	57
30 + 10 kg N/fed. (100 % N)	8.05	7.82	7.63	7.83	90	41	24	51.7
Mean	8.03	7.86	7.30		83.4	36.2	20.6	

Nitrogen (N) L.S.D.	(0.05)	0.34	0.253	0.27	6.78	2.55	1.63
	(0.01)	0.46	0.343	0.37	9.20	3.46	2.21
Inoculation (I) L.S.D.	(0.05)	0.56	0.398	0.443	4.78	2.65	2.29
	(0.01)	-	0.732	-	8.77	4.88	-
(N) x (I) L.S.D.	(0.05)	0.48	0.360	0.385	9.59	3.61	2.30
	(0.01)	0.66	0.480	0.523	13.01	4.89	3.12

Table 3: Effect of *Azospirillum* inoculant in combination with N fertilizer plant height and dry matter in Giza 15 variety of sorghum, 1998 season.

Treatment	Plant height (cm/plant)				Plant dry matter yield (g/plant)			
	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean
No Azospirillum treatment								
0 kg N fed (zero % N)	139.5	106.8	72.0	106.1	1.52	0.41	0.12	0.68
5 + 5 kg N/fed. (25 % N)	158.3	131.5	75.0	121.6	2.22	0.71	0.13	1.02
0 + 10 kg N/fed. (50 % N)	168.3	150.5	78.3	132.4	2.66	0.96	0.13	1.25
0 + 10 kg N/fed. (75 % N)	172.3	165.8	79.0	139.0	3.13	1.34	0.14	1.54
0 + 10 kg N/fed. (100 % N)	176.5	178.3	81.0	145.3	3.56	1.62	0.17	1.78
Mean	162.98	146.58	77.06		2.62	1.01	0.14	
Azospirillum treatment								
kg N fed (zero % N)	145.8	126.3	73.8	115.3	1.93	0.58	0.19	0.90
+ 5 kg N/fed. (25 % N)	156.3	142.0	81.3	126.5	2.45	0.87	0.19	1.17
0 + 10 kg N/fed. (50 % N)	171.3	156.8	81.8	136.6	3.00	1.14	0.19	1.44
0 + 10 kg N/fed. (75 % N)	196.3	190.8	83.8	156.9	4.87	1.95	0.22	2.35
0 + 10 kg N/fed. (100 % N)	184.3	172.8	82.8	7 146.6	3.67	1.46	0.23	1.79
Mean	170.8	157.74	80.7		3.18	1.20	0.20	

Nitrogen (N) L.S.D.	(0.05)	4.21	6.68	2.94	0.269	0.135	0.012
	(0.01)	5.71	9.07	3.99	0.365	0.183	0.16
Inoculation (I) L.S.D.	(0.05)	4.89	5.84	1.67	0.313	0.036	0.008
	(0.01)	7.25	10.73	3.07	-	0.066	0.015
(N) x (I) L.S.D.	(0.05)	5.95	N.S	N.S.	0.380	N.S.	N.S.
	(0.01)	8.08	-	-	0.520	-	-

Table 4: Effect of *Azospirillum* inoculant in combination with N fertilizer on crude protein and N uptake in Giza 15 variety of sorghum, 1998 season.

Treatment	Plant crude protein yield (%)				N uptake by plant, mg/plant			
	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean
o Azospirillum treatment								
kg N fed (zero % N)	7.02	6.39	6.65	6.69	18	5	1	8.0
+ 5 kg N/fed. (25 % N)	8.39	7.43	6.79	7.54	32	9	1	14.0
+ 10 kg N/fed. (50 % N)	8.49	8.35	7.67	8.17	39	14	2	18.3
+ 10 kg N/fed. (75 % N)	9.01	8.72	8.47	8.73	48	20	2	23.3
+ 10 kg N/fed. (100 % N)	10.09	9.77	9.03	9.63	61	27	3	30.3
Mean	8.60	8.13	7.72		39.6	15.0	1.8	
zospirillum treatment								
kg N fed (zero % N)	10.69	9.09	8.56	9.45	35	9	3	15.7
+ 5 kg N/fed. (25 % N)	11.36	9.77	9.29	10.14	48	15	3	22.0
+ 10 kg N/fed. (50 % N)	12.40	9.99	10.10	10.83	64	19	3	28.7
+ 10 kg N/fed. (75 % N)	15.02	13.11	11.77	13.30	125	44	5	58.0
+ 10 kg N/fed. (100 % N)	13.82	11.91	10.96	12.23	87	30	4	40.3
Mean	12.66	10.77	10.14		71.8	23.4	3.6	

Nitrogen (N) L.S.D.	(0.05)	0.183	0.261	0.244	6.21	2.72	0.239
	(0.01)	0.248	0.355	0.331	8.43	3.69	0.325
Inoculation (I) L.S.D.	(0.05)	0.053	0.299	0.267	6.93	2.33	0.183
	(0.01)	0.096	0.549	0.491	12.72	4.28	0.335
(N) x (I) L.S.D.	(0.05)	0.259	0.369	0.345	8.79	3.85	0.339
	(0.01)	0.351	0.502	0.468	11.92	5.22	0.460

Table 5: Effect of *Azospirillum* inoculant in combination with N fertilizer on plant height, tillers number and panicle length in Giza 176 variety of rice, 1997 season.

Treatment	Plant height (cm)			No. of tillers/hill			Panicle length (cm)
	60th day	120th day	Mean	Total	Productive	Mean	
No Azospirillum treatment							
0 kg N fed (zero % N)	79	101.0	90.0	22	16	19.0	16.1
5 + 5 kg N/fed. (25 % N)	79	109.5	94.25	22	21	21.5	16.5
10 + 10 kg N/fed. (50 % N)	80	109.5	94.75	25	23	24.0	18.4
20 + 10 kg N/fed. (75 % N)	85	111.5	98.25	30	25	27.5	19.0
30 + 10 kg N/fed. (100 % N)	87	112.0	99.50	31	25	28.0	19.5
Mean	82	108.7		26	22		17.9
Azospirillum treatment							
0 kg N fed (zero % N)	84	108.5	96.25	27	21	24.0	17.2
5 + 5 kg N/fed. (25 % N)	86	109.0	97.50	29	25	27.0	18.0
10 + 10 kg N/fed. (50 % N)	89	112.5	100.75	33	28	30.5	20.2
20 + 10 kg N/fed. (75 % N)	92	116.5	104.25	46	33	39.5	21.5
30 + 10 kg N/fed. (100 % N)	88	115.0	101.50	42	30	36.0	20.9
Mean	87.8	112.3		35.4	27.4		19.6

Nitrogen (N) L.S.D.	(0.05)	2.74	1.91	2.30	1.37	0.77
	(0.01)	3.71	2.59	3.12	1.86	1.05
Inoculation (I) L.S.D.	(0.05)	1.90	1.84	3.54	1.43	0.72
	(0.01)	3.49	3.39	4.12	2.63	1.32
(N) x (I) L.S.D.	(0.05)	N.S.	2.69	3.26	1.94	N.S.
	(0.01)	-	3.66	4.42	-	-

Table 6: Effect of *Azospirillum* inoculant in combination with N fertilizer on yield and yield components in Giza 176 variety of rice, 1997 season.

Treatment	Grain yield (g/hill)	Straw yield (g/hill)	Grain crude protein yield (%)	N uptake by grain (mg/hill)	N uptake by straw (mg/hill)	Total N-uptake (mg/plant)
No Azospirillum treatment						
0 kg N fed (zero % N)	29.54	26.16	3.12	155	105	14.46
5 + 5 kg N/fed. (25 % N)	33.26	31.71	3.41	191	130	15.00
10 + 10 kg N/fed. (50 % N)	38.91	34.12	3.41	223	151	15.73
20 + 10 kg N/fed. (75 % N)	50.16	47.49	4.66	424	288	24.11
30 + 10 kg N/fed. (100 % N)	55.89	47.96	5.03	438	298	26.56
Mean	41.55	37.49	3.93	286.2	194.4	19.17
Azospirillum treatment						
0 kg N fed (zero % N)	41.81	36.58	3.40	239	160	17.31
5 + 5 kg N/fed. (25 % N)	44.06	38.13	3.67	272	182	17.16
10 + 10 kg N/fed. (50 % N)	49.60	39.40	5.20	434	291	24.32
20 + 10 kg N/fed. (75 % N)	65.02	57.66	5.75	628	421	28.18
30 + 10 kg N/fed. (100 % N)	58.83	56.27	4.95	489	328	27.13
Mean	51.86	45.61	4.59	412.4	276.4	22.82

Nitrogen (N) L.S.D.	(0.05)	2.79	2.33	0.031	21.45	2.41
	(0.01)	3.79	3.16	0.042	29.10	3.27
Inoculation (I) L.S.D.	(0.05)	0.68	2.13	0.014	15.16	0.449
	(0.01)	1.25	3.91	0.026	27.85	0.826
(N) x (I) L.S.D.	(0.05)	3.95	N.S.	0.044	30.34	3.41
	(0.01)	5.36	-	0.059	41.16	4.62

Table 7: Effect of *Azospirillum* inoculant in combination with N fertilizer on plant height, tillers number and panicle length in Giza 176 variety of rice, 1998 season.

Treatment	Plant height (cm)			No. of tillers/hill			Panicle length (cm)
	60th day	120th day	Mean	Total	Productive	Mean	
No Azospirillum treatment							
0 kg N fed (zero % N)	79.0	90.3	84.65	20	20	20	18.67
5 + 5 kg N/fed. (25 % N)	83.8	97.8	90.80	22	22	22	18.83
10 + 10 kg N/fed. (50 % N)	86.5	101.0	93.75	27	27	27	18.90
20 + 10 kg N/fed. (75 % N)	88.3	104.0	96.15	29	29	29	18.96
30 + 10 kg N/fed. (100 % N)	90.3	105.3	97.80	30	30	30	20.20
Mean	85.58	99.68		25.6	25.6		19.11
Azospirillum treatment							
0 kg N fed (zero % N)	90.8	96.0	93.4	23	23	23	19.09
5 + 5 kg N/fed. (25 % N)	92.5	97.5	95.0	26	26	26	20.54
10 + 10 kg N/fed. (50 % N)	93.3	103.3	98.3	28	28	28	21.33
20 + 10 kg N/fed. (75 % N)	97.3	106.0	101.7	34	34	34	21.74
30 + 10 kg N/fed. (100 % N)	94.8	104.0	99.4	31	31	31	21.64
Mean	93.74	101.4		28.4	28.4		20.87
Nitrogen (N) L.S.D. (0.05)		1.06	3.21		1.46	1.49	0.92
(0.01)		1.43	4.36		1.97	2.02	1.25
Inoculation (I) L.S.D. (0.05)		1.17	N.S.		1.84	2.13	1.24
(0.01)		2.15	-		-	-	-
(N) x (I) L.S.D. (0.05)		1.49	N.S.		N.S.	N.S.	N.S.
(0.01)		2.03	-		-	-	-

Table 8: Effect of *Azospirillum* inoculant in combination with N fertilizer on yield and yield components in Giza 176 variety of rice, 1998 season.

Treatment	Grain yield (g/hill)	Straw yield (g/hill)	Grain crude protein yield (%)	N uptake by grain (mg/hill)	N uptake by straw (mg/hill)	Total N-uptake (mg/plant)
No Azospirillum treatment						
0 kg N fed (zero % N)	27.3	28.8	3.40	156	106	13.10
5 + 5 kg N/fed. (25 % N)	32.4	29.1	3.44	187	127	14.30
10 + 10 kg N/fed. (50 % N)	41.5	32.2	3.55	248	169	15.45
20 + 10 kg N/fed. (75 % N)	47.3	49.7	4.18	333	227	19.31
30 + 10 kg N/fed. (100 % N)	59.5	52.6	4.66	466	317	26.10
Mean	41.6	38.48	3.85	278	189.2	17.65
Azospirillum treatment						
0 kg N fed (zero % N)	30.4	38.7	4.01	205	137	14.87
5 + 5 kg N/fed. (25 % N)	34.8	47.3	4.02	235	159	15.16
10 + 10 kg N/fed. (50 % N)	49.2	49.8	5.94	491	328	29.25
20 + 10 kg N/fed. (75 % N)	65.2	60.0	5.02	550	369	27.03
30 + 10 kg N/fed. (100 %N)	59.4	57.9	4.89	488	326	26.26
Mean	47.8	50.74	4.78	393.8	263.8	22.51
Nitrogen (N) L.S.D. (0.05)		2.22	0.67	0.018	17.47	2.58
(0.01)		3.01	0.91	0.024	23.69	3.49
Inoculation (I) L.S.D. (0.05)		2.11	0.85	0.011	11.34	1.16
(0.01)		3.88	1.56	0.021	20.82	2.13
(N) x (I) L.S.D. (0.05)		3.14	0.95	0.025	24.70	3.64
(0.01)		4.26	1.28	0.034	33.51	4.94

Table 9: Effect of *Azospirillum* inoculation and N-fertilization on *Azospirillum* spp. population in rhizosphere (S) and root surface (R) soils of sorghum (C_4) and rice (C_3) plants (averaged over seasons).

Treatment (kg N/fed.)	Population (10^2/g dry soil)						R/S ratio
	Rhizosphere soil (S)			Root surface soil (R)			
	Inoculated	Uninoculated	Mean	Inoculated	Uninoculated	Mean	
Sorghum (C_4)							
0	18	3.5	10.8	32	10.8	21.4	1.98
10	32	18	25	376	25	200.5	8.02
20	32	25	28.5	2360	376	1368	48.0
30	376	32	204	11000	2360	6680	32.8
40	2360	376	1368	18000	9360	13680	10.0
Mean	563.6	90.9	327.3	6353.6	2426.4	4389.9	
Rice (C_3)							
0	369	3.5	186	2360	4.3	1182	6.35
10	720	3.5	362	2860	25	1443	3.99
20	720	17.8	369	7860	376	4118	11.16
30	720	376	548	9360	376	4868	8.88
40	2360	376	1368	11500	2360	6930	5.07
Mean	977.8	155.4	566.6	6788.0	628.3	3708.2	

However, these results are supposedly attributable to the synergetic effects due to the association of plant with *Azospirillum lipoferum* which play an important role in the superiority of N uptake by *Azospirillum* inoculated plants. At first hand, the findings of Martin *et al.* (1989) that, the application of *Azospirillum brasilense* onto the roots of young wheat plants grown in soil increased the number of lateral roots, the total root length and the number of root hairs, a similar results were obtained after application of IAA. This suggest that IAA is an important factor responsible for the effects observed after inoculation with *A. brasilense* and that the increase in root surface may improve acquisition of nutrients and enhance growth of plants.

At second hand, the observations of Heulin *et al.* (1987) that, the *A. lipoferum* and *A. brasilense* colonized in the rhizosphere of rice caused an increase in exudations, + 36 and + 17 per cent, respectively compared with sterile control. Similarly, Okon and Kapulnik (1986), stated that inoculation of several cultivars of wheat, maize, sorghum and *Setaria italica* with *Azospirillum* caused morphological changes in root starting immediately after germination. Root length, and surface area were differentially affected according to bacterial age and inoculum level. They also reported that the number of root hairs, root hair branches and lateral roots were increased by inoculation. They also found that the rate of NO_3^-, K^+ and $H_2PO_4^-$ uptake was greater in inoculated seedlings and in the field, dry matter, N, P and K accumulated at faster rates, and water content was higher in *Azospirillum*-inoculated maize, sorghum, wheat and *S. italica*.

However, concerning that remarkable effect of N fertilization on such ratio, these results indicated that raising N application level up to 30 kg/fed. increased the N uptake of the *Azospirillum*-inoculated plants under the conditions of this study. With respect to the influence of N levels on N uptake, it was found that N application resulted in a significant increase which continued till 40 kg/fed. in the case of uninoculated treatments and till 30 kg/fed. in the case of *Azospirillum lipoferum*- inoculated ones either by sorghum or rice, plant.

Regarding sorghum plant (Tables 2 and 4), the increase in the amounts of N uptake due to mineral N addition reach 1.35, 1.57, 1.87 and 2.27 times that in the control treatments for 10, 20, 30 and 40 kg N/fed., respectively. The corresponding values of these were 1.06, 1.13, 1.58 and 1.91 times, respectively by rice plant (Tables 6 and 8). Concerning plant and/or grain crude protein yield in both plants, results behaved a similar trend like that of N uptake. Thus, our findings indicated the possibility of saving nitrogen fertilizer to the extent 10 kg N/fed. without effecting the accumulative dry matter and/or grain yield of forage sorghum and rice crop by the application of *Azospirillum lipoferum* inoculant. The economics of the results showed that bacterial inoculation of both different cereal crops with *A. liporferum* in combination with reduced N fertilizer level was profitable. The response of sorghum with reduced level of N fertilizer (30 kg N/fed.) in combination with *Azospirillum* inoculation was however, better than rice plant.

Population of *Azospirillum* spp. in rhizosphere and root surface soils: The results of the study made on the influence of bacterial inoculation in combination with different levels of N fertilization on *Azospirillum* spp. populations at harvest stage in both rhizosphere and root surface soils of the two physiologically different plants are presented in Table 9. The changes in populations of these N_2-fixing bacteria were clearly noted. The results revealed that the population was responded well to the inoculation

The MPN of *Azospirillum* spp. in rhizosphere soil of each plant species averaged over sampling seasons and plant species as well as N fertilizer levels was highest in the inoculated treatment to the extent 6.3 times more than that of uninoculated one (Table 9). Regarding the root surface soil, the MPN amounted 10.8 and 2.6 times of those for sorghum and rice uninoculated plants. Hence, the results of the present study suggest that root exudations, generally play an important role in the colonization of the rhizosphere and root surface region by *Azospirillum* spp. The observations of Balandreau (1975) Baldani *et al.* (1986) and Martin *et al.* (1989) that, the extra nitrogenase activity and more establishment of inoculated *Azospirillum* spp. in the rhizosphere soil and roots of several grasses which are roughly proportional to the concentration of the exuded photosynthates and definitely to the microbial population density and nitrogen fixing microorganisms in particular, supports such a possibility.

Concerning the N fertilizer effect, the changes in population of *Azospirillum* spp., irrespective of the soil sampling site, were in agreement with changes in levels of N fertilizer giving the maximum mean values with the highest level of N for both plant species. Between the C_4 (sorghum) and C_3 (rice) plants, the rhizosphere and/or the root surface soil effect on MPN of *Azospirillum* spp. is moderately pronounced. In general, the sorghum plant exhibited greater number than rice. Irrespective of N fertilizer level and soils sampling site, the sorghum plant recorded on an average 47.2 x 10^4 cells/g dry soil, while the MPN of *Azospirillum* spp. bacteria amounted only 42.7 x 10^4 cells/g of dry soil of rice plant (Table 9).

This considerable increase could be attributed to that the sorghum posses the C_4 dicarboxylic acid pathway of photosynthesis, less carbohydrate is consumed in photorespiration, and thus more may be available for root exudation to enhance the growth and changes in rhizosphere and root surface microflora including nitrogen fixing bacteria in particular. That the production of photosynthate by plant is responsible for supporting growth and activity by free-living N_2 fixers in many rhizosphere and phyllosphere systems has been reported earlier by Balandreau *et al.* (1975). Changes in plant genome are known to bring about changes in rhizosphere microflora (Dobereiner and Campelo, 1971; Hatch *et al.*,1971 and Zelitch, 1971).

References

Balandreau, J., 1975. Mesure de l, active nitrogenasique de microorganismes fixateurs libres d'azote de la rhizosphere equlques graminees. Rev. Ecol. Biol. Sol., 12: 273-290.

Balandreau, J., G. Rinaudo, I. Faris Hamad and Y. Dommergues, 1975. In "Nitrogen Fixation by Free-Living Microorganisms" W.D.P. stewart, Ed., Cambridge Univ. Press, Cambridge.

Baldani, V.L.D., M.A. de B. Alvarez, J.I. Baldani and J. Dobereiner, 1986. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. Plant and Soil, 90: 35-46.

Black, C.C. Jr., 1973. Photosynthesis carbon fixation in relation to net O_2 uptake. ann. Rev. Plant Physiol., 24: 283-286.

Chapman, H.D. and F.P. Parker, 1981. Methods of Analysis of Soil, Plant and Water. Univ. of California, August 1981, 2nd Printing.

Dart, P.J., 1976. Non symbiotic nitrogen fixation in soil. In: Soil Microbiology, Ed., N. Waker, Butterworths, London, pp: 225-252.

Dobereiner, J., 1980. Forage grasses and grain crops. In: Methods for Evaluation of Biological Nitrogen Fixation. Bergersen, F.J. (ed.) John Wiley and Sons, pp: 535-556.

Dobereiner, J. and A.B. Campelo, 1971. In: "Biological Nitrogen Fixation in Natural and Agricultural Habitats," T.A. Lie and E.G. Mulder, Eds Martinus Nijhoff, the Hague, 1971, pp: 457.

Dobereiner, J. and J.M. Day, 1974. Associative symbiosis in tropical grasses: characterization of microorganisms and dinitrogen fixation sites. International Symp. on Nitrogen fixation, Pullman, Washington, U.S.A.

Hammouda, F.M. and M.I. Zidan, 1991. Yield and yield components of rice (*Oryza sativa* L.) as affected by bacterization and N fertilizer. Egypt. J. Agric. Res., 69: 1991.

Hardy, R.W.F., R.C. Burns and R.D. Holsten, 1973. Application of the acetylene ethylene reduction assay for measurement of nitrogen fixation. soil Biol. Biochem., 5: 47-81.

Hatch, M.D., C.B. Osmond and R.O. Slatyer, 1971. Photosynthesis and photorespiration," Wiley-Interscience, New York, 1971.

Heulin, T., A. Guckert and J.B. Balandreau, 1987. Stimulation of root exudation of rice seedlings by Azospirillum strains: Carbon budget under gnotobiotic conditions. biology and Fertility of Soils, 4: 9-14.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) 1984. Response to inoculation with nitrogen fixing bacteria. Patancheru, A.P. 502324, India ICRISAT. 38 pp.

Kipe-Nolt, J.A., U.K. Avalakki and P.J. Dort, 1985. Root exudation of sorghum and utilization of exudates by nitrogen-fixing bacteria. Soil Biol. and Biochem., 17: 859-863.

Knowles, R., 1974. Factors Affecting Dinitrogen Fixation by Bacteria in Natural and Agricultural Systems. In: W.E. Newton and C.J. Nyman, eds. Proceedings of the 1st International Symposium on Nitrogen-Fixation. Vol. 2, Pullman, Washington, U.S. 1974. Washington State University Press. 539-555.

Martin, P., A. Glatzle, W. Kolb, H. Omay and W. Schmidt, 1989. N_2 fixing bacteria in the rhizosphere: quantification and hormonal effects on root development. Zeitschrift fur Pflanzenernahrung und Bodenkunde, 152: 237-245.

Martinez-Toledo, M.V.; T. de la Rubia and J.L. Gonzalez, 1988. Root exudates of *Zea mays* and the acetylene-reduction activity of *Azotobacter chroococcum*. Soil Biology and Biochem., 20: 961-962.

Okon, Y. and Y. Kapulnik, 1986. Development and function of Azospirillum-inoculated roots. Plant and Soil, 90: 3-16.

Paul, E.A., R.J.K. Myers and W.A. Rice, 1971. Nitrogen fixation in grassland and associated cultivated ecosystems. Pl. Soil (special volume), 495-507.

Purushothaman, D., C. Kasirajan and G. Rangaswami, 1976. Nitrogen fixation by *Azotobacter* occurring in the rhizosphere of C_4 plant species. Curr. Sci., 45:807-809.

Raju, P.N., H.J. Evans and R.J. Seildler, 1972. An asymbiotic nitrogen fixing bacterium from the root environment of Corn. Proc. Natl. Acad. Sci., USA, 69: 3474-3478.

Rangaswami, G., 1975. Bacterial fertilizer prospects and limitations. Proc. FAI-FAO Seminar on Optimizing Agric. Production under Limited Availability of Fertilizers. New Delhi, pp: 453-458.

Sanathanakrishna, P. and G. Oblisami, 1980. The Response of Maize and Sorghum to Azotobacter Inoculants page 95-97. In: Aspects of Biological Nitrogen Fixation No. 28. Univ. Agricultural Sciences, Hebbal, Bangalore, India.

Snedecor, G.W. and W.G. Cochran, 1967. Statistical methods, 6th ed. Iowa State Univ. Press. Ames., Iowa.

Subba Rao, N.S., 1981. Response of crops of Azospirillum inoculation in India. Associative N_2-fixation. Volume 1 (edited by Vose, P.B., Ruschel, A.P.). 1981, 137-144.

Vincent, J.M., 1970. A Manual for the Practical Study of Root Nodule Bacteria, IBP Handbook No. 15, Blackwell Sci. Publications, Oxford and Edinburgh. 58-72.

Wani, S.P., S. Chandvapalaiah and P.J. Dart, 1985. Response of pearl millet cultivars to inoculation with nitrogen-fixing bacteria. Experimental Agriculture 21: 175-182.

Wani, S.P., 1986. Nitrogen fixation associated with cereals and forage grasses. Page227-240 in Soil biology: Proceedings of the National Symposium on Current Trends in Soil Biology, 25-27 Feb. 1985, Hisar, Haryana, India. Hisar, Haryana, India: Haryana Agricultural University (C.P. 229).

Zelitch, I., 1971. "Photosynthesis, Photorespiration, and Plant Productivity," Academic Press, New York, 1971.