

EFFECT OF AZOSPIRILLUM SPP. ON THE PHYSIOLOGICAL, YIELD AND BIOCHEMICAL PARAMETERS OF WHEAT CULTIVAR, SHATABDI

*F Rahman¹, K C Das¹, S Nasrin¹ and Z U M Khan²

Present address

¹National Institute of Biotechnology, Savar, Dhaka Bangladesh ²Dept. of Botany Jahangirnagar University, Savar, Dhaka, Bangladesh

Correspondence

*fahmida_shimul@yahoo.com

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Abstract

Abstract: Inoculation effect of 9 *Azospirillum* spp. on the physiological, yield and biochemical parameters of the wheat cultivar, Shatabdi was studied. Here, emergence of seedlings, plant height, panicle length and fresh & dry weights were considered as physiological parameter. On the other hand, yield parameters include panicle length, no. of grains per panicle, 100 grains weight and biochemical parameters include nitrogen & protein content of wheat grain. All of the parameters were improved significantly in the wheat plants inoculated with *Azospirillum* strains compared to the uninoculated control plants and ultimately wheat yield was increased. *Azospirillum brasilense* (ATCC No. 29145) was used as a reference strain to compare the performance of the 9 isolated strains (F-1-F-9). Finally, it was observed that all of the *Azospirillum* strains had positive impact on the wheat cultivar, Shatabdi to increase yield and F-2 was found to be the best among the 9 strains.

Keywords: *Azospirillum*, Inoculation, Wheat, Seedling emergence

Introduction

Heavy input of inorganic fertilizers is required to increase the food production through intensive agriculture. However, the energy crisis has hampered chemical fertilizer production and availability with the result that attention is being increasingly paid to alternate ways of increasing crop yields. In recent, scientists have identified two major research thrusts to augment crop yields. One of them is strengthening research on biological nitrogen fixation.

Augmentation of food production in future lies in increasing yield per unit of land, which can only be achieved by the cultivation of nutrient responsive high yielding grain crops. Nitrogen is an important input in maximum yields. In this context, the nitrogen gap in populous developing country like Bangladesh will be difficult to bridge especially in the wake of energy crisis unless a major breakthrough could be made in finding cheaper alternative sources for nitrogen supply. The approaches involving the manipulation of the ecological *milieu* of the root environment of crop plants so as to bring about maximum biological nitrogen fixation by microorganism coupled with judicious selection of both the nitrogen fixed as well as the higher plant for maximum nitrogen fixing potential are bound to offer dividends. Inoculation of plants with *Azospirillum* spp. has been found to cause significant increases in growth and yield of different crops including rice and wheat. This organism has wide adaptability to different environmental conditions.

Sandy soils with more than 90 percent sand require high fertilizer input. The use of biofertilizer such as nitrogen fixing bacteria can reduce chemical fertilizer requirement and consequently lower production cost (Saad *et al.*, 1999). Under such stress condition *Azospirillum* spp. might be naturally adapted to attain remarkable efficiency in fixing atmospheric nitrogen

and in enhancing plant growth by this and by some other ways like production of growth promoting substances and influencing root development and causing increased uptake of nutrients from the land. So, the present work was undertaken to study the inoculation effect of 9 *Azospirillum* spp. isolated from the different locations of Bangladesh on physiological, yield and biochemical parameters of the wheat cultivar, Shatabdi.

Materials and Methods

Azospirillum strains

In the present study, 9 *Azospirillum* spp., collected from the Department of Botany, Jahangirnagar University, Savar, Dhaka, which were previously isolated and identified by Rahman (2002) from three locations, Bhuapur of Tangail at the bank of Jamuna; Bhagyakul of Munshiganj at the bank of Padma and Srikanthapur of Dinajpur at the bank of Atrai on the basis of colony, cell morphology and biochemical tests. The best *Azospirillum* spp. were selected for further study by Rahman (2002) from initially isolated *Azospirillum* colonies on the basis of their ability to grow better and faster in Nfb semi solid medium. The reference strain, *Azospirillum brasiliense* (ATCC No. 29145) was collected from the American type culture collection to compare the performance of these 9 strains.

Collection of wheat seeds and soil

Fresh and healthy seeds of 'Shatabdi' variety of wheat were collected from Wheat Research Center of Bangladesh Agriculture Research Institute (BARI). The germination rate of the seeds was 96%. The soil for pot experiment was collected from Dhamrai. Such soil was selected because the soil had almost neutral pH value. Thirty three pots with height of 12 inches

were taken for 11 treatments and filled with collected soil that is homogenous. Each pot contained 6.5 kg of soil. Each treatment had three replications.

Preparation of inoculum

The organisms (reference strain, selected strains) were grown in 250 ml Erlenmeyer flask containing 250 ml malate liquid medium supplemented with 0.25% NH₄Cl and 0.1% yeast extract. The culture was incubated for 48 hours at 30°C±2°C with agitation (100 rpm) in a water bath.

Sterilization of seeds

Surface sterilization of the wheat seeds was accomplished by treatment with 0.1% HgCl₂ for 2/3 minutes. The seeds were washed 4 times with sterilized distilled water.

Inoculation and seed sowing

Inoculation was performed by immersing of the surface sterilized seeds for 3 hours with inoculum concentration of 10⁷ cfu/ml. The inoculated seeds were sown (25 seeds/pot) in the soil in earthen pot. The pots were watered regularly in a way that the pots never overflowed or to prohibit the water logging conditions. Seeds immersed in sterile mediums for 3 hours were sown in control pots.

Cultural practice

Thinning was done 15 days after emergence of seedlings. Fifteen seedlings were kept. It was made in such a way that healthy seedlings of uniform size and vigor were allowed to grow. Regular watering and weeding were performed to ensure equal environmental condition throughout the pot. No chemical fertilizer was applied.

Observation and data collection

During the present investigation some growth, yield and biochemical parameters were noted in the field. To study the sequence of morphological and physiological changes, some characteristics were studied at different stages of growth and development of plants. Five randomly selected plants were used from each pot for data collection. Most of the results were statistically analyzed arranging data under Completely Randomized Design (CRD).

(a) Enumeration of Azospirillum spp. in root sample of wheat plant

For the determination of *Azospirillum* spp. population, the root samples of wheat plant growing in pot culture (at pre-flowering stage) were processed as described by Watanabe and Brotonegro (1981) and the populations in root sample were estimated by the most probable number (MPN) method of Alexander (1995).

(b) Physiological parameters or plants growth status

The following parameters were considered as plant growth status-

(i) Emergence of seedlings (ii) Plant height (iii) Panicle length and (iv) Fresh and dry weight of plant.

Germination records were taken up to 15 days of sowing. For each measurement, data were recorded at 15 days interval from seedlings to late mature stage and data were taken with the help of centimeter scale and fine balance.

(c) Yield parameters

During harvest, 5 randomly selected plants were uprooted for collecting post harvest data on the following yield parameters-

(i) Panicle weight (ii) No. of grains per panicle and (iii) 100 grains weight

Number of grain per panicle were counted after removing them from plant

(d) Biochemical parameters

(i) Determination of nitrogen content of wheat grain

The basic principle of this procedure involves digestion of wheat grain with concentrated sulfuric acid (H₂SO₄) and then with digestion mixture, which causes oxidation and destruction of protein and ultimately causes conversions of the organic nitrogen to ammonia. The ammonia remains in the acid mixture as ammonium sulfate.



The amount of ammonia nitrogen is determined by making the digest alkaline followed by distillation of the liberated ammonia into standard acid solution and estimated titrimetrically.

The clear dried 500 ml kjeldahl digestion flasks were taken and about 1 g dried sample was taken in each kjeldahl flask. 20 ml of concentrated H₂SO₄ and 5 g of digestion mixture (CuSO₄ · 5 H₂O + K₂SO₄ with ratio of 10:1) were added and then heated in digestion chamber until the content become cleared. After completing the digestion the flask was cooled and the digest was transferred in 100 ml volumetric flask and diluted up to the mark with distilled water. 10 ml of the diluted solution was transferred a microkjeldahl distillation apparatus and after adding of 5 to 15 ml of 30% NaOH, the solution was distilled for 5 minutes. The distillate was collected in excess of 4% boric acid solution with indicator and was titrated by 0.1 N HCl solution.

(ii) Determination of protein content of wheat grain

The total N₂-content was converted into crude protein by multiplying with the factor of 5.67 (Anonymous, 1971).

$$\% \text{ of crude protein} = \text{N} \times 5.67$$

Statistical analysis

The experiment was conducted in Completely Randomized Design (CRD) and analyzed statistically using ANOVA.

Results and discussion

Inoculation of plants with *Azospirillum* spp. can cause a significant change in various plant growth parameters, which may or may not affect in crops yield. Most studies of the *Azospirillum*-plant association have been conducted on cereals and grasses (Patriquin *et al.*, 1983). The following responses to *Azospirillum* inoculation in wheat were reported: increase in total plant dry weight, in the amount of nitrogen in shoots and grains, earlier heading and flowering time, increased number of grain per panicle, increased grain weight, greater plant height, leaf size and higher germination rates (Bashan, 1986; Millet and Feldman, 1986; Warembourg *et al.*, 1987). The effect of *Azospirillum* inoculation on the total yield increase of field grown plants is generally ranged from 10-30% (Kapulnik *et al.*, 1987; Watanabe and Lin, 1984). A few reports indicated extremely higher values, 50-70% over uninoculated controls. Even moderate yield increases (up to 20%) attributed to inoculation with *Azospirillum* are considered commercially valuable to modern agriculture, if obtained consistently (Bashan and Levanony, 1990). The significant effect of *Azospirillum* spp. inoculation on the grain yield of rice, wheat, oat, barley and sorghum has been reported (Subba Rao *et al.*, 1979). In the present investigation, the pot experiment was undertaken to observe the effect of *Azospirillum* spp. inoculation on the colonization in root, some physiological parameters, yield parameters and some biochemical parameters of wheat cultivar (Shatabdi). Effect of inoculation was found significant in almost all the parameters.

(a) Populations of *Azospirillum* spp. in wheat root after inoculation

The populations of *Azospirillum* spp. in the root samples of inoculated wheat plant at pre-flowering stage were studied. F-2 showed maximum colonization and the log₁₀ of cells/g root dry weight was found to be 8.68. The least population was found in F-4, the log₁₀ of cells/g root dry weight was 7.85. It is indicated that *Azospirillum* strains could colonize well in the roots of wheat plants as a result of inoculation (Fig. 1).

(b) Physiological parameters

(i) Emergence of seedlings

Inoculation of wheat seeds with different *Azospirillum* strains showed significant variations on seedling emergence in the pot culture (Table 1). Markus (1988)

reported the similar results in case of wheat expressing that *Azospirillum* inoculation increases the emergence of wheat seedlings. Emergence started from the 5th day after sowing (DAS) and completed on 16th DAS. During the course of emergence, seeds inoculated with the strain F-2 showed maximum (90%) performance and were followed by the seeds inoculated with F-5 (87.5%), F-7 (85.77%), F-8 (85.40%), F-6 (85.21%), F-3 (82.30%), F-9 (81.40%), F-4 (63.00%), and F-1 (62.2%), respectively. The reference strain *Azospirillum brasilense* (ATCC No. 29145) (Ab) caused 87.36% emergence (Table 1) on 16th day after sowing.

(ii) Plant height

In pot culture, inoculation of wheat with different *Azospirillum* strains significantly affected plant height at different stages of growth i.e. initial stage, pre-flowering stage and flowering stage (Table 2). At initial stage, plants inoculated with F-2 strain showed maximum height (7.15 cm) and was followed by the plants inoculated with the strains F-5 (7.00 cm) and reference strain *A. brasilense* (Ab) (6.87 cm). In all inoculated pots, plant height was found to be higher than that of control pot.

At pre-flowering stage, plants inoculated with selected strain F-2 showed maximum height (10.59 cm) (Fig. 2A) and was followed by the plants inoculated with F-5 (10.13 cm), F-6 (10.03 cm) and *A. brasilense* (Ab) (9.62 cm) respectively. At flowering stage, plants inoculated with F-2 strain showed maximum height (31.99 cm) and *A. brasilense* (Ab) showed height of 29.95 cm. The plant height was shortest in the control (24.76 cm).

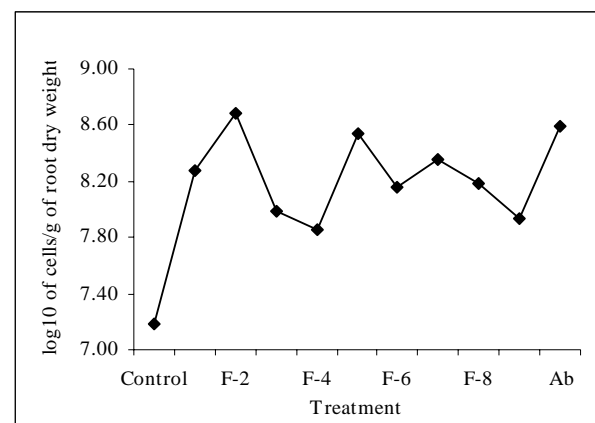


Fig.1. Population of *Azospirillum* spp. in the root samples of wheat plant determined at pre-flowering stage after inoculation with various *Azospirillum* strains (average of 3 replications; Ab: *Azospirillum brasilense*, ATCC No. 29145)

Table 1. Effect *Azospirillum* spp. inoculation on the emergence (%) of wheat seedlings (average of 3 replications)

Treatment	% emergence at different days after sowing (DAS)											
	5 th	6 th	7 th	8 th	9 th	10 th	11 th	12 th	13 th	14 th	15 th	16 th
Control	0.00	0.00	8.00	15.00	30.30	38.30	45.09	50.25	55.00	59.01	60.10	60.10
F-1	0.00	4.00	9.33	16.00	35.91	40.00	48.00	52.70	59.33	60.10	62.20	62.20
F-2	6.68	9.33	25.33	43.60	69.75	77.75	82.10	86.60	88.10	90.00	90.00	90.00
F-3	4.00	6.47	13.67	20.90	45.00	55.70	65.92	75.00	80.01	82.30	82.30	82.30
F-4	1.33	4.12	10.67	16.00	36.00	39.00	50.62	55.33	60.22	62.10	63.00	63.00
F-5	5.33	8.52	20.33	39.00	60.00	71.00	81.45	83.45	85.29	86.60	87.50	87.50
F-6	5.31	8.25	17.30	25.33	55.33	69.15	77.23	82.00	84.33	85.21	85.21	85.21
F-7	2.67	5.33	16.00	23.00	54.00	65.77	78.22	81.23	82.34	85.20	85.20	85.77
F-8	2.90	6.67	17.30	25.33	57.00	66.00	79.02	82.10	85.23	85.23	85.23	85.40
F-9	4.00	7.00	15.25	23.20	51.22	56.11	67.34	76.00	80.00	81.40	81.40	81.40
Ab	5.33	9.33	22.00	38.90	61.00	74.00	81.00	85.35	86.01	87.00	87.10	87.36
	Sig.											Sig.
LSD at 5% level	1.02											5.50

Ab: *Azospirillum brasilense* (ATCC No. 29145)Table 2. Effect of inoculation with various strains of *Azospirillum* spp. on the height of wheat plant at different stages of growth (average of 3 replications)

Treatment	Plant height (cm)					
	Initial stage		Pre-flowering stage		Flowering stage	
	Height	Height increased over control	Height	Height increased over control	Height	Height increased over control
Control	4.96	0.00	7.46	0.00	24.76	0.00
F-1	5.46	0.50 ^{ns}	7.95	0.49 ^{ns}	25.66	0.90 ^{ns}
F-2	7.15	2.19 ^{**}	10.59	3.13 ^{**}	31.99	7.23 ^{**}
F-3	6.87	1.19 ^{**}	9.47	2.01 ^{**}	29.24	4.48 ^{**}
F-4	5.74	0.78 ^{**}	8.03	0.57 ^{ns}	26.97	2.21 ^{**}
F-5	7.00	2.04 ^{**}	10.13	2.67 ^{**}	31.06	6.30 ^{**}
F-6	6.94	1.98 ^{**}	10.03	2.57 ^{**}	30.99	6.23 ^{**}
F-7	6.70	1.74 ^{**}	8.24	0.78 ^{ns}	28.57	3.81 ^{**}
F-8	6.77	1.81 ^{**}	8.30	0.84 ^{ns}	28.67	3.91 ^{**}
F-9	5.49	0.53 [*]	7.76	0.30 ^{ns}	26.03	1.27 ^{ns}
Ab	6.87	1.19 ^{**}	9.62	2.16 ^{**}	29.95	5.19 ^{**}
	Sig.		Sig.		Sig.	
LSD at 5% level	0.51		0.90		1.36	
LSD at 1% level	0.69		1.22		1.84	

Ab: *Azospirillum brasilense* (ATCC No. 29145); * Significant at 5% level; ** Significant at 1% level; ^{ns}Non significant

In the present investigation, *Azospirillum* spp. inoculation was found to increase plant height significantly at initial, pre-flowering and flowering stages. F-2 was found to be the most effective in increasing the plant height and next to better results were shown by F-5 and F-6 (Table 2). Kapulnik *et al.* (1983) found similar results. They observed 13% increase of plant height of wheat due to inoculation with *Azospirillum*. Mubassara *et al.* (2001) also reported the similar result expressing that inoculation with *Azospirillum* strains isolated from Bangladeshi

soil caused significant increase in height (18.28-40.00%) of wheat plant.

(iii) Panicle length

Table 3 represents the length of panicle/plant in pot experiment. At flowering stage as per efficiency of strains in increasing panicle length, 10 strains can be arranged as F-2>Ab>F-5>F-8>F-6>F-7>F-3>F-9>F-4>F-1. The results were highly significant as compared to uninoculated control plants. At post flowering stage, F-3, F-6 and F-8 strains followed F-5, *A. brasilense* (Ab) and F-2 strains (Table 3, Fig. 2B). Mubassara *et al.* (2001), Wedad and Vlassak (1988) reported the

Table 3. Effect of *Azospirillum* spp. inoculation on panicle length, fresh and dry matter of wheat plant on the height of wheat plant at different stages of growth (average of 3 replications)

Treatment	Panicle length (cm)/plant				Plant weight of (g)			
	Flowering stage		Post flowering stage		Fresh weight/plant		Dry weight/plant	
	Length	Length increased over control	Length	Length increased over control	Weight	Weight increased over control	Weight	Weight increased over control
Control	6.57	0.00	7.60	0.00	3.90	0.00	1.67	0.00
F-1	7.93	1.36**	8.92	1.32**	4.80	0.90 ^{ns}	2.57	0.90**
F-2	9.71	3.14**	10.71	3.11**	9.03	5.13**	5.13	3.46**
F-3	8.41	1.84**	9.42	1.82**	6.67	2.77**	3.77	2.10**
F-4	8.04	1.47**	9.08	1.48**	5.47	1.57*	2.87	1.20**
F-5	9.23	2.66**	10.21	2.61**	7.90	4.00**	4.50	2.83**
F-6	8.71	2.14**	9.71	2.11**	8.27	4.37**	4.07	2.40**
F-7	8.53	1.96**	9.20	1.60**	6.87	2.97**	3.83	2.16**
F-8	8.96	2.39**	9.95	2.35**	7.47	3.57**	4.10	2.43**
F-9	8.32	1.75**	8.99	1.39**	7.00	3.10**	3.83	2.16**
Ab	9.30	2.73**	10.29	2.69**	8.31	4.23**	5.03	3.36**
		Sig.		Sig.		Sig.		Sig.
LSD at 5% level		0.47		0.53		1.23		0.50
LSD at 1% level		0.65		0.72		1.67		0.68

Ab: *Azospirillum brasilense* (ATCC No. 29145); *Significant at 5% level; **Significant at 1% level; ^{ns}Non significant

increase of panicle length due to the inoculation of *Azospirillum* spp.

(iv) Fresh and dry weight of plant

Fresh weights per plant have been presented in Table 3. F-2 strain showed better results than the other strains. The highest fresh weight per plant (9.03 g) was recorded from F-2 treated plant and lowest fresh weight (3.90 g) was recorded from uninoculated control plant. Only F-1 strain could cause no significant difference in fresh yield compared with uninoculated control plants. Other strains in general were better than control but inferior to F-2 strain (Table 3).

Inoculation performed significant role to increase dry matter yield of wheat plants (Table 3). All of the strains induced higher dry production as compared to the control. F-2, F-5, F-6, F-8 and reference strain *A. brasilense* were found highly promising in increasing dry matter content over control. The effect of other strains F-1, F-3, F-4, F-7 and F-9 were more or less identical. Similar increase of plant dry weight was reported by Zambre and Konde (1990) as a result of inoculation with *Azospirillum* spp. Bashan *et al.* (1990) reported that *Azospirillum* inoculation significantly improved wheat growth by increasing root and shoot dry weight and root surface area.

(c) Yield parameters

All the strains including reference strain *Azospirillum brasilense* significantly improved wheat yield by

increasing panicle weight, number of grains per panicle and grain weight.

(i) Panicle weight

The plants inoculated with the strains F-2, F-5, F-6, F-8 and the reference strain *A. brasilense* (Ab) caused 0.30, 0.27, 0.24, 0.20 and 0.27 g increase in panicle weight over uninoculated control plants respectively. Rest of the plants inoculated with F-1, F-3, F-4 and F-9 showed non-significant increase over control (Table 4).

(ii) No. of grains per panicle

Number of grains per panicle was significantly influenced due to the inoculation with various strains of *Azospirillum* spp. F-2 treated plant produced highest no. of grains per panicle and it was statistically identical to F-5 and *A. brasilense* (Ab). Among inoculated plants, the lowest no. of grains 17.78/panicle was obtained from F-4 treated plant which was not statistically significant. The other strains showed significant increasing of grain no. per panicle over the control (Table 4).

(iii) 100 grains weight

Significant increase of grain weight was found in the plants which received F-2 strain and in this case mean weight of 100 grain was 5.10 g. F-5, F-8, F-3, F-6, F-9, F-7 and the reference strain *A. brasilense* (Ab) showed the same level of significance which caused increase in 100 grain weights by 1.80, 1.60, 1.40, 1.34, 1.34, 1.07

Table 4. Effect of inoculation with various strains of *Azospirillum* spp. on the height of wheat plant at different stages of growth (average of 3 replications)

Treatment	Panicle weight (g)/plant		No. of grains/panicle		100 grains weight (g)	
	Weight	Weight increased over control	Number	Number increased over control	Weight	Weight increased over control
Control	1.03	0.00	16.05	0.00	3.03	0.00
F-1	1.17	0.14 ^{ns}	18.25	2.20*	3.50	0.47**
F-2	1.33	0.30**	24.36	8.31**	5.10	2.07**
F-3	1.13	0.10 ^{ns}	19.62	3.57**	4.43	1.40**
F-4	1.10	0.07 ^{ns}	17.78	1.73 ^{ns}	3.63	0.60**
F-5	1.30	0.27**	21.99	5.94**	4.83	1.80**
F-6	1.27	0.24**	19.12	3.07**	4.37	1.34**
F-7	1.20	0.17*	17.94	1.89*	4.10	1.07**
F-8	1.23	0.20**	19.92	3.87**	4.63	1.60**
F-9	1.13	0.10 ^{ns}	18.44	2.39**	4.37	1.34**
Ab	1.30	0.27**	20.62	4.57**	4.97	1.94**
		Sig.		Sig.		Sig.
LSD at 5% level		0.14		1.74		0.34
LSD at 1% level		0.19		2.37		0.46

Ab: *Azospirillum brasilense* (ATCC No. 29145); *Significant at 5% level; **Significant at 1% level; ^{ns}Non significant

and 1.94 g over control respectively. F-1 and F-4 strains were found less efficient in increasing grains weight. Similar results have been reported by Mubassara *et al.* (2001) about increasing panicle weight, grains per panicle, grain weight of wheat due to inoculation with *Azospirillum brasilense*.

Table 5. Effect of inoculation with various strains of *Azospirillum* spp. on the total nitrogen and protein content of wheat grains (average of 3 replications)

Treatment	N ₂ -content of grain (%)	Protein content of grain (%)
Control	1.32	7.48
F-1	1.40	7.94
F-2	2.10	11.91
F-3	1.68	9.53
F-4	1.48	8.39
F-5	2.09	11.85
F-6	1.96	11.11
F-7	1.84	10.43
F-8	1.70	9.64
F-9	1.82	10.32
Ab	2.10	11.91

Ab: *Azospirillum brasilense* (ATCC No. 29145)

(d) Biochemical parameters

(i) Nitrogen content of wheat grain

Inoculation with various *Azospirillum* strains enhanced the N₂-content of wheat grain as compared to the control. The N₂-content ranged from 1.32 to 2.10%. From the table 5, it is clear that F-2 and reference strain *A. brasilense* (Ab) treated plants gained highest total nitrogen followed by the plants

inoculated with F-5 and F-6. Increase of nitrogen content in grain as a result of inoculation with *Azospirillum* spp. was reported by Zambre and Konde in 1990.

(ii) Protein content of wheat grain

Increase of protein content in wheat grain was observed as a result of inoculation of various *Azospirillum* strains with wheat (Table 5). Protein content was higher in the grains of all inoculated plants that in those of uninoculated plants. F-2, reference strain *A. brasilense* (Ab), F-5, F-6 were found highly promising in increasing protein content of the wheat grain.

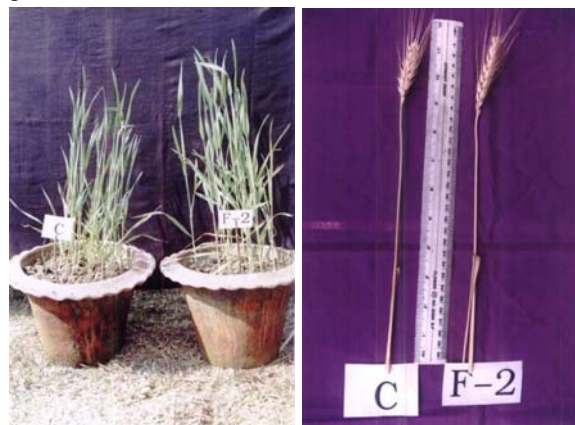


Fig. 2. A. Pre-flowering stage of wheat plant inoculated with *Azospirillum* strain F-2; B. Effect of inoculation with *Azospirillum* strain F-2 on panicle length of wheat, Shatabdi.

Conclusion

In the present investigation, 9 *Azospirillum* spp. were used to observe their role in seedling emergence, enhancing the plant growth and yield of wheat cultivar, Shatabdi. Moreover, their affect in nitrogen and protein content in grain was also observed. It was detected that all the 9 *Azospirillum* strains have contribution in all the parameters mentioned and of them, F-2 was superior to other 8.

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