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Full Length Research Paper

Improvement of yield potential of rice through combined application of biofertilizer and chemical nitrogen

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A field experiment was conducted to evaluate the suitable combination of plant growth promoting rhizobacteria (*Azospirillum* biofertilizer strain BM9 and BM11) along with different nitrogenous fertilizer levels (0, 20, 40, 60, 80 and 100% N) on rice variety Binadhan 4 at Bangladesh Institute of Nuclear Agriculture farm, Mymensingh in Aman season of 2006. Roots of rice seedlings were dipped in broth culture of bacterial strains in Luria-Bertani (LB) medium for half an hour before transplanting. Data were recorded on plant growth, yield, yield components and nutrient uptake. Results showed a significant increase in growth parameter like plant height, shoot dry weight, root length and dry weights, grain and straw yields, effective tillers/hill and panicle length, and nitrogen, phosphorus and potassium uptake over uninoculated under most nitrogen levels except 100% N. Number of grains per panicle and 1000 grain weights were increased considerably compared to uninoculated control. Strains BM9 and BM 11 along with 80% N produced similar grain and straw yield to that with 100% nitrogen fertilizer applied alone. Results revealed greater scope of *Azospirillum* biofertilizer application for supplementing fertilizer nitrogen for achieving optimum yield of rice in Bangladesh soil and environmental condition.

Key words: Rhizobacteria, *Azospirillum* biofertilizer strain, rice growth, nitrogenous fertilizer.

INTRODUCTION

Biofertilizers are involved in symbiotic and associative microbial activities with higher plants. These are natural mini-fertilizer factories that are economical and safer source of plant nutrition for increasing the agricultural production improving soil fertility. The microorganisms colonize on roots of rice, wheat, maize, sugarcane and form root nodules in leguminous plants. Different biofertilizer have been shown nitrogen fixing, phosphate solubilizing and phytohormone product ability and are used for increasing the agricultural productivity (Hafeez et al., 2002; Nuruzzaman et al., 2005). *Azospirllum* is considered as potential plant growth promoting rhizobacteria. The mechanism of plant growth promoting rhizobacteria is mobilization of nutrients, production of phytohormones and non-symbiotic nitrogen fixation. Increased uptake of nutrient such as N, P and K was suggested as one of the mechanisms by which biofertilizer increased crop yield (Lucy et al., 2004; Nuruzzaman et al., 2003). Significant increase in crop yield following application of plant growth promoting bacteria has been documented under diverse field condition (Bashan, 1998). There have widely been reported to fix atmospheric nitrogen with grasses and cereals (Dobereiner, 1997) and increase nutrient uptake (Lin et al., 1983; Murty and Ladha, 1987; Sarig et al., 1988; Kapulnik, 1991; Bashan and Holguin, 1997). Biofertilizer can be used with higher doses of chemical nitrogen in integrated nutrient supply system (Dilip et al., 2003). With this aim an experiment was designed to

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Abbreviations: LB, Luria-bertani; **PGPB,** plant growth promoting bacteria; **PGPR,** plant growth promoting rhizobacteria.

Table 1. Effect of plant growth promoting bacteria (PGPB) and nitrogen on shoot and root growth of rice as affected by nitrogen fertilizer.

Treatment	Plant height (cm)	Shoot dry weight (g/plant)	Root length (cm)	Root dry wt. (g/plant)	Panicle length (cm)
PGPB					
PG_0	94.77b	12.50b	32.57b	3.02	24.64b
BM11	98.24a	13.94a	35.18a	3.43	25.69a
BM9	98.49a	13.91a	35.36a	3.46	25.65a
Sig. level	*	**	**	**	*
Nitrogen					
N_0	89.96d	9.43e	28.82d	2.51d	22.92d
N_{20}	93.37cd	11.11d	31.97c	2.63d	23.46cd
N_{40}	97.00bc	12.71c	34.34b	2.99c	24.93bc
N_{60}	98.00b	14.49b	35.89ab	3.42b	26.03ab
N_{80}	101.34ab	16.35a	37.72a	3.99a	27.01a
N_{100}	103.33a	16.60a	37.43a	4.04a	27.59a
Sig. level	**	**	**	**	**
% CV	**4.75**	**3.70**	**7.63**	**8.09**	**5.09**

In a column, same letter (s) do not differ significantly at 1% (**) and 5% (*) level of probability as per DMRT, ns = non significant.

evaluate some *Azospirillum* strains in combination with graded levels of nitrogen to find out suitable combination of biofertilizer and chemical nitrogen in cultivation of rice.

MATERIALS AND METHODS

A field experiment was conducted at Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh in Aman season of 2006 to evaluate the efficiency of two *Azospirillum* strains for production of biofertilizer in rice cultivation. Soil used was silt loam in texture containing organic matter 1.06%, pH 6.6, Nitrogen 0.06%, P 13ppm and K 40 meq/100 g soil. Biofertilizer treatments comprised of *Azospirillum* strains BM9, BM11 and uninoculated control. Chemical nitrogen was used at the rate of 0, 20, 40, 60, 80 and 100% recommended nitrogen dose. *Azospirillum* cultures were prepared through inoculation of cultures in Luria-Bertani (LB) broth medium. Roots of twenty five days old rice seedlings (Binadhan 4) were dipped in *Azospirillum* broth for half an hour and seedlings were transplanted to the field. The experiment was laid out in split plot design. Biofertilizer treatments were placed in sub plots and chemical nitrogen levels in main plots with three replications. The rice plants were cut down at ground level at ripening stage and data on growth, yield and yield contributing characters were recorded. Grain and straw samples were analyzed chemically for estimation of N (Bremner and Mulvaney, 1982), P (Olsen and Sommer, 1982) and K (Knudsen et al., 1982). The data were statistically analyzed using the method of Gomez and Gomez (1984).

RESULTS

Shoot and root growth

Biofertilizer application increased plant height, shoot dry weight, root length, root dry weight and panicle length significantly over uninoculated control. Both strains showed significantly higher plant growth parameters whereas BM9 resulted the highest plant height (98.49 cm), root length (35.36 cm) and root dry weight (3.46) g/plant) (Tables 1 and 2) . The highest panicle length and shoot dry weight were recorded due to inoculation of BM11. Nitrogen application showed significant increase in all the growth parameters that is, plant weight, root length, root dry weight and panicle length. The 100% recommended nitrogen application gave the highest results in plant growth parameters except root length. A non significant interactive effect of biofertilizer and chemical nitrogen was observed on plant height, root length, root dry weight and panicle length. Interaction effect was significant among biofertilizer and chemical nitrogen on shoot dry weight of rice.

Yield components

Azospirillum inoculation showed better results in yield contributing characters of rice like effective tiller/hill, grains/panicle and 1000 grain weight (Tables 3 and 4). Statistically higher number of effective tillers was produced in inoculated treatments over uninoculated control. Strain BM11 recorded the highest number of effective tillers/hill (10.01) followed by BM9 (9.84). Treatment 100% N level exhibited the highest effective tiller (11.51/hill) and decreased gradually toward lower nitrogen rate. Grains/panicle was increased considerably due to inoculation. BM11 resulted in the highest number of grains/panicle (128.64). Nitrogen showed treated plots showed significantly higher grains/panicle. The 1000

Table 2. Interaction effect of plant growth promoting bacteria (PGPB) and nitrogen fertilizer on shoot and root growth of rice.

Treatment	Plant height (cm)	Shoot dry weight (g/plant)	Root length (cm)	Root dry wt. (g/plant)	Panicle length (cm)
PGPB×N					
N^0 PG_0	86.73	8.15g	26.23	2.45	22.17
N^o BM11	91.33	9.99f	29.70	2.62	23.27
N^o BM9	91.80	10.15f	30.53	2.45	23.33
N_{20} PG_0	90.40	10.22f	29.70	2.31	22.31
N_{20} BM11	94.43	11.49e	33.54	2.80	24.10
N_{20} BM9	95.27	11.62e	32.67	2.76	23.97
N_{40} PG_0	95.07	11.37e	32.23	2.64	24.00
N_{40} BM11	97.27	13.70d	35.10	2.89	25.37
N_{40} BM9	98.67	13.06d	35.70	3.43	25.43
N_{60} PG_0	96.53	13.39d	34.27	3.31	25.53
N_{60} BM11	99.27	14.94c	36.50	3.85	26.43
N_{60} BM9	98.20	15.15c	36.90	3.76	26. 13
N_{80} PG_0	96.90	15.55bc	36.47	3.63	26.43
N_{80} BM11	103.60	16.42ab	37.93	4.11	27.33
N_{80} BM9	103.53	17.08a	38.77	4.23	27.27
N_{100} PG_0	103.00	16.30ab	36.50	3.77	27.40
N_{100} M11	103.53	17.12a	38.23	4.29	27.63
N_{100} BM9	103.47	16.38ab	37.57	4.15	27.73
Sig. level	ns	*	ns	ns	ns
% CV	4.75	3.70	7.63	8.09	5.09

ns = non significant.

Table 3. Effect of plant growth promoting bacteria (PGPB) and nitrogen on yield and yield contributing characters of rice.

Treatment	Effective tiller/hill (No.)	Grains/panicle (No.)	1000 grain wt. (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
PGPB					
PG_0	9.16b	122.99	24.12	3.80b	7.21b
BM11	10.01a	128.26	24.93	4.32a	8.05a
BM9	9.84ab	128.64	24.79	4.28a	7.90a
Sig. level	*	ns	ns	**	**
Nitrogen					
N_0	7.43c	108.08d	25.13	2.90e	5.48e
N_{20}	8.43bc	116.48cd	24.81	3.25d	6.58d
N_{40}	9.55ab	125.09bc	24.42	3.92c	7.43c
N_{60}	9.81ab	131.48ab	24.20	4.43b	8.36b
N_{80}	11.27a	137.89ab	24.53	5.09a	9.18a
N_{100}	11.51a	140.42a	24.59	5.21a	9.28a
Sig. level	**	**	ns	**	**
%CV	8.03	8.97	7.25	5.73	5.96

In a column, same letter (s) do not differ significantly at 1% (**) and 5% (*) level of probability as per DMRT, ns = non significant.

grain weight was considerably affected by inoculation. A 2.78 to3.36% increment was observed due to inoculation of *Azospirillum* culture. Interactive effect of biofertilizer and chemical nitrogen showed non-significant in all the yield contributing characters, that is effective tillers/hill, grains/panicle and 1000 grain weight of rice.

Table 4. Interaction effect of plant growth promoting bacteria (PGPB) and nitrogen fertilizer on yield and yield contributing characters of rice.

Treatment	Effective tiller/hill (No.)	Grains/panicle (No.)	1000 grain wt. (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
PGPB×N					
N_0 PG_0	6.88	104.30	24.58	2.49f	4.92e
N_0 BM11	7.63	110.87	25.05	3.05def	5.91d
N_0 BM9	7.77	109.07	25.77	3.14def	5.61de
N_{20} PG_0	7.87	111.53	24.62	2.83ef	6.06d
N_{20} BM11	8.73	118.67	25.22	3.50cde	7.27c
N_{20} BM9	8.70	119.23	24.60	3.41c-f	6.43d
N_{40} PG_0	8.86	120.53	23.87	3.77b-e	7.31c
N_{40} BM11	9.63	127.27	25.03	4.18bc	7.44c
N_{40} BM9	10.16	127.47	24.35	3.82bc	7.54c
N_{60} PG_0	9.13	127 .60	24.25	3.88bcd	7.30c
N_{60} BM11	10.87	133.46	24.13	4.69ab	8.80ab
N_{60} BM9	9. 43	134.47	24.22	4.72ab	8.97ab
N_{80} PG_0	10.88	13 4.13	23.82	4.63ab	8.34b
N_{80} BM11	11.53	139.27	25.28	5.30a	9.64a
N_{80} BM9	11.40	140.27	24.52	5.35a	9.56a
N_{100} PG_0	11.33	139.87	23.56	5.24a	9.32a
N_{100} M11	11.63	140.07	24.92	5.20a	9.26a
N_{100} BM9	11.56	141.33	25.28	5.18a	9.27a
Sig. level	ns	ns	ns	*	**
% CV	**8.03**	**8.97**	6.9	**5.73**	**5.96**

In a column, same letter (s) do not differ significantly at 1% (**) and 5% (*) level of probability as per DMRT, ns = non significant.

Grain and straw yield

Grain and straw yield of rice were increased significantly due to inoculation of biofertilizer. Both the strains produced statistically higher grain over uninoculated. BM 11 showed higher grain and straw yield (4.32 and 8.05 t/ha, respectively) over BM9 (4.28 and 7.9 t/ha, respectively) (Table 2). Treatment where 100% of recommend nitrogen was applied resulted the highest grain and straw yield (5.21 and 9.28 t/ha, respectively) among the nitrogen treatments. Chemical nitrogen and bifertilizer showed significant interaction effect on grain and straw yield of rice. An 80% nitrogen with biofertilizer inoculation recorded statistically equal grain and straw yield with that of 100% chemical nitrogen alone applied. Eighty percent nitrogen + BM9 exhibited the highest grain yield (5.35 t/ha) whereas the highest straw yield (9.64 t/ha) with BM11 at same N rate was observed.

Nutrient uptake

Biofertilizer influenced N, P and K uptake in grain and straw of rice significantly. Both the *Azospirllum* strains showed significantly higher nitrogen, phosphorus and potassium uptake in grain and straw of rice (Tables 5 and 6). BM11 explored the highest N uptake in both grain (56.54 kg/ha) and straw (24.72 kg/ha) and P and K in straw (4.15 and 66.1kg/ha, respectively) where BM9 showed the highest P and K uptake in grain (13.08 and 11.68 kg/ha, respectively). The 100% nitrogen (level) application exhibited the highest amount of N, P and K uptake in grain and straw except P in grain. Interaction effect of biofertilizer and chemical nitrogen was observed significant on N, P and K uptake in grain and straw of rice. BM11 along with 80% N accumulated the highest amount of N in grain and straw (69.39 and 28.93 kg/ha, respectively) where the highest P and K were accumulated in grain (16.58 and 16.56 kg/ha, respectively) and straw (4.88 and 80.28 kg/ha, respectively) by strain BM9 along with 80% N application. Higher N, P and K uptake was observed by biofertilizer applied treatments over uninoculated at all the nitrogen levels except N_{100}-

DISCUSSION

Biofertilizer increased plant height, root length, root dry weight and panicle length due to *Azospirillum* inoculation through N fixation, growth promoting hormone secretion and mineral nutrient uptake by more elongation of root, uptake of nutrient and water was done as a result plant height and panicle length were increased. Information on secretion of growth promoting substances and nitrogen

Table 5. Effect of plant growth promoting bacteria (PGPB) and nitrogen fertilizer on N, P and K uptake of rice.

Treatment	N uptake (kg ha^{-1})		P uptake (kg ha^{-1})		K uptake (kg ha^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw
PGPB						
PG_0	49.68b	21.84b	11.15b	3.63b	9.84b	59.24b
BM11	56.54a	24.72a	12.91a	4.15a	11.52a	66.10a
BM9	55.55a	24.62a	13.08a	4.07a	11.68a	65.70a
Sig. level	**	**	**	**	**	**
Nitrogen						
N_0	38.13e	17.92d	8.90e	2.88e	7.67d	43.82e
N_{20}	42.66d	20.63c	9.76d	3.43d	7.89d	54.22d
N_{40}	51.41c	22.79b	11.91c	3.77c	9.94c	62.24c
N_{60}	58.52b	26.20a	13.16b	4.33b	10.79b	69.40b
N_{80}	66.35a	27.26a	15.30a	4.54ab	14.52a	75.63a
N_{100}	66.46a	27.56a	15.07a	4.74a	15.27a	76.76a
Sig. level	**	**	**	**	**	**
%CV	5.67	5.98	5.69	5.95	5.88	5.79

In a column, same letter(s) do not differ significantly at 1 and 5% level of probability as per DMRT.

Table 6. Interaction effect of plant growth promoting bacteria (PGPB) and nitrogen fertilizer on N, P and K uptake of rice.

Treatment	N uptake (kg ha^{-1})		P uptake (kg ha^{-1})		K uptake (kg ha^{-1})	
PGPB×N	**Grain**	**Straw**	**Grain**	**Straw**	**Grain**	**Straw**
N_0 PG_0	32.96i	15.73e	7.49j	2.61f	6.49i	39.39f
N_0 BM11	40.31gh	19.49d	9.46hi	3.13def	8.03ghi	46.07ef
N_0 BM9	41.14gh	18.51d	9.74ghi	2.92ef	8.48fgh	46.00ef
N_{20} PG_0	37.11hi	18.78d	8.22ij	3.15def	7.09hi	49.06e
N_{20} BM11	45.89efg	22.54bc	10.51gh	3.85bc	8.41fgh	59.61cd
N_{20} BM9	44.97fg	20.56cd	10.56gh	3.28cde	8.18fgh	53.98cd
N_{40} PG_0	49.05ef	22.65bc	11.32fg	3.66bcd	9.81c-f	63.10cd
N_{40} BM11	55.13d	22.33bc	12.95ef	3.80bc	10.44b-e	61.04cd
N_{40} BM9	50.04def	23.38b	11.46fg	3.84bc	9.56d-g	62.59cd
N_{60} PG_0	51.22de	22.64bc	11.25 fg	3.66bcd	9.32efg	59.89cd
N_{60} BM11	61.43bc	28.15a	13.59 cde	4.57a	11.25bc	73.89ab
N_{60} BM9	62.93bc	27.80a	14.62 b-e	4.75a	11.80b	74.42ab
N_{80} PG_0	60.65c	24.18b	13.43 de	4.03b	11.11bc	67.52bc
N_{80} BM11	69.39a	28.93a	15.89ab	4.73a	15.89a	79.07a
N_{80} BM9	69.02a	28.68a	16.58 a	4.88a	16.56a	80.28a
N_{100} PG_0	67.07ab	27.04a	15.19 abc	4.66a	15.19a	76.45ab
N_{100} M11	67.08ab	26.87a	15.08 a-d	4.82a	15.08a	76.88a
N_{100} BM9	65.23abc	28.77a	15.53 ab	4.73a	15.53a	76.94a
Sig. level	*	*	**	**	**	**
% CV	5.67	5.98	5.69	5.95	5.88	5.79

In a column, same letter (s) do not differ significantly at 1% (**) and 5% (*) level of probability as per DMRT.

fixation by plant growth promoting bacteria in hands (Islam et al., 2005; Hafeez et al., 2006). Similar results were described in cereals and tomato seedlings where *Azospirillum* inoculation enhanced root growth (Okon, 1985; Hadas and Okon, 1987). Dobbelarere et al. (1999) reported that secretion of plant growth promoting substances such as auxins, gibberellins and cytokines by the bacteria seem to be responsible for these effect.

Growth promoting effects of biofertilizer inoculation are mainly derived from morphological and physiological changes in inoculated sorghum roots and enhancement in water and plant nutrient uptake was described by Sarig et al. (1988).

Effective tillers/hill, grains/panicle and 1000 grain weight of rice were enhanced due to plant growth promoting bacterial inoculation. Increment of yield components through *Azospirillum* inoculation was stated by several workers (Mathews et al., 2006; Reddy and Kumar, 2006). *Azospirillum* biofertilizer increased grain and straw yield of rice significantly due to combined effect of nitrogen fixation, growth promoting substances secretion and nutrient uptake. It was reported that significant increment of rice yield through *Azospirillum* inoculation (Ashrafuzzaman et al., 2009; Govindan and Varma, 2004; Subashini et al., 2007). They reported that 80% recommended nitrogen application along with *Azospirillum* inoculation resulted statistically similar grain and straw yield of rice through 100% N applied with inoculation. Similar results were also described by Govindan and Varma, 2004. Biofertilizer application showed significantly higher N, P and K uptake in grain and straw of rice. This higher nutrient uptake was occurred due to the activity of *Azospirillum* inoculants along with nitrogen fertilizer application which was reported by Lucy et al. (2004) where they reported that increased uptake of nutrient such as N, P and K which considered as one of the mechanisms by which PGPR increased crop yield.

Conclusion

Results of the present study indicated that *Azospirillum* biofertilier can be used in combination with 80% chemical nitrogen for achieving greater crop yield and supplementing chemical nitrogen in rice cultivation.

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