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Effect of diazotrophs on the mineralization of organic nitrogen in the rhizosphere soils of rice (*Oryza sativa*)

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ABSTRACT

A field experiment was conducted during 2002 to investigate the effect of inoculation of 2 non-symbiotic N_2-fixing bacteria, *Azotobacter* (strain AS_8) and *Azospirillum* (strain AM_1), in presence of 50 kg N ha^{-1} and 5 t FYM ha^{-1} on the performances of the diazotrophs with respect to transformation of organic nitrogen in the rhizosphere soils of rice (*Oryza sativa* L, cv. PNR-381). Inoculation of the diazotrophs substantially increased the content and availability of different fractions of organic (hydrolysable and non-hydrolysable) nitrogen in the rhizosphere soils resulting in greater yield of the crop. In general, combined inoculation of the diazotrophs was most stimulative in augmenting the nitrogen status of the rhizosphere soils, which was comparable to the effects under the uninoculated soils receiving 100 kg N ha^{-1} as urea-N. Between the two organisms, *Azotobacter* was more effective than *Azospirillum* in relation to accumulation and transformation of different fractions of organic nitrogen content in the rhizosphere soils. All the fractions of hydrolysable organic nitrogen (hydolysable NH_4^+, hexosamine, amino acid and unidentified hydrolysable organic-N) decreased with a concomitant increase in non-hydrolysable fraction with the age of the crop.

***Key words* :** Amino acid-N, diazotrophic inoculation, hexosamine-N, hydrolysable NH_4^+, nitrogen uptake, organic-N, rhizosphere soil, rice yield, unidentified organic-N.

Biological nitrogen fixation by heterotrophic microorganisms plays an important role in nitrogen economy of rice (*Oryza sativa* L) soils. Besides blue-green algae (Ghosh and Saha, 1997), high potential nitrogen fixation by free-living *Azotobacter* and root associative microaerophilic *Azospirillum* in rhizosphere soil of rice has been well documented (Das and Saha, 2000). Ammonia is an inhibitor of nitrogenase enzyme, but incorporation of small amounts of inorganic nitrogen does not inhibit N_2-fixation by non-symbiotic N_2-fixing bacteria (Kanungo et al., 1998). Rather it enhances their population, resulting in a greater fixation of atmospheric nitrogen in soil (Vendan and Sundaram, 1997). In addition, association of N_2-fixing bacteria also improves nitrogen transformation and contributes a significant amount of growth-promoting substances to the standing crop (Rao et al., 1998) resulting in greater grain yield (Das and Saha, 2003). However, limited information is available on enhancement of growth of cereals under field condition. Therefore, an experiment was conducted to find out the effect of inoculation of heterotrophic non-symbiotic N_2-fixing fixing bacteria, viz *Azotobacter* and *Azospirillum*, either alone or in combination, in presence of partial application of inorganic nitrogen, on content of different fractions of organic nitrogen in rhizosphere soils of rice in relation to yield of crop.

MATERIALS AND METHODS

A field experiment was conducted during 2002 in microplots (7 m by 7 m) following a randomized block design (RBD) in the experimental farm of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, India. The soil was classified as Typic Fluvaquent having the general characteristics: textural class, clay loam; density, 1.08 g cm^{3-1}; water holding capacity, 64.2%; pH (1:2.5 w/v) in water, 7.4; CEC, 15.0 cmol (p^+) kg^{-1}; EC, 0.35 dS m^{-1}; organic C, 7.18 g kg^{-1}; total N, 0.75 g kg^{-1}; C:N ratio, 9.6; available N, 84.6 mg kg^{-1}; hydrolysable organic N, 443.3 mg kg^{-1}; non-hydrolysable organic N, 220.4 mg kg^{-1}; available P, 12.3 mg kg^{-1}. Farmyard manure (FYM) at the rate of 5 t ha^{-1} and fertilizers consisting 25 kg ha^{-1} urea-N, 22 kg ha^{-1} single superphosphate-P and 42 kg ha^{-1} potassium chloride-K were mixed thoroughly with the soil during land preparation. Thirty-day old rice seedlings (*Oryza sativa* L, variety PNR-381) previously raised in a seedbed receiving 25 t ha^{-1} FYM, 60 kg ha^{-1} urea-N, 13 kg ha^{-1} single superphosphate-P and 25 kg ha^{-1} potassium chloride-K, were uprooted and the soil adhering to the roots was washed off carefully in clean water. The seedlings were inoculated with 2 efficient strains of *Azotobacter* (strain AS_8) and *Azospirillum* (strain AM_1), either alone or in combination, previously isolated from roots of same rice variety (Das and Saha, 2003), by dipping rice roots in bacterial suspensions containing viable cell numbers 10.4×10^9 ml^{-1} for 1 hr, followed by air drying in shade for 30 min. Seedlings inoculated with bacterial cultures were then transplanted separately at 4 seedlings/hill, with a spacing of 15 cm × 20 cm between hill and row. There was an uninoculated control. Thirty days after transplanting, another 25 kg ha^{-1} urea-N was applied as a top dressing to all the plots. Thus nitrogen was applied at 50 kg ha^{-1}, which was 50% of recommended field rates for the crop. There was also an uninoculated treatment receiving 100 kg ha^{-1} urea-N but no FYM. All the treatments were replicated 3 times. The crop was cultivated following usual cultural practices.

Rhizosphere soil samples were collected from each plot at maximum tillering [35 days after transplanting (DAT)], flowering (70 DAT), and maturity (105 DAT) stages of the crop growth, by uprooting plants carefully as outlined by Das and Mukherjee (2000). After the pieces of plant roots had been removed, rhizosphere soils from replicated plots of each treatment were analyzed immediately to estimate hydrolysable and non-hydrolysable fractions of organic nitrogen following the method of Stevenson (1996). The crop was harvested at the age of 115 days, and grain and straw yields were measured. The harvested plants were then analyzed to estimate total nitrogen content (Bremner, 1996) to determine N-uptake by the rice plants. Results were evaluated by 2-way analysis of variance and the statistical significance of effects within the factors (diazotrophs and sampling days) and their interactions were evaluated.

RESULTS AND DISCUSSION

Effect on organic nitrogen

The uninoculated soil receiving 100 kg N ha^{-1} significantly accentuated the accumulation of hydrolysable organic nitrogen in the rhizosphere soils of rice (Figs. 1-2). Similar trend was recorded with the inoculated soils receiving FYM and partial amount of fertilizer-N. This pointed out that induced diazotrophs fixed higher amount of dinitrogen to be released from the cells together with growth promoting substances (Arshad and Frankenberger, 1998). These substances contained high amount of hydrolysable organic nitrogen. Moreover, the higher availability of plant nutrients as induced by enhanced microbial activities stimulated the growth of rice plants which released a significant amount of root exudates containing greater amount of hydrolysable organic nitrogen in the rhizosphere soils (Das and Saha, 2004). The decrease in the accumulation of different fractions of hydrolysable organic nitrogen in the rhizosphere soils with the age of the crop was due to its conversion to non-hydrolysable form (Das and Saha, 2003) together with its rapid meneralization. This was evidenced by the existence of linear relationship between different fractions of organic nitrogen in soil (Table 2). In general, the accumulation of hydrolysable NH_4^+-N and amino acid-N were comparatively higher than that of hydrolysable hexosamine-N (Fig. 1) in the rhizosphere soils of rice. This was also in agreement with the earlier reports (Mukhopadhyay et al., 1985). In the present study inoculation with *Azotobacter* and *Azospirillum* in combination mineralized 41.2, 36.8 and 30.3% hydrolysable NH_4^+-N, hexosamine-N and amino acid-N, respectively during the period of crop growth. The corresponding values for single inoculation of *Azotobacter* and *Azospirillum* were 50.2, 40.5, 32% and 51.4, 38.7, 38.6%, respectively. Thus combined inoculation of *Azotobacter* and *Azospirillum* mineralized lesser amounts of hydrolysable organic nitrogen and resulted in greater accumulation of hydrolysable organic nitrogen in soil than single inoculations. A significant amount of hydrolysable organic nitrogen could not be identified (Fig. 2A) and the accumulation of unidentified hydrolysable organic nitrogen in general, was decreased up to the maturity of the crop. Among the inoculated soils, combined inoculation of *Azotobacter* and *Azospirillum* accounted highest amount of unidentified hydrolysable organic nitrogen followed by the soils inoculated with *Azotobacter* and *Azospirillum*, respectively.

Amongst the different treatments, the uninoculated soils receiving 100 kg N ha^{-1} accounted highest amount of total hydrolysable organic nitrogen (Fig. 2B) followed by the combined inoculated soils. The single inoculation of the diazotrophs, on the other hand, not only increased the mineralization of hydrolysable organic nitrogen, but also significantly increased their accumulations in the rhizosphere soils. Inoculation of diazotrophs also significantly stimulated the content of non-hydrolysable organic nitrogen in the rhizosphere soils of rice (Fig. 2C). The results also showed that the increased accumulation of non-hydrolysable organic nitrogen in the rhizosphere soils was concomitant with the decrease in total hydrolysable fraction (Fig. 2B) with the age of the crop. The decrease in the accumulation of hydrolysable organic nitrogen in the rhizosphere as the crop reached maturity was due to its conversion to non-hydrolysable form (Saha and Mukhopadhyay, 1986; Ghosh et al., 1990), together with its rapid mineralization. Among the inoculated treatments, the highest amount of non-hydrolysable organic nitrogen was recorded uniformly in the rhizosphere soils under the combined inoculation of the diazotrophs followed by the uninoculated soils receiving full dose (100 kg ha^{-1}) of fertilizer-N.

Effect on yield of the crop

Inoculation of *Azotobacter* and *Azospirillum*, either alone or in combination highly stimulated the availability of different fractions of organic nitrogen in the rhizosphere soils of rice which resulted in an increased utilization of available nitrogen by the rice plants for their growth and development, leading to higher yield of the crop (Table 1). The results also showed that combined inoculation of the diazotrophs performed better than their single inoculation. Similar response on N-uptake, plant growth and yield of rice due to inoculation of *Azotobacter* and/or *Azospirillum*, in the presence of low applications of inorganic nitrogen, were also reported by Vendan and Sundaram (1997). Inoculation of *Azotobacter* and *Azospirillum* in presence of partial application of fertilizer-N and FYM yielded less grain, by 12.7, and 18.3%, and less straw, by 10.6 and 17.1%, respectively, while the combined inoculation of the diazotrophs increased grain and straw yields by 4.5 and 5.8%, respectively than the optimum yields recorded in the soil treated with 100 kg N ha^{-1} only.

Table 1 : Effect of inoculation with non-symbiotic N_2-fixing bacteria on yield of rice and nitrogen uptake by the rice plants (means ± s.e.)

Treatments	Grain yield (kg $plot^{-1}$)	Straw yield (kg $plot^{-1}$)	N uptake (mg $hill^{-1}$)
N_{50} + FYM (uninoculated control)	8.4 ± 1.2	20.1 ± 1.4	407.2 ± 11.8
N_{50} + FYM + Azotobacter (AS_8)	11.0 ± 1.1	26.2 ± 1.4	448.4 ± 10.8
N_{50} + FYM + Azospirillum (AM_1)	10.3 ± 1.3	24.3 ± 1.5	439.9 ± 13.2
N_{50} + FYM + (AS_8) + (AM_1)	13.2 ± 1.1	31.1 ± 2.1	495.9 ± 12.3
N_{100} (uninoculated)	12.6 ± 1.3	29.3 ± 1.9	471.4 ± 14.9
Mean	11.1	26.2	452.6
LSD (P = 0.05)	**0.2**	**0.6**	**19.6**
(P = 0.01)	**0.3**	**0.9**	**28.5**

N_{50} and N_{100} denote fertilizer-N applied at 50 and 100 kg ha^{-1} respectively as urea; FYM applied at 5 t ha^{-1}; AS_8 and AM_1 indicate *Azotobacter* and *Azospirillum* strains used as inocula

Table 2 : Linear relationships (r values) between different fractions of soil organic nitrogen, nitrogen uptake and yield of rice influenced by diazotrophic inoculation

PRM	HEA	AMA	UIH	TOH	TNH	NUP	GRY	STY
HYA	0.980**	0.947*	0.956*	0.977**	0.978**	0.975**	0.990**	0.993**
HEA	-	0.967**	0.977**	0.976**	0.995**	0.980**	0.986**	0.988**
AMA	-	-	0.972**	0.977**	0.993**	0.930*	0.954*	0.961**
UIH	-	-	-	0.992**	0.972**	0.917*	0.951*	0.950*
TOH	-	-	-	-	0.977**	0.931*	0.970**	0.960**
TNH	-	-	-	-	-	0.979**	0.987**	0.991**
NUP	-	-	-	-	-	-	0.988**	0.991**
GRY	-	-	-	-	-	-	-	0.999**

*, ** Indicate significant 'r' values at P = 0.05 and 0.01 levels of significance, respectively

PRM = Parameters, HYA = Hydrolysable NH_4^+, HEA = Hexosamine N, AMA = Amino acid N, UIH = Unidentified hydrolysable organic N, TOH = Total hydrolysable organic N, TNH = Total non-hydrolysable organic N, NUP = N-uptake, GRY = Grain yield, STY = Straw yield

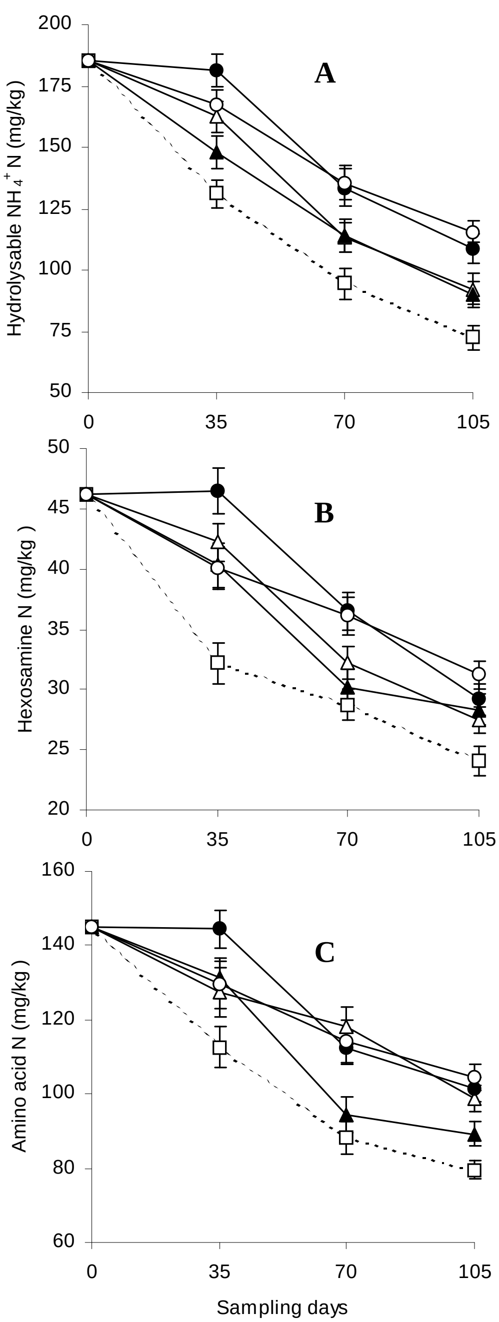

Fig. 1 : Effect of inoculation of diazotrophs on status of different fractions of organic nitrogen in the rhizosphere soils of rice. Treatments: □, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) (uninoculated control); ○, Fertilizer-N (100 kg ha^{-1}) (uninoculated); △, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) + *Azotobacter* (AS_8); ▲, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) + *Azospirillum* (AM_1); ●, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) + (AS_8) + (AM_1). Values are means ± s.e

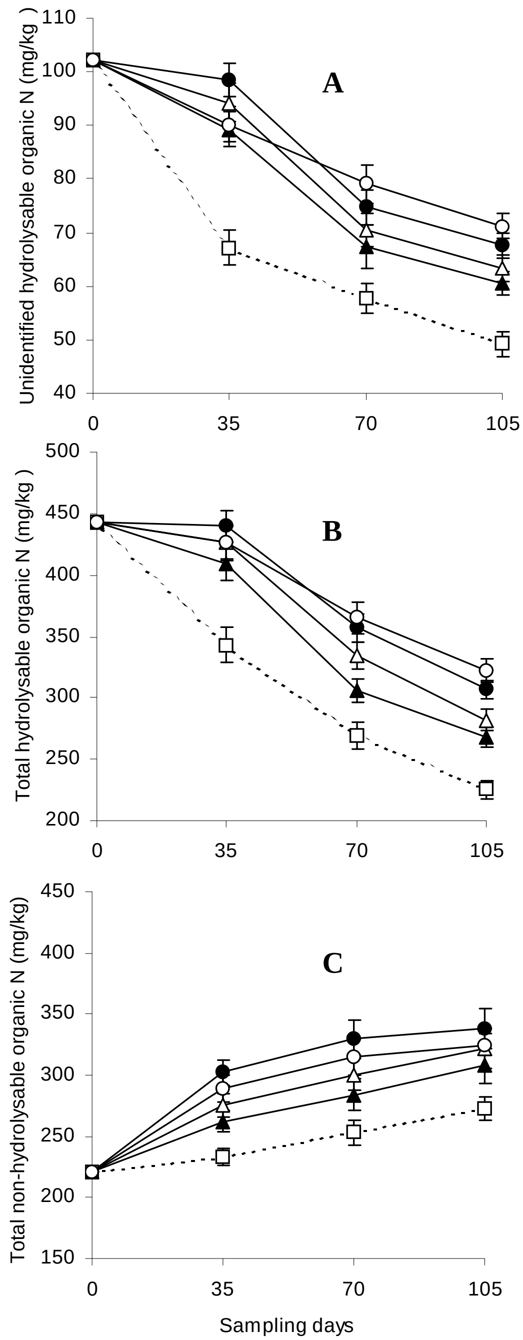

Fig. 2 : Effect of inoculation of diazotrophs on status of unidentified hydrolysable, total hydrolysable and total non-hydrolysable organic nitogen in the rhgizosphere soils of rice. Treatments: □, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) (uninoculated control); ○, Fertilizer-N (100 kg ha^{-1}) (uninoculated); △, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) + *Azotobacter* (AS_8); ▲, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) + *Azospirillum* (AM_1); ●, Fertilizer-N (50 kg ha^{-1}) + FYM (5 t ha^{-1}) + (AS_8) + (AM_1). Values are means ± s.e.

The results of the present investigation clearly indicated that inoculation of *Azotobacter* and *Azospirillum*, either alone or in combination, and in the presence of a partial application of fertilizer-N and FYM, stimulated the availability of organic nitrogen in the rhizosphere soil, resulting in greater uptake of nitrogen by the standing crop and subsequently the yield of the crop was increased, which was comparable to yields under full dose (100 kg N kg^{-1}) of fertilizer-N. The results also indicated that combined inoculation of the diazotrophs was more stimulative than their single inoculation in stimulating the activities of the diazotrophs as well as improving the nitrogen status of the rhizosphere soils beneficial to the crop as a whole. Of the two organisms, *Azotobacter* was more effective than *Azospirillum*.

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