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SHORT COMMUNICATION

SCREENING OF RICE CULTIVARS AND DIAZOTROPHS COMBINATION FOR BETTER N_2-FIXING SYSTEM

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In a pot culture experiment three rice cultivars were inoculated with eight different N_2-fixing bacterial strains with the objective to find out effective nitrogen fixer and rice cultivar combination for better nitrogen nutrition. Dry weight of shoot and N-accumulation data showed that effect of diazotrophs was more in IR 50 than the other two varieties, MW 10 and CR 544-1-1. Among the 'variety x diazotroph' combinations, *Azospirillum* (Ap18) appeared to be the best followed by *Pseudomonas* (P12) and *Azotobacter* (A7) when inoculated to rice variety IR-50. In this study some isolates (D2, P3 and P4) failed to show any encouraging result although they had better acetylene reduction activity (ARA) under pure culture conditions.

Key words **: Diazotrophs, N-uptake, plant biomass, rice.**

It has long been reported that most of the bacteria (80%) found in rice roots and rhizosphere are N_2-fixing bacteria (Watanabe and Barraquio 1979) but all of these are not effective N_2-fixers and do not colonize roots effectively (Nayak *et al.* 1986). Rice germplasms having different genetic background also govern the quality and quantity of root exudates and types of root system, which ultimately regulate the colonization of diazotrophs and their efficiency. This 'genotype x diazotroph' interaction is again influenced by the environmental factors specially the edaphic factors. Because of these complex phenomena inoculation of diazotroph to a particular rice variety do not always perform well. Hence, selection of better rice 'variety x diazotroph' combination is an important aspect to make an inoculation program successful. Thomas-Bauzon *et al*. (1982) introduced a new approach to select 'rice cultivar x diazotroph' combination for better N_2-fixation using axenic rice seedling in pankurst tube inoculated with diazotrophs without providing carbon and nitrogen from outside. This approach can not be used always (Charyulu *et al.* 1985) probably due to some edaphic factors which were not considered during selection of efficient 'rice x diazotroph' system. It is therefore, logical to select rice cultivars and N_2-fixing bacteria in an unsterilized soil environmental condition.

Sandy loam soil (pH, 6.2; Organic C, 10.5 g kg^{-1}; total N, 0.87 g kg^{-1}; available P, 12.5 mg kg^{-1}; CEC, 7.46 cmol kg^{-1}) was collected from University farm, Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal for pot experiment. Three rice cultivars; IR 50, CR 544-1-1 and MW 10 were used for the experiment. Soil was passed through a 2 mm sieve and mixed to homogeneity. Each of the earthen pot (28 cm diameter and 25 cm height) lined with polyethylene sheet was filled with 8.0 kg soil. Each pot was fertilized with N, P_2O_5, K_2O and ammonium molybdate @ 20 kg, 40 kg, 40 kg, and 0.2 kg ha^{-1}, respectively. Manuring was done @ 5000 kg FYM ha^{-1} as a source of organic carbon to all the treatments in addition to fertilizer. Nitrogen was applied two weeks after transplantation. Nitrogen fixing isolates, A6, A7, Ap3, Ap18, D2, P3, P4 and P12 were used for inoculation. All the eight strains were isolated from the wetland paddy field of Terai zone of West Bengal. Among the

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diazotrophs, A6, A7 and P12 were identified as *Azotobacter beijerinckia, Azotobacter chroococcum* and *Pseudomonas*, respectively. Ap3 and Ap18 were *Azospirlillum*, strains (Choudhury, 2000).

All the diazotrophs were grown separately in 500 ml flasks containing 200 ml Rennie's Combined Carbon broth (Rennie, 1981). After incubation the bacterial cells were centrifuged at 8000 rpm for 10 minutes and finally suspended in the same Combined Carbon medium at a concentration of approximately 10^8 cells ml^{-1}. Three rice cultivars were inoculated with eight diazotrophs and six replications were kept for each treatment. Roots of seedlings, freed from seedbed soil by washing with distilled water, were dipped into said bacterial suspension for 24 h before transplantation. Appropriate control was maintained by inoculating rice seedlings with heat-killed bacteria. Fifteen days after transplantation, 5.0 ml freshly prepared above bacterial suspension was again inoculated into each pot according to treatment. After 45 days of transplantation observations were recorded on dry weight of shoot and total N-uptake per pot. Total N was estimated by Kjeldahl method. Rhizosphere and rhizoplane samples at tillering stage were collected as described by Watanabe *et al*. (1979). Total aerobic nitrogen fixer, *Azotobacter*, *Azospirillum* and *Pseudomonas* like organisms were enumerated using combined carbon medium (Renni 1981), Jensen medium (Jensen 1951), semisolid malate yeast extract medium (Dobereiner 1980) and semisolid glucose yeast extract medium (Watanabe *et al*. 1979), respectively. *Azospirillum* and *Pseudomonas* like organisms were enumerated by MPN method with 10 fold dilution and 5 tubes per dilution (Alexander 1982).

Total biomass production per pot varied significantly with rice varieties and diazotrophs. The maximum effect was observed for isolate A7 and Ap18 (Table1). Among the varieties, effect of diazotrophs was more pronounced in IR 50 and inoculation could increase the dry mater production up to 55.0% over uninoculated control. Interaction effect showed that maximum benefit was derived when IR 50 was inoculated with Ap18 (12.7 g/pot) and A7 (12.68 g/pot). Significant variations in plant N-uptake were observed among varieties and N_2-fixers as well as their interactions. Considering the varietal averages, the ranking of N-accumulation induced by eight diazotrophs was in the decreasing order of Ap18 (107.7 mg N pot^{-1}), Ap3 (105.9 mg N pot^{-1}), P12 (91.3 mg N pot^{-1}) and A7 (85.4 mg N Pot^{-1}). Observation on percentage increase in N-uptake over control revealed

Table 1. Response of rice varieties inoculated with different diazotrophs on dry matter production.

Treatments	Shoot dry weights (g/pot)			
	CR 544-1-1	IR 50	MW 10	Mean
Control	8.95	8.20	7.00	8.05
A6	9.88 (10.39)	10.33 (25.97)	9.41 (34.42)	9.87
A7	9.41 (5.13)	12.68 (54.63)	8.66 (23.71)	10.25
Ap3	9.68 (8.15)	12.53 (52.80)	7.70 (10.00)	9.97
Ap18	10.08 (12.62)	12.71 (55.00)	7.48 (6.85)	10.09
D2	8.96 (0.11)	11.78 (43.66)	7.23 (3.28)	9.32
P3	7.63 (-14.75)	11.73 (43.04)	6.32 (-9.71)	8.56
P4	9.35 (4.47)	11.20 (36.58)	7.80 (11.42)	9.45
P12	9.71 (8.49)	10.93 (33.29)	8.00 (14.28)	9.55
Mean	9.30	11.34	7.73	-
	Variety	Diazotroph	Variety x Diazotroph	
$CD_{0.05}$	0.55	0.95	1.66	

Figure within parentheses indicate % increase over control

that the effect of inoculum was more pronounced in IR 50 followed by MW 10 and CR 544-1-1 (Table 2). Interaction between varieties and diazotrophs depicted that the best combination in respect of N-uptake was IR 50 with Ap18. In this study, differences in diazotrophic population were observed, both in rhizosphere and rhizoplane and variety IR 50 harboured the maximum dinitrogen fixing bacteria (Table 3). *Azotobacter* was found only in rhizosphere sample and MPN count of *Pseudomonas*-like organisms was more as compared to *Azospirillum*.

Table 2. N-uptake of different rice varieties inoculated with different diazotrophs.

Treatments	N uptake (mg N/pot)			
	CR 544-1-1	IR 50	MW 10	Mean
Control	69.15	88.15	60.70	72.66
A6	76.75 (11.0)	96.86 (9.88)	69.06 (13.77)	80.89
A7	72.70 (5.13)	102.88 (16.71)	80.60 (32.78)	85.39
Ap3	82.43 (19.20)	151.78 (72.18)	83.38 (37.36)	105.86
Ap18	79.78 (15.37)	159.20 (80.60)	84.23 (38.76)	107.73
D2	74.55 (7.80)	120.93 (37.18)	59.28 (-2.33)	84.92
P3	64.80 (-6.29)	88.86 (0.81)	66.05 (8.81)	73.23
P4	70.28 (1.63)	88.65 (0.57)	82.51 (35.93)	80.48
P12	83.00 (20.20)	123.45 (40.04)	67.41 (11.05)	91.28
Mean	74.83	113.44	72.58	-
	Variety	Diazotroph	Variety x Diazotroph	
$CD_{0.05}$	3.79	6.56	11.37	

Figure within parentheses indicate % increase over control

Table 3. Population of aerobic and microaerophilic diazotrophs in rhizosphere and on rhizoplane of three rice cultivars.

Variety	Putative diazotrophs on CCM ($x10^5$)	*Azotobacter* ($x10^2$)	MPN of *Azospirillum* ($x10^5$)	PMN of putative *Pseudomonas* ($x10^6$)
CR 544-1-1				
a	32.7	12.4	7.2	17.8
b	28.0	-	11.5	22.8
IR 50				
a	65.8	28.7	18.6	67.12
b	132.6	-	21.6	92.6
MW 10				
a	30.5	5.6	7.9	15.1
b	35.2	-	11.5	17.8

a – CFU per gram of dry rhizosphere sample
b - CFU per gram of dry rhizoplane sample
CCM – Combined carbon medium

Earlier report about the correlation of total N-uptake and plant biomass with biological N_2-fixation among different genotypes (Ladha *et al*. 1988) suggested to give more emphasis on N-uptake and plant biomass production to select effective diazotroph and rice cultivar. Mechanisms contributing to such differences in N-uptake include differences in (i) specific nutrient uptake rates (per unit root surface), (ii) modification of the rhizosphere by root metabolites, and (iii) exudation, including the stimulation of associative N_2 fixation in the rhizosphere (Ladha *et al*. 1998; Malarvizhi and Ladha 1999). In the concluding part of this experiment, it can be highlighted that IR-50 was the best variety with respect to plant biomass, N-uptake and stimulation of diazotrophic population in the rhizosphere. Irrespective of varieties studied, *Azospirillum* like organism has emerged out as the best inoculum for rice in this experiment. Among the 'Variety x Diazotroph' combinations, *Azospirillum* (Ap18), *Pseudomonas*, (P12) and *Azotobacter* (A7) appeared to be the best when inoculated to IR 50. In the present study, the isolated D2, P3 and P4 failed to produce any encouraging result although they had better ARA activities under cultural condition (Choudhury 2005). On the contrary, P18 showed better result in spite of having low ARA. Differences may be due to various degrees of establishment of inoculum in the rice rhizosphere. The better result of IR 50 can be explained with its higher root biomass as compared to CR 544-1-1 and MW 10, which may be attributed to derepression of nitrogenase enzyme through depletion of available nitrogen by vigorous root system. The above results provide justification for careful screening of diazotrophs for their use as inoculants for a specific crop cultivar.

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