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Nitrogen Fixation in the Rhizosphere of Rice

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The free-living nitrogen-fixing bacteria, *Azotobacter* and *Beijerinckia* were more in the rhizosphere of the two rice varieties, IR 8 and Bhavani than in the non-rhizosphere region. At boot-leaf and flowering stages, the population of these bacteria was very high. In general, IR 8 harboured more number of these bacteria than Bhavani. The anaerobic, nitrogen-fixing bacterium, *Clostridium pasteurianum* was more in the rhizosphere than in the non-rhizosphere soil. In IR 8, flowering stage had registered maximum clostridial population whereas in Bhavani it was at the tillering stage. The nitrogenase activity of the two varieties was maximum at the reproductive phase of the crop. Both rhizosphere and the rhizoplane recorded appreciable nitrogenase activity. The different rice varieties tested at the flowering stage showed quite a variation in the nitrogenase activity. In rhizosphere, non-rhizosphere and rhizoplane samples, the nitrogenase activity was more under illuminated conditions than under darkness, illustrating the role of photoautotrophs in nitrogen fixation.

Key Words: **N_2 Fixation, Free-living bacteria, Rhizosphere, Nitrogenase, Diazotrophs, Rhizoplane**

Introduction

The yield of rice depends chiefly on the nitrogen availability in soils. On an average, rice plants take 75 kg N/ha of soil nitrogen (Shiga & Ventura 1976). That rice field soils continue to give a consistent grain yield over many years without any additional application of fertilizer strongly suggests the fixation of atmospheric nitrogen and its subsequent mineralisation (Yoshida & Ancajas 1973). At present, we remain well-informed about the myriad groups of microorganisms that take part in dinitrogen fixation under rice field conditions (Balandreau et al. 1975, Purushothaman et al. 1976 and Yamaguchi 1976). The occurrence and the role of microorganisms like *Azotobacter*, *Beijerinckia*, *Clostridium*, *Derxia*, *Rhodopseudomonas capsulata*, *Rhodospirillum rubrum*, *Flavobacterium* and blue green algae in nitrogen fixation are increasingly realised (Kobayashi 1975). Rice-rhizosphere serves as a potential microbiological niche attracting not only the heterotrophic flora but also the nitrogen-fixing microorganisms

(Yoshida 1975). In the present investigation, the occurrence of a few nitrogen-fixing microorganisms in the rhizosphere of two rice varieties grown under field conditions and their role in dinitrogen fixation have been examined.

Materials and Methods

A field experiment with two popular rice varieties, IR 8 and Bhavani was undertaken at the Tamil Nadu Agricultural University, Coimbatore. The crop was raised with the recommended dose of fertilizer viz., N-60, P-40 and K-40 kg/ha. The soil was maintained under water-logged conditions throughout the period of study. When the seedling were 30 days old, they were transplanted in the main field (Plot size 5×4 m). The clay-loam field soil contained 0.129% total nitrogen, 1.25% organic carbon, 285 kg/ha available nitrogen, 11.2 kg/ha available phosphorus, 480 kg/ha available potash with a *p*H of 7.8 and an E.C of 10 mmhos/cm.

At different growth stages like seedling, tillering, boot-leaf and flowering of the crop, the rhizosphere and non-rhizosphere soil samples (Alexander 1961) were collected as per the standard procedures (Pramer & Schmidt 1966). The rhizoplane (washed root tissue) samples were also collected. The free-living nitrogen-fixing bacteria were enumerated in the samples. The population of *Azotobacter* was determined by plating the samples on Waksman's medium No. 77 (Rangaswami 1966).

The population of *Beijerinckia* was enumerated by using the Becking's medium (Pramer & Schmidt 1966). The anaerobic nitrogen fixing bacterium, *Clostridium pasteurianum* was enumerated by the anaerobic culture technique using the medium of Mishustin and Yemtseve (1975). The samples were incubated at 28°C for 15 days under anaerobic jars. Anaerobiosis was achieved by alkaline pyrogallol placement and burning a candle inside the jars.

Assay of nitrogenase: The acetylene reduction technique (Hardy et al. 1973) was followed for assaying nitrogenase. A Perkin Elmer Model F. 33 Gas Chromatograph fitted with a Porapak-R column served the purpose. The samples were taken in 500 ml Erlenmeyer flasks fitted with rubber needle puncture stoppers. Air in the vessels was driven-off by flushing it with a gas mixture of nitrogen and oxygen (4 : 1). A known quantity (10% of the total gas volume) of pure acetylene was injected with a Hamilton's gas-tight syringe. The vessels containing rice roots (rhizoplane) were maintained at an acetylene concentration of 0.3 atm.—the partial pressure at which rice roots exhibit higher nitrogenase activity (Yoshida & Ancajas 1973). In order to study the contribution of photosynthetic microorganisms towards nitrogen fixation, one set of samples was incubated under daylight (Ca. 30,000 lux) for 6 hr. All samples were incubated for a period of 12 hr at 28 ± 1°C.

Results and Discussion

The lowland rice field soils have been studied in greater details, for the distribution of various kinds of nitrogen-fixing microorganisms. These soils are unique as they experience aerobic, microaerophilic and anaerobic conditions which favour the activities of the respective group of organisms. The rhizosphere of rice has been recognized to be a potential site for many biochemical reactions notably nitrogen fixation (Yoshida & Ancajas 1973).

The data on the occurrence of *Azotobacter* (table 1) in rice field soils illustrate that rhizosphere of the two cultivars harbours more population than the non-rhizosphere region. Moreover, boot-leaf and flowering stages registered maximum population of *Azotobacter*. In general, IR 8 registered more population of *Azotobacter* than the cultivar Bhavani.

Considering *Beijerinckia,* the population was relatively low: however the rhizosphere of the two cultivars recorded more counts than the non-rhizosphere samples. Here again the boot-leaf and flowering stages of the crop registered more population of *Beijerinckia* (table 2). In Bhavani, the rhizosphere harboured greater number of clostridia than the non-rhizosphere at all stages of the crop growth while in IR 8 the colonization of rhizosphere appeared to begin from the tillering stage (table 3).

Table 1 *Population* of* Azotobacter *in rice field soil* (10^3/*g of soil*)

Stage of the crop	Bhavani		IR 8	
	Rhizosphere	Non-rhizosphere	Rhizosphere	Non-rhizosphere
Seedling	6.0±0.25	5.5±1.0	13.5±2.5	8.2±3.0
Tillering	11.2±0.6	8.5±0.5	15.2±1.4	10.5±1.0
Boot-leaf	14.0±2.0	6.2±0.6	14.2±3.1	8.5±0.6
Flowering	18.0±4.0	8.2±2.0	85.0±6.5	6.2±2.5

Table 2 *Population* of* Beijerinckia *in the rice field soil* (10^2/*g of soil*)

Stage of the crop	Bhavani		IR 8	
	Rhizosphere	Non-rhizosphere	Rhizosphere	Non-rhizosphere
Seedling	4.1±1.8	2.5±1.5	6.9±2.4	6.2±3.0
Tillering	9.5±1.2	7.0±1.2	4.8±1.8	6.3±1.9
Boot-leaf	26.0±3.6	3.8±0.5	14.5±7.3	7.2±4.1
Flowering	11.5±1.8	6.5±0.8	16.2±2.6	5.1±3.0

Table 3 *Population* of* Clostridium *in rice field soil* (10^2/*g of soil*)

Stage of the crop	Bhavani		IR 8	
	Rhizosphere	Non-rhizosphere	Rhizosphere	Non-rhizosphere
Seedling	95.8±5.0	50.0±5.2	36.8± 6.0	50.4± 3.6
Tillering	112.0±3.6	37.6±12.0	72.6± 7.5	48.5±11.0
Boot-leaf	70.5±11.0	46.8± 6.5	58.6±14.0	45.0±16.0
Flowering	97.6± 8.0	69.0± 4.0	118.6± 8.5	66.5± 7.5

*Data represent mean of three determinations

These results clearly confirm that rice rhizosphere irrespective of the variety, harbours a fairly rich flora of nitrogen fixing bacteria. Rice roots in the earlier stages of development are characterized by more oxidative activities; as they grow older, they tend to become more reductive (Mitsui 1955). Moreover, a rich supply of carbohydrates and organic nitrogenous compounds in the form of root exudations is available only during boot-leaf and flowering stages of the crop. These physiological and biochemical variations of the rhizosphere might explain the differential colonization of the diazotrophs in the rhizosphere at different growth phases.

The role of *Beijerinckia* in N_2 fixation under rice paddies has been reemphasized by Sen (1972) and Ishizawa et al. (1975). In India, not much work has been done on the anaerobic bacterium. Mishustin and Yemtseve (1975) claimed that Ca. 38 kg N/ha could be fixed by *C. pasteurianum.*

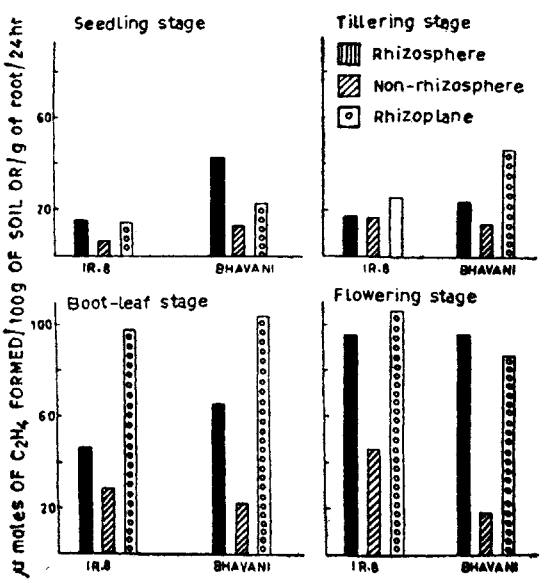

Figure 1 Nitrogenase activity in rice field soils and roots

Table 4 *Nitrogenase activity of certain rice varieties (At boot-leaf stage)*

Rice varieties	Nitrogenase activity*
Co.20	22.50
Co.40	23.50
TKM 4	44.00
TKM 6	27.00
Jaya	42.50
Basumathi	77.50
SLO 16	22.50
T.2910	31.00
T.2912	113.00
DGWG	24.50

*μmoles of C_2H_4 formed/g of root/12 hr. The acetylene assay was done according to Hardy et al., 1973

The nitrogen fixing potential of the cultivars has been presented in figure 1. It is revealing that the activity was more at boot-leaf and flowering stages of the crop. Besides, the rhizoplane of the cultivars also recorded appreciable nitrogenase activity. Table 4 sets out the nitrogenase activity of different rice cultivars assayed at the boot-leaf stage. The data point out the wide variations among the cultivars in nitrogenase activity. The observations of Lee and Yoshida (1977) merit our consideration. According to them the N_2-fixing bacteria are found closely associated with the root system of rice and hence in our study even the washed roots (rhizoplane) recorded appreciable nitrogenase activity.

It is quite obvious that light favours the activities of photosynthetic organisms. Incubation of samples under illuminated conditions enhanced the nitrogenase activity (table 5). Investigating the effect of light on the nitrogenase activity of rice, Balandreau et al. (1975) recorded that the rice rhizo-

Table 5 *Contribution of photosynthetic micro-organisms in nitrogen fixation*

Rice variety	Rhizosphere*		Rhizoplane**	
	Darkness	Illumination	Darkness	Illumination
IR 8	42.00	76.00	56.00	112.00
Bhavani	36.00	92.00	85.00	126.00

* μ moles of C_2H_4 formed/100 g of soil/12 hr
** μ moles of C_2H_4 formed/g roots/12 hr
Acetylene assay was done according to Hardy et al. 1973

sphere responded well to light intensities. The occurrence of photosynthetic N_2-fixing organisms in rice field soils has been studied in greater details (Kobayashi 1975). From the results it appears that rice roots posses a greater potential for biological N_2 fixation and obviously greater the root surface greater would be the N_2-fixing activity. Therefore the various agronomic and cultural practices stimulating a greater root spread would eventually enhance N_2 fixation.

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