EPIPHYTIC NITROGEN FIXATION ON LOWLAND RICE PLANTS

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ABSTRACT

Epiphytic nitrogen fixation on the submerged part of the rice stems was examined by:

- *studying the distribution of acetylene-reducing activity (ARA) and epiphytic algae among the hills at tillering stage;*
- *enumerating and identifying epiphytic microorganisms on the outer and inner leaf sheaths;*
- *measuring ARA and evaluating algal populations at seedling, tillering, heading and maturity stages of rice growth.*

Dark and light ARA (nmole C_2H_4 h^{-1} $hill^{-1}$) exhibited a log-normal distribution (L-shaped histogram; mean = standard deviation) while the total algal flora had an assymmetrical histogram, indicating the presence of several dominant epiphytic species.

Total and N_2-fixing algal populations on the outer parts of the stems (3.5 x 10^5 and 1.2 x 10^5 cells (g fresh weight)$^{-1}$ respectively) were about twenty times higher than those of the inner parts. A similar distribution was observed with N_2-fixing bacteria (outer parts: 2.9 x 10^7 cells (g fresh weight)$^{-1}$; inner parts: 1.0 x 10^5 (g fresh weight)$^{-1}$) where the dominant types were related to the Enterobacteriaceae, associated with Azospirillum*-like organisms. A macroscopic epiphytism by* Gloeotrichia *sp. was observed at seed-*

fresh weight was determined of the epiphytic algae dislodged from their host. At tillering, a visible growth was still present but insufficient for direct weighing. Biomass was calculated from chlorophyll measurements on algal material removed from the stems. Chlorophyll was measured after acetone extraction using MacKinney's (1941) specific absorption coefficient. Fresh weight was calculated using a ratio of 30.5 mg chlorophyll-*a* per gram fresh weight determined from the same algal material. These measurements were done separately on 35 hills, harvested from the same plot, in order to study the variability of algal epiphytism among rice hills.

At heading and maturity stages where epiphytism was not observable by the naked eye, algal enumerations were done on BG11 media (Allen & Stanier, 1968) with and without combined nitrogen for total and N_2-fixing algae respectively, as described earlier (Kulasooriya *et al.*, 1981).

Bacteria. Bacterial enumerations were conducted by the MPN method as described by Watanabe *et al.* (1979) for N_2-fixing Enterobacteriaceae and *Azospirillum*-like organisms. Total heterotrophic bacteria were enumerated by plating according to Watanabe & Barraquio (1979).

Host biomass measurements

After harvesting the whole plant, the root system and the aerial parts above the flood water level were cut off; the remaining material was used for ARA and fresh weight measurements and algal enumeration.

ARA measurements

Light and dark ARA measurements were carried out in the laboratory as previously described (Kulasooriya *et al.*, 1981) using cut rice stems. At seedling stage, parallel measurements were done *in situ* and in the laboratory to compare ARA under these different conditions.

At tillering stage, cut stems of 35 rice hills from the same plot were separately incubated to study the variability of the ARA among rice hills.

At heading and harvesting stages ARA measurements were done on 10 g triplicates randomly selected from the mixed material from the entire harvest of a plot of 35 hills.

At heading stage, the outermost leaf sheaths (outer parts) were separated from the inner parts of the tillers. Samples from these two sets were used separately for ARA measurements and enumerations of epiphytic microorganisms.

RESULTS

Epiphytic organisms

Of the epiphytic algae, *Gloeotrichia* sp. produced a visible growth on the rice stems at seedling and tillering stage. This growth could be observed irrespective of whether the host material was living or dead. Furthermore *Gloeotrichia* colonization was also observed on synthetic material such as nylon strings.

Gloeotrichia epiphytism decreased from seedling to tillering stage, mainly due to algal masses getting detached from their hosts as a result of gas bubble formation within the colonies. It was also noticed that colonies attached to the living parts were more easily dislodged than those attached to the dead parts.

At heading and harvesting stages, algal epiphytism was observable only under the microscope and during these stages the dominant N_2-fixing species was *Nostoc*, together with *Calothrix*, *Tolypothrix* and *Gloeotrichia* as associated species. At heading stage, N_2-fixing blue-green algae constituted 36% of the total epiphytic algal flora (Table 1).

Bacterial enumerations done at heading showed the presence of N_2-fixing acid-gas producing bacteria (Enterobacteriaceae) as well as *Azospirillum*-like organisms. The presence of these bacteria on rice has been already reported by Watanabe *et al.* (1979).

Results of the comparison of epiphytism on outer and inner leaf sheaths (Table 1) indicated that both ARA and microbial colonization of the outer parts was much higher than on the inner parts irrespective of the type of microorganisms. Experiments using labelled N_2-gas have also shown a higher N_2-fixing activity on the outer surface of stems than on the inner parts (Ito *et al.*, in press).

In the case of algae this may be related to light availability. N_2-fixing algae present on the inner leaf sheaths (5.3×10^3 (g fresh weight)$^{-1}$) were mainly spores or inactive forms as demonstrated by the negligible difference between dark and light ARA measurements on the inner leaf sheaths. The much higher density of bacteria on the outer parts may be

Table 1. Distribution of ARA (nmole C_2H_4 (g fresh weight)$^{-1}$ h^{-1}) and epiphytic microorganisms (number (g fresh weight of host material)$^{-1}$) between outer and inner parts of rice stem at heading stage

	Outer sheath	Inner sheath	Whole stem (leaf sheaths + culm)
ARA Light	2.5	0.14	1.9
Dark	0.5	0.11	0.47
Total algal flora	3.5×10^5	1.7×10^4	1.4×10^5
N_2-fixing algae	1.2×10^5	5.3×10^3	4.8×10^4
Total aerobic heterotrophs	4.7×10^8	3.0×10^6	1.8×10^8
N_2-fixers on glucose (Enterobacteriaceae)	2.0×10^7	9.5×10^4	7.5×10^6
N_2-fixers on malate (*Azospirillum*-like)	9.5×10^6	9.5×10^3	3.7×10^6

Variation of epiphytism among rice hills

Light and dark ARA among 35 hills from the same plot are depicted in Fig. 1A and B, in the form of histograms. Both histograms exhibited a characteristic L shape; mean and standard deviation of the variables were very close to one another. These features indicate a log-normal distribution of ARA in the light and in the dark. Similar results have been reported for ARA by soil algae and bacteria (Roger *et al.*, 1977).

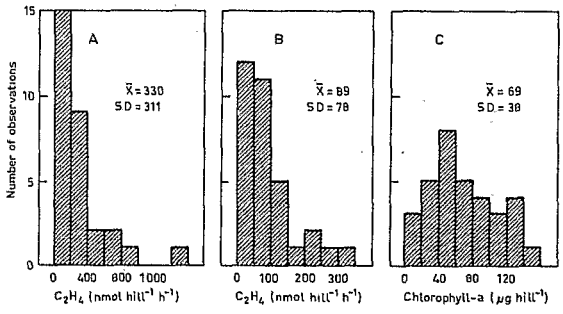

Fig. 1. Histograms showing the variations of: (A) light ARA; (B) dark ARA and (C) epiphytic algal chlorophyll, among 35 hills from a rice field at tillering stage.

This large variability of ARA among the hills implied that subsequent measurements should be done on replicates obtained from mixed material from the complete harvest of a plot and not on a few randomly selected hills. The distribution of epiphytic algae on the rice plants, determined as chlorophyll-*a* per hill was not log-normal (Fig. 1C). The assymmetrical histogram indicates that algae other than *Gloeotrichia* had also contributed to these pigment measurements. This was confirmed by plating dislodged algal material, which showed the presence of several associated blue-green algae, mainly *Oscillatoria*, *Pseudoanabaena* and *Nostoc*.

Variations of epiphytism and ARA along the cultivation cycle

A remarkable change was found in the algal epiphytism along the developmental cycle of the rice plant, with a corresponding change in the light ARA (Table 2). At seedling stage, when the rice stems had an epiphytic *Gloeotrichia* biomass of about 2 t fresh weight ha^{-1}, ARA in the light was 51 µmole C_2H_4 m^{-2} h^{-1}. At tillering, when this biomass had diminished to 0.5 t fresh weight ha^{-1} it still had an activity of 15 µmole C_2H_4 m^{-2} h^{-1}. The algae exhibited the same specific activity at these two stages (about 2.4 nmole C_2H_4 (mg protein)$^{-1}$ min^{-1}). A similar specific activity was reported by Finke & Seeley (1978) for *Gloeotrichia*

Najas (Kulasooriya *et al.*, 1981).

- colonies on living, smooth rice stems get detached more easily than those on dead plant material which has rough surfaces as demonstrated by Howard-Williams *et al.* (1978).
- colonization was observed even on old, rough nylon strings but not on new smooth ones placed into the flood water. Similar colonization on polyethylene strips has been reported by Finke & Seeley (1978).

In the case of "microscopic epiphytism" it was also observed that most of the isolated epiphytic strains grew adherent to the surface of the culture vessels and rarely formed floating colonies. The results obtained do not permit confirmation of either the existence or the absence of biotic relationships between the algae and the host, but indicate that both a mechanical effect in relation to the roughness of the support and an ability of certain strains to grow attached to a support are involved in algal epiphytism. From the ARA measurements of these experiments the N_2 input by organisms epiphytic on rice can be evaluated as a few (2-3) kg ha^{-1} $crop^{-1}$, mainly due to the activity of *Gloeotrichia*.

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