SHORT COMMUNICATION

Effect of applied nitrogen upon acetylene reduction in the rice rhizosphere

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While it has been well established that nitrogen fixation by free-living microorganisms is greatly increased in the rhizosphere of rice plants growing in flooded soils (Yoshida and Ancajas, 1971, 1973a and 1973b; Rinaudo, Balandreau and Dommergues, 1971; Dommergues, Balandreau, Rinaudo and Weinhard, 1973) very little appears to be known about the effect of applied fertilizer N upon the extent of N2 fixation in this zone. The present study was aimed at determining the effect of two forms of inorganic N upon the C2H2 reducing capacity of the rhizosphere microfloras of rice seedlings grown in flooded and non-flooded soil.

Japonica type rice seedlings were grown in tubes (15 × 2 cm) of flooded soil in the greenhouse for 20 days after germination. Nitrogen in the form of (NH4)2SO4 or KNO3 was added to some of the tubes at the time of planting at the rates of 50 and 200 mg/g soil. At the end of the growth period the root systems were removed from the tubes, the leaves cut off at the base and the root systems and adhering rhizosphere soil placed in 25 ml serum bottles. The bottles were capped with serum caps, secured by aluminium compression caps and 2 ml of the headspace air removed by means of gas tight syringe and needle. Two ml of purified C2H2 was added to the vials which were then incubated for 4 days at 30°C. After incubation the headspace atmosphere was sampled by gas tight syringe and the amount of C2H4 and C2H2 determined by gas chromatography. The planting and sampling of plant rhizosphere techniques were similar to those described by Rinaudo et al. (1971), except that the plant tops were removed before C2H2 reduction assay.

The soil used had the following characteristics: pH, 7-2; C, 1-7% clay, 29%; NH4-N, 38 mg/g; NO3-N, 222 mg/g. Tubes of unplanted soil were also incubated in the greenhouse to represent non-rhizosphere soil samples for later C2H2 reduction tests. Nine replicate tubes were prepared for each treatment and each tube contained 14 g soil. The tubes of flooded soils were placed in a large container of flooded soil and the tubes of non-flooded soil in a container of non-flooded soil so that the soil and water surfaces coincided.

C2H4 and C2H2 assays were performed with a Perkin-Elmer F11 gas chromatograph, having a single stainless steel column (2 m × 5 mm o.d.) packed with Porapak N (80–100 mesh) and a single flame ionization detector.

When the C2H4 and C2H2 assays were completed the dry weights of soil and plant roots were determined. From the dry weights of soil, the bulk density of the soil, and the moisture content of the soil the headspace volume of each assay vial was computed. The plant root dry weights ranged from 10 to 50 mg for plants grown in non-flooded soil and from 15 to 60 mg for plants grown in flooded soil.

The results obtained in the experiment (Table 1) show a clear rhizosphere effect with respect to non-symbiotic N2-fixing microorganisms in rice seedlings grown in both flooded and non-flooded soil. The effect was stronger in the flooded soil.

<table>
<thead>
<tr>
<th>Form of nitrogen</th>
<th>Application rate parts/100</th>
<th>C2H4 production* (nmole/g soil/d)</th>
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<tbody>
<tr>
<td><strong>Flooded</strong></td>
<td></td>
<td></td>
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<tr>
<td>&quot;Native&quot;</td>
<td>59.00 (0.03)</td>
<td>22.40 (0.04)</td>
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<tr>
<td>NH4+-N</td>
<td>6.20 (0.03)</td>
<td>0.93 (0.03)</td>
</tr>
<tr>
<td>NH2+-N</td>
<td>0.35 (0.03)</td>
<td>0.65 (0.03)</td>
</tr>
<tr>
<td>NO3--N</td>
<td>0.47 (0.03)</td>
<td>0.73 (0.03)</td>
</tr>
<tr>
<td>NO3--N</td>
<td>2.92 (0.18)</td>
<td>0.48 (0.03)</td>
</tr>
<tr>
<td>LSD 49.75</td>
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</table>

* Figures in parentheses represent C2H2 reduction activity in non-rhizosphere soil.

The critical F value obtained by statistical analysis of the results showed that the nitrogenase activity in the rhizosphere of rice seedlings grown in unfertilized flooded soil was significantly different (1 per cent level) from all the rhizosphere samples from N-amended soil. Differences among the non-flooded soil samples proved to be not significant.

The results agree with earlier findings that non-symbiotic N2 fixation is stimulated in the rice rhizosphere. They also indicate that applied N can significantly inhibit non-symbiotic N2 fixation in the rice rhizosphere during the early phases of the rice plant development. The inhibition by applied N occurred even though initially the unamended soil contained a high amount of inorganic N.

The use of prolonged incubation periods in the C2H2 reduction assay is undesirable, particularly in studies of aerobic nitrogen-fixing microbes (Hardy, Burns and Holsten, 1973). However, because of the relatively small root sample per assay vial in the present study, a 4-day incubation period was found necessary to obtain detectable amounts of C2H2. The use of detached root systems proved convenient in this study but raises questions regarding the nature of the rhizosphere effect. Obviously, shorter term, whole-plant studies involving controlled illumination are required.

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REFERENCES


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