

Effect of Combined Nitrogen on the Activity of Non-symbiotic Diazotrophs

T. A. KALININSKAYA

Institute of Microbiology of the USSR Academy of Sciences, Moscow /USSR/

Combined nitrogen compounds greatly influence the activity of non-symbiotic nitrogen fixation.

Doses of mineral nitrogen, completely inhibiting nitrogen fixation, vary depending on soil conditions, the presence of accessible carbon sources and other factors.

Rice is cultivated with the application of high doses of nitrogen fertilizers (120-240 kg N/ha). The studies of the influence of different doses of nitrogen fertilizers on nitrogen fixation activity and the development of different groups of diazotrophs in soils under rice are therefore of great interest. Particular attention was given to the behavior of *Azospirilla*, which is one of the leading groups of diazotrophs in rice rhizosphere and in paddy soils.

The influence of combined nitrogen on *Azospirilla* has been investigated in pure cultures, in association with rice plants and in soils under rice. Cultivation of *Azospirilla* was carried out on semi-solid malate medium.

In pure *Azospirilla* cultures ammonium nitrogen in concentrations of 56-70 mg N/l was shown to depress nitrogen fixation completely.

The inhibiting effect of nitrates is less manifest and complete inhibition of nitrogen fixation usually ensured at doses, exceeding 210 mg N/l.

Azospirilla strains, capable of denitrification, were shown to be less sensitive to high nitrate doses.

The influence of nitrates and ammonium salts on associative symbioses of *Azospirilla* with rice plants was studied in water culture with different concentrations of mineral nitrogen maintained in the nutrient solution. Rice seeds served as a source of *Azospirilla*, proliferating on rice roots and in the nutrient solution. Nitrogen fixation activity was determined by the acetylene reduction method.

Data on the nitrogen fixation activity of two-week-old rice seedlings are presented in Table 1.

The results recorded were in agreement with those obtained in experiments with pure cultures of *Azospirilla*.

The inhibition of nitrogen fixation by ammonium nitrogen was manifest as soon as its concentration, was equal to 14 mg/l. Complete inhibition of nitrogen fixation ensured at the dose of NH_4^+/N equal to 56 mg/l. The inhibiting effect of nitrates appeared at considerably higher concentrations.

Table 1
Influence of ammonium and nitrate nitrogen on N₂ fixation by Azospirilla in rice rhizosphere

Doses of nitrogen mg/l	Fixed nitrogen µg/30 plants/day	In % of control
Control /without nitrogen application/ <u>With ammonium salts applied</u>	87.7	100.0
14	23.7	27.0
28	19.3	22.0
56	0.2	0.2
<u>With nitrates applied</u>		
56	42.7	48.7
112	11.6	13.2
224	7.8	8.9

Table 2
Influence of nitrogen fertilizers on nitrogen fixation activity and Azospirilla number in soil under rice

Fertilizers, kg N/ha	Nitrogen fixation activity µg N ₂ / kg of soil/day	Number of nitrogen fixers thousand cells per 1 g of soil		
		anaerobes malate	aerobes on medium	Azo- spirilla
Controll	355	1400	2400	250
120	277	4500	6000	600
240	164	950	6000	130

Table 3
Influence of high doses of nitrogen fertilizers on the growth of different groups of diazotrophs in the pot experiment with rice /average number during vegetation period, thousand cells/1 g of soil

Variants	Anaerobes	Aerobes on malate medium	Azo- spirilla	Azoto- bacter
Control	3690	7160	4170	105
P ₁₅₀ K ₁₅₀	3660	21120	1580	128
N ₁₅₀ P ₁₅₀ K ₁₅₀	1460	27820	36	4
N ₃₀₀ P ₁₅₀ K ₁₅₀	31506	13870	15	2

Depending on the phase of plant growth the dose equal to 56 mg of $\text{NO}_3^- \text{N/l}$ did not exert a depressing effect or reduce nitrogen fixation activity to 50% or less.

In the plant rhizosphere considerable nitrogen fixation went on which still kept the nitrate nitrogen content of the soil on a level equal to 112-227 mg/l.

The effect of nitrogen fertilizer on Azospirilla growth in soil was of great interest.

In experiments with the rice cultivar "Spalchik", carried out under field conditions on a meadow-chernozem soil in the Krasnodar region the doses of nitrogen fertilizers /120-240 kg N/ha/, applied in the form of urea, had no unfavourable effect on the growth of nitrogen fixing microorganisms, including Azospirilla, and did not considerably inhibit nitrogen fixation activity in the soil /Table 2/.

The influence of higher nitrogen doses was tested in pot experiments. Nitrogen in the form of urea was applied to soil in doses of 150 and 300 mg/kg, that corresponded to 450 and 900 kg N/ha, respectively.

High doses of nitrogen gave rise to long-lasting inhibition of nitrogen fixation up to the 70-90th day of rice cultivation. In the variant with 300 mg N-dose per 1 kg the inhibition only came to a complete end in the ripening period.

The inhibition of nitrogen fixation in the rhizoplane, measured by acetylene reduction in whole rice plants, was shown to be less intense than in the soil.

Thus, after two weeks the nitrogen fixation of rice seedlings in the 150 and 300 mg N/kg variants was 34 and 13%, respectively, of the activity recorded in the control treatment, whereas in the 4th week it had increased to 78 and 54%.

Different groups of diazotrophs reacted in different ways to the application of high nitrogen doses to the soil /Table 3/. Thus, the total number of facultative and obligate anaerobes was the highest in the maximum nitrogen dose treatment. No considerable decrease of the total number of aerobic diazotrophs, growing on semi-solid malate medium was observed.

At the same time a sharp drop in the number of Azotobacter and Azospirilla was observed.

High nitrogen doses depressed, first of all, the growth of more specialized and high active nitrogen fixers - Azospirilla and Azotobacter. As a result the groups of diazotrophs with lesser nitrogen-fixing activity grew predominant.

The data obtained show that in a meadow-chernozem soil most of the diazotrophs can defy competition with the microflora which does not fix nitrogen, even during the application of high doses of mineral nitrogen to the soil.

Azotobacter and Azospirilla turned out to be the most sensitive to high doses of nitrogen fertilizers.

Industrial doses of nitrogen /up to 240 kg/ha/ did not depress the growth of any groups of heterotrophic free-living nitrogen fixers.