ความสัมพันธ์ระหว่างอโซโตแบคเตอร์และคลอสตริเดียม ในการเพิ่มผลผลิตของข้าวและปริมาณการดูดซึมไนโตรเจน

Interrelationship between Azotobacter and Clostridium in Increasing the Yield of Paddy and Nitrogen Uptake

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The conversion of nitrogen in the form of gas into nitrogen compound by biochemical processes is rather small, but the processes continue throughout the year, if the climate and other factors such as nutrients, temperature, moisture, pH, aeration, number and strains of micro-organisms are suitable.

Bhat and Palacios reported in 1949 that nitrogen fixation in the soil was favoured by moisture and absence of direct sunlight. Gupta (1956) manured alkaline clay and acid sandy soils and found that manured soil showed greater nitrogen fixation than the unmanured soils. In 1958 Sharma and Bhattacharya noted that in alkaline soil, the presence of calcium phosphate alone or with glucose or starch promoted the oxidation of organic matter and increased nitrogen fixation. Vojinovic (1956) reported that most important factors, were nature or organic matter and the phosphate level. De and Mandal (1956) observed the application of molybdenum with phosphorus caused greater nitrogen fixation than phosphorus alone.

In 1953 Neuhuber found that rhizosphere stimulated soil algae especially blue-green algae. This probably depended on amino acid root excretion. Wallace and Lochhead (1950) reported that the number of amino acid-requiring micro-organisms were higher in the rhizosphere, due to excretion of amino acids by root. Riviere (1958) observed that rhizosphere effects stimulated nitrogen fixing bacteria.

In 1957 Nilsson found that number of Azotobacter increased in the rhizosphere of lucerne and red clover soils which were rich in potash. Fedorov and Savkina (1960) found that the rhizosphere bacteria increased growth and nitrogen by Azotobacter. Daraseliya (1961) found that Azotobacter was always accompanied by Clostridium in the soil. Parker (1957) reported that the nitrogen content of cut grass was about 100 lbs. per acre and concluded that very active biological fixation of nitrogen had taken place by either Clostridium or Azotobacter. Van (1958) reported that nitrogen fixation by Azotobacter in culture was stimulated by some rhizosphere bacteria, but inhibited by others. Golovacheva (1957) showed in field and pot experiments that when maize and wheat were

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sown. Clostidium added to the soil multiplied intensively in the rhizosphere and increased the grain yield.

In the submerged anaerobic condition in the rhizosphere of rice, Clostridium may help to improve the soil for multiplication of Azotobacter. The Azotobacter accompanied by Clostidium may increase nitrogen fixation, nitrogen uptake by rice when molybdenum and phosphate are applied to the soil, and yield of rice.

This investigation was undertaken to determine the effect of Clostridium and Azotobacter, both alone and in combination, upon the yield of rice where molybdenum and phosphate had or had not been applied.

MATERIALS AND METHODS

Loamy sand soil which contained 0.67 per cent of organic carbon, 0.09 per cent of nitrogen and having pH 8.4 was used in this experiment. Double superphosphate, ammonium sulphate and sodium molybdate free from toxic substance to Azotobacter were used. Azotobacter culture which fixed 13.64 mg. of nitrogen per one gram of sucrose was isolated from the soil in Jensen media as described by Jensen (1944). It was inoculated in Kaolin media and incubated at temperature 30°C for 3 weeks before using. Clostridium culture was isolated from the soil in Winogradsky media at temperature 28°C, for 5 days in anearobic jar as described by McClung and Lindberg (1957).

Ten pounds of the air-dry soil was placed in Mitcherlich pots. Double superphosphate was applied at the rate of 8 lbs. of P_2O_5 per acre and mixed thoroughly with the soil. Sodium molybdate was applied at the rate of one ounce per acre in the form of solution. Ammonium sulphate was applied at the rate of 40 lbs. of nitrogen per acre the day of planting. This experiment was a randomized block design. It was carried out with 8 treatments, 4 replications and the total number of 32 pots. Three week old seedlings (*Oryza sativa* strain No. NP130 obtained from the Indian Agricultural Research Institute, New Delhi) were carefully pulled out and the roots were washed with running tap water and dipped for ten minutes in a media consisting of ten grams of Kaolin media containing Azotobacter mixed with 500 ml. of normal saline water and 500 ml. of cellulose decomposing organism. The seedling were then planted. 100 ml. of Clostridium culture was mixed with 250 ml. of sterilized normal saline water and mixed with 50 ml. of the cellulose decomposing organism. Selected seedlings of rice were dipped into the Clostridium suspension for about 10 minutes and then planted. The number of Azotobacter in the rhizosphere soil of rice was determined three times, the first was two weeks after planting, the second was at flowering stage and the last at harvesting stage. Jensen media was used for planting. The grain and straw were dried to constant weight in the oven at 55-60°C for two days. Nitrogen content of the soil samples were determined by the Kjeldahl digestion method. The nitrogen content of grain and straw was determined by the micro-Kjeldahl

digestion method as described by Jackson (1958).

RESULTS AND DISCUSSION

The count of Azotobacter in the rhizospere of the soil, the yield of straw and grain, and nitrogen uptake by the plant are given in Table 1.

Table 1: Azotobacter count, plant yield and nitrogen uptake.

No.	Treatment	Number of Azotobacter*			Yield of Paddy		Nitrogen Uptake
		Two weeks	Flowering	Harvesting	Straw gm./pot	Grain gm./pot	
1	Clost.	2.581	2.304	2.288	6.225	4.425	59.125
2	Azot.	3.565	3.493	3.076	5.575	3.825	45.450
3	Clost. + Azot.	3.384	3.422	3.145	6.200	4.475	59.325
4	P_2O_5 + Mo + Clost.	2.434	3.267	2.773	7.250	5.125	65.525
5	P_2O_5 + Mo + Azot.	2.716	3.405	3.231	6.575	4.975	59.500
6	P_2O_5 + Mo + Clost. + Azot.	2.979	3.358	3.446	7.100	4.575	57.800
7	Ammonium sulphate	1.563	2.405	2.297	9.100	5.750	67.400
8	Control	1.477	2.368	2.105	6.175	4.700	56.150
	L.S.D. 0.05	0.329	0.079	0.174	1.500		
	0.01	0.446	0.107	0.236	2.040		

*Given as the log. of the count

The data indicate that the highest count of Azotobacter was obtained from the Azotobacter treatments at the planting and flowering stages, these may be due to the accompanying of cellulose decomposing micro-organisms and Azotobacter. The same results were reported by Chulakov (1956) and Mekhtiev (1959). Azotobacter is an aerobic bacteria. It is usually difficult to grow in the anaerobic conditions of the flooded rhizosphere soil of paddy. The thin layer of oxidation in paddy soil undoubtedly provides a good condition for its growth. The benefits devided from the accompanying cellulose decomposing micro-organisms by Azotobacter may be due to the fact that Azotobacter can convert cellulosic materials in the form of sugars at the planting stage of paddy.

At the harvesting stage however, the highest count of Azotobacter was observed from the treatment of P_2O_5 + Mo + Clostridium + Azotobacter. Bu-

rokova (1957) and Bilodub (1960) have obtained similar results. The application of P_2O_5 + Mo to the paddy may have stimulated growth of Azotobacter at the harvesting stage.

Clostridium in association with Azotobacter may provide proper conditions for the growth of Azotobacter also at the planting and flowering stages when hydrolysed cellulose is available, but when the cellulose is exhausted, the organisms have to depend upon the excretory substances from the roots. When the paddy become too old, the excretory substances were less, so that lowest count was obtained.

The depression in the number of Azotobacter also occured under ammonium sulphate treatment. Any form of combined nitrogen is known to cause depression in the activity and number of nitrogen fixing micro-organisms. The control soil shows the mininum number of Azotobacter not very different from that in the case of ammonium sulphate treatment. Application of P_2O_5 + Mo resulted in an increase in the counts of Azotobacter which is significantly greater than those where no P_2O_5 + Mo were applied. There was no significant difference in the count of Azotobacter between the treatments of Azotobacter and Azotobacter + Clostridium. This may be due to Clostridium not competing with or stimulating Azotobacter in the rhizosphere soil.

It is evident from the data that the highest yield of paddy straw was obtained from the treatment with ammonium sulphate. Ammonium sulphate gave significantly higher yield of straw over other treatments at the 5 per cent level. The inoculation with Clostridium in the presence of P_2O_5 + Mo has given yields which differ from control by values near the critical difference. The data indicate further that there was no significant difference in yield of grain or of nitrogen uptake due to treatment. Though there were differences in the Azotobacter counts at different stages of growth of the plant, there seems to be no correlation between the Azotobacter counts and the yield of the crop. These results again support them views expressed by Rothamsted worker Jackson et al (1960) that inoculation may result in the highest count of Azotobacter, but it may not mean an increase in the yield of the crop.

CONCLUSIONS

1. Ammonium sulphate failed to increase the number of Azotobacter over the control.
2. Combined application of phosphate and molybdenum resulted in significant in the count of Azotobacter in rhizosphere soil.
3. Clostridium in association with Azotobacter failed to increase the growth of Azotobacter under anaerobic condition.
4. There was no correlation between number of Azotobacter and nitrogen taken up by paddy or its yields.

SUMMARY

During the study, paddy (*Oryza sativa*) was grown under inoculation with Azotobacter (Indian 41) and *Clostridium* sp. in Delhi soil with and without treat-

ment with molybdenum and phosphate. The number of Azotobacter in the rhizosphere and non-rhizosphere soils was estimated by plating at post-planting, flowering and at the harvesting stages of the plant. The yields of grain and straw were analysed for their nitrogen contents to obtain the amount of nitrogen taken up by the plants from the soil during their growth. The soil was also analysed for total nitrogen. The results of the study could be summerized as follows:

1. Ammonium sulphate failed to increase the number of Azotobacter over the control.

2. Combined application of phosphate and molybdenum resulted in significant in the count of Azotobacter in rhizosphere soil.

3. Clostridium in association with Azotobacter failed to increase the growth of Azotobacter under anaerobic condition.

4. There was no correlation between number of Azotobacter and nitrogen taken up by paddy or its yields.

สรุป

ในการศึกษาครั้งนี้ได้ทดลองปลูกข้าว (*Oryza satine*) ในดินเคมี เติมโมลิบดินั่มกับฟอสเฟทกับไม่เติม พร้อมด้วยเชื้ออโซโตแบคเตอร์ (อินเดียน 41) และคลอสตริเดียม ได้หาจำนวนอโซโตแบคเตอร์ทั้งในดินและในไรโซสเพียร โดยวิธีทำเพลทเมื่อหลังปลูก เมื่อออกดอกและเมื่อเก็บเกี่ยว ได้วิเคราะห์หาไนโตรเจนในเมล็ดข้าวและในฟาง เพื่อดูปริมาณไนโตรเจนที่พืชได้จากดินขณะเติบโต ทั้งได้วิเคราะห์ปริมาณไนโตรเจนทั้งหมดในดินด้วย ผลสรุปได้ดังนี้.–

1. ที่ใส่ปุ๋ยแอมโมเนียมซัลเฟต ไม่ทำให้ปริมาณของอโซโตแบคเตอร์ เพิ่มปริมาณมากขึ้นกว่ากระถางที่ไม่ได้ใส่

2. การใส่ปุ๋ยฟอสเฟตและโมลิบดินัมทำให้ปริมาณของอโซโตแบคเตอร์ในไรโซสเพียของข้าวเพิ่มขึ้น

3. ในสภาพอนาโรบิค คลอสตริเดียมที่ใส่ร่วมกับ อโซโตแบคเตอร์ ไม่ทำให้ปริมาณของ อโซโตแบคเตอร์เพิ่มขึ้น

4. ปริมาณของ อโซโตแบคเตอร์กับปริมาณไนโตรเจนที่ถูกดูดซับไม่มีความสัมพันธ์กัน

REFERENCES

1. Baht, J.V. and G. Palacios, 1949.
Influence of some bacterial cultures in the nitrogen status of soil. IV Influence of Aerobacter aerogenes in the nitrogen status of soil. J. Univ. Banbay. 17B, 84-87.

2. Bilodub, H., 1960.
Investigation on Azotobacter in the experimental plots of the Laskowise Olawskie Experimental Station. Prace Zakl. Upraw. Roli Plodoz., 2, 55-63.

3. Burakova, V.V., 1957.
The microflora of soils of the north Yenisie and means of increasing their activity, Trydy nauck-issled. Inst. Sel. Khoz. Krain., 5, 39-68.

4. Chulakov, Sh. A., 1956.
The distribution of Azotobacter in some soils of the Kryl-Ordin irrigation massif. Vestn Akad. Nauk Kajakh., SSR. No. 9, 38-44.

5. Daraseliya, N.A., 1961.
Nitrogen-fixing organisms in Krasnozen soils of Georgia, Pochvovedenie, 4, 111-114.
6. De, P.K. and L.N. Mandal, 1956.
Fixation of nitrogen by algae in :ice soils. Soil Sci., 81, 453-458.
7. Fedorov, M.V. and E.A. Savkina, 1960.
Interaction between Azotobacter and typical rhizosphere bacteria of maize. Mikrobiologiya, 29, 862-867.
8. Golovacheva, R.S., 1957.
The effect of **Clostridium pasteurianum** on the yield of agricultural plants. ENSV. tea-duste Akad. toimetised. Boilseer, 6, 29-73.
9. Gupta, S.R.S., 1956.
A new factor in the study of nitrogen fixation in soil. Indian J. mycol. Res., 2, 29-36.
10. Jackson, M.L., 1958.
Soil chemical analysis. Constable and Co. Ltd, London.
11. Jensen, H.L., 1944.
Symbiotic nitrogen fixation. Austral. J. Sci., 6, 162-165.
12. McClung, L.S. and Lindberg, 1957.
The study of obligately anaerobic bacteria, Manual of Microbiological Methods. McGraw-Hill Book Co., Inc., New York.
13. Mekhtiev, S. Ya., 1959.
Some data of the microflora of various Moldavian soils. Mikrobiologiva, 28, 743-774.
14. Neuhuber, J., 1953.
Building-up soil fertility by planting burdok. Bodenkulture, 7, 100-107.
15. Nilsson, P.E,. 1957,
Influence of crop on biological activities in soil potassium. Lanthy. Hogsk. 23, 175-218.
16. Parker, G.A., 1957.
Non-symbiotic nitrogen fixing bacteria in soil. III. Total nitrogen changes in a field soil. Sci., 8 48-59.
17. Riviere, J., 1958.
Micrological study of the rhizosphere of wheat. Ann. Agron. Paris, 44, 75-82.
18. Rothamsted Experiment Station Report, 1960.
Soil microbiology depertment, p. 92.
19. Sharma, S.S. and A.K. Bhattacharya, 1958.
Influence of calcium phosphate incorporated with energy rich materials on nitrogen fixation Part I. Proc. Nat. Acad. Sci. India., 27, 265-267.
20. Van, Ts. F., 1958.
Interaction between Azotobacter and rhizoshere bacteria of wheat. Dokl. S.-kh. Akad. Timiryazeva 34, 94-100.
21. Vojinovic, Z.D., 1956.
The occurrence of Azotobacter in the soils of Serbia. Arh. Poljopr. Nauke 9. 26, 97-112.
22. Wallace, R.H. and A.G. Lochhead, 1950.
Qualitative studies of soil micro-organisms. IX. Amino acid requirements of rhizosphere bacteria. Canad. J. Res. 28c, 1-6.