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Studies on the *Azotobacter* Inoculation of Crop Plants

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ABSTRACT

Rhizosphere and rhizosplane population build-up of *Azotobacter* in inoculated rice, chilli and ragi was estimated. Maximum respone to *Azotobacter* inoculation was seen with chilli crop followed by ragi and rice. The population build-up due to inoculation was maximum at the vegetative stage of growth in all the three crops. The yield increase due to inoculation ranged from 12-39 per cent and was comparable to the yields with application of recommended doses of nitrogen fertilizer.

INTRODUCTION

Inoculation of crops with *Azotobacter* has been practiced extensively in Soviet Union for many years. The claimed increases in yields due to inoculation ranged from 6-20 per cent (Mishustin, 1970). However, early attempts to reproduce these results in other countries have failed. But, of late such investigations have been revived in many countries outside Soviet Union (Brown, 1974; Balandreau *et al.*, 1975; Hardy and Havelka, 1975). Studies carried out in these countries have indicated the beneficial effect of free living N_2-fixing bacteria in the rhizosphere on crop growth. Dobereiner (1974) has demonstrated the natural occurrence of commensular association between N_2 fixing bacteria and root surfaces of grasses. In our prelimi-nary studies on *Azotobacter* inoculation of crop plants, yield increases due to inoculation ranging from 6-40 per cent were obtained. It was therefore contemplated to study if the rhizospheres and the rhizoplanes of the inoculated plants permitted the establishment of inoculant strains of *Azotobacter chroococcum*.

MATERIALS AND METHODS

Ragi (*Eleusine coracana* Gaertn) and chilli (*Capsicum annuum* L.) crops were inoculated with *Azotobacter chroococcum* isolated from red soil from Main Research Station of the the University of Agricultural Sciences, Bangalore through seed seedling inoculation and rice (*Oryza sativa* L.) seedlings were likewise, inoculated with isolate from rice soil. Inoculated seeds/seedlings were sown or transplanted as the case may be, in the respective fields with four replications. The following treatments were used:-

1. Control - no *Azotobacter* and no nitrogen.

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2. With N but without *Azotobacter* (N applied at 60 kg/ha for chilli, 40 kg/ha for ragi and 50 kg/ha for rice as ammonium sulphate).

3. With half the above dose of nitrogen but without *Azotobacter*.

4. *Azotobacter* alone.

In case of rice, inoculation was made once as root - dip before planting and second time as soil inoculation one month after transplanting. Similarly, nitrogen fertilizer was applied in two split doses. The crops were irrigated at regular intervals. *Azotobacter* populations in the soil, rhizosphere and rhizoplane at three intervals namely, at the time of planting, one and half month after planting and at harvest were estimated. The soil and rhizosphere population was determined following the method of Shantaram and Rangaswami(1967). For rhizoplane population root samples used for the estimation of rhizosphere population were washed vigorously in four changes of sterile distilled water. The samples were then separately macerated using sterile mortor and pestle. The macerated material was made up to 100 ml in sterile distilled water. *Azotobacter* population was determined following serial dilution method using Waksman 77 N - free medium. Similarly, rigorously washed root bits from each treatment were plated on N - free medium. *Azotobacter* growth (+) or no growth (-) around the plated root bit was recorded.

RESULTS AND DISCUSSION

The data on *Azotobacter* population in the soil, rhizosphere and rhizoplane of the three crops are presented in Table I. Maximum build-up of the population of *Azotobacter* in soil, rhizosphere and rhizoplane was seen only after six weeks of crop growth i. e., during the active vegetative growth period. The population declined to almost negligible number at harvest. The population build - up was particularly marked in the rhizosphere. The fact that the build-up of the population was markedely noticeable in the rhizosphere and rhizoplane of the inoculated plants only, indicated that the inoculant strain was well established. There were no such marked changes in the uninoculated control or in the uninoculated nitrogen control plots. The increased number of *Azotobacter* in inoculated rice soil as compared to other two crops was presumably due to the second application of *Azotobacter* to the crop one month after planting. There was also some increase in the population of native strains of *Azotobacter* at the maximum vegetative growfh stage of the crops in the uninoculated controls. Further, it was noted that the rhizosphere population of the inoculated plants was several fold higher than the rhizoplane population. Similarly washed root bits of the inoculated plants placed on N-free agar gave a heavy confluent growth of *Azotobacter* originating from root surface. Similar root - bit plating from uninoculated plants showed only anegligible growth. Besides, such growth from free plated root – bits on the N-agar medium was seen only with the plants sampled at vegetative growth phase.

The enhaced *Azotobacter* population during vegetative growth phase appeared to be due to intense photosynthate production and its excretion and due to the probable increased root sloughing in the rhizosphere. Such reasoning has been provided by Dommergues *et al.*, (1973) and Dobereiner and Campelo (1971). Free - living nittrogen fixing bacteria are said to require plentiful supply of carbon source and low nitrogen environment to thrive best in competition with other heterotrophic soil microflora. Rovira (1965) has noted that N_2 fixation abruptly stopped when the C/N ratio in the environment fell below 70 to 40. He has further noted (Rovira, 1969) that the roots formed the major sinks for the photosynthate produced during vegetative growth phase up to reproductive growth phase. In the present studies, the uninoculated plants when supplied with two levels of nitrogen showed negligible increase in the *Azotobacter* population during active vegetative growth phase, whereas, the

TABLE I. Crop response to *Azotobacter* inoculation and nitrogen fertilizer application

Crops	*Azotobacter* population x 10^{2}/g of dry sample in different treatments*											
	Control (without *Azotobater* or N)			*Azotobacter* alone			Half N alone			Full Nalone		
	A	B	C	A	B	C	A	B	C	A	B	C
RAGI												
Soil	0.00	0.70	0.00	0.00	3.80	1.40	0.00	0.80	0.00	0.00	0.70	0.00
Rhizosphere	0.00	2.70	0.00	0.00	470.00	0.50	0.00	5.00	0.70	0.00	0.50	0.00
Rhizoplane	0.00	6.70	0.00	0.00	130.00	0.70	0.00	4.00	0.00	0.00	0.00	0.00
Root Plating	—	+	—	—	++	—	—	—	—	—	—	—
RICE												
Soil	0.00	0.26	0.25	0.00	370.00	40.00	0.00	3.70	0.50	0.00	0.50	0.00
Rhizosphere	0.00	2.80	0.00	0.00	500.00	8.00	0.00	3.50	0.20	0.00	0.00	0.00
Rhizoplane	0.00	0.00	0.00	0.00	80.00	0.00	0.00	1.50	0.00	0.00	0.00	0.00
Root Plating	—	—	—	—	++++	—	—	++	—	—	—	—
CHILLI												
Soil	0.00	0.56	0.00	0.00	12.00	0.12	0.00	0.73	0.45	0.00	0.57	0.00
Rhizosphere	0.00	7.60	0.17	0.00	1200.00	80.00	0.00	50.00	0.17	0.00	0.80	0.00
Rhizoplane	0.00	8.30	0.50	0.00	800.00	5.20	0.00	80.00	2.00	0.00	0.00	0.00
Root Plating	—	+	—	—	++++	+	—	+	—	—	—	—

A = At the time of planting; B = 45 days after planting; C = After harvesting
—: = No grawth; +: = growth present

TABLE II. Effect of *Azotobacter* inoclution on the grain/green fruit yield compared to nitrogen application

Treatment	Yiled of crop plants expressed in Kg plot @		
	Ragi Yield/plot	Rice Yield/plot	Chilli Yield/plot
Control (without *Azotobacter* & N)	15.15	0.54	55.50
Azotobacter alone	21.15	0.62	61.50
Half N alone	20.15	0.64	62.50
Ful lN alone	22.25	0.84	65.50
C. D. (P=0.05)	1.78		

@ Average of four replications

inoculated plants not supplied with nitrogen showed several fold increase n *Azotobacter* population. The absence of *Azotobacter* population build *Azotobacter* up in the uninoculated plant rhizosphere supplied with fertilizer nitrogen appears to be due to the intense competition from other heterotrophic bacteria in the presence of plentiful supply of nitrogen in addition to carbon sourec. *Azotobacter*, because of its ability to fix atmospheric nitrogen is likely to thrive best in the practical absence of combined nitrogen and in the presence of large quantity of carbohydrates.

The data on the increase in the final grain or green fruit yield as the case may be due to inoculation paralleled the increase in the population of *Azotobacter* in the rhizosphere and rhizoplane. The yield increase due to inoculation ranged from 10-39 per cent, the least being with rice and the highest being with chilli. The increased yields were statistically significant. The studies indicated the possible need for the photosynthate for the establishment of such association of free-living nitrogen fixing bacteria in rhizosphere and rhizoplane. Hardy and Havelka (1975) have considered the availability of photosynthate as a key factor in the fixation of nitrogen by *Azotobacter*. Bagyaraj and Rangaswami (1968) noted reduced number of *Azotobacter* in the rhizosphere of *Eleusine coracana* with the application of ammonium sulphate. Balasubramanian and Rangaswami (1969) noted reduction in the root exudation of sugars in sorghum when urea or nitrate was applied as foliar spray. They further reported increased exudation of sugars in sorghum by the roots with the application of phosphorus. Their results further indicated the need for plentiful supply of carbohydrates and low nitrogen environment if *Azotobacter* were to thrive in the rhizosphere in competition with other heterotrophic microorganisms and fix nitrogen. It is not also unlikey that the growth promoting substances produced by *Azotobacter* contributed to increased yields as suggested by Brown (1974).

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