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The role of *Azotobacter* in the production of oil Seeds crops

Tej Prakash[1*] and N K Singh[2]
[1]P.G. Department of Botany, Dr Ashok Kumar Smarak PG College,
Akbarpur, Ambedkar Nagar (U.P.), India
[2]P.G. Department of Botany, M.L.K.P.G College, Balrampur (U.P.), India
*Corresponding author: Tej Prakash

Abstract

In the present context, the best alternative of chemical fertilizer is necessary because of its advance effects on the soil health. There are several alternatives available to enhance the soil fertility one of them *Azotobacter*. It is face-living N_2- Fixer diazatroph that has several beneficial effects on the crop growth and yield. It helps in the synthesis of growth regulating substances like. It stimulates the Rhizospheric microbes, Protects the plant form phyto-pathogens improve nutrient uptake and utility boost up biological nitrogen fixation.

Keywords: Biofertilizer, diazatroph, *Azotobacter*, phyto-pathogens.

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Introduction

Azotobacter an aerobic soil bacterium, capable of fixing molecular nitrogen was discovered by Beijerinck, in 1901. An attempt to use its capacity to fix free nitrogen, for increasing crop production work first made by Gerlach & Vogel (1902). The considerable work that he accumulated in this connection since then has been reviewed from time to time by several workers. *Azotobacter* is considered to be free living, however, there are some reports showing its associative symbiosis. *Azotobacter* activates rhizosphere and favours plants growths. The fixation of nitrogen by *Azotobacter* is brought about by a system of enzymes designated as “azotase”. Nitrogenase, one of the components of this system is capable of combining directly with elementary nitrogen. Use of seeds treated with *Azotobacter* has been shown to increase the yield in a variety of crops including rice, wheat and pea.

Azotobacter chroococcum, a widely distributed species, is found in almost all cultivable soils. It is considered more useful for agriculture than other species. Its different strains vary in their nitrogen fixing capabilities which ranges from 0-25 mg N/g sucrose consumed. Till recently the strains capable of fixing more than 10mg N/g sucrose consumed were selected for the purpose of inoculation. However, since many strains fixing ever lower amount of nitrogen were found to enhance growth and yield in some crops with nitrogen fixing capacity is now not taken as sole criterion for selection of strains for inoculation.

Materials and Methods

Determination of population of *Azotobacter* in rhizosphere and non rhizosphere soils

A number of sites were identified in and around Ayodhya and a number of agricultural fields raising diverse crops were marked out at each site. Roots of different crops along with adhering soil were collected from different spots in a field and merged into a composite sample.

Roots of five plants were selected at – random form each composite sample and processed for estimating of *Azotobacter* in the rhizosphere soil.

Samples of non-rhizosphere soil were collected aseptically from non-cropped areas of the fields leaving surface soil upto a depth of 5 cm. The samples of each fields were composited and mixed thoroughly. A working sample was drawn from the composite sample and processed for estimating the population of *Azotobacter* in the non-rhizosphere soil.

Samples of roots and non-rhizosphere soil were collected when the crops were at their seedling, vegetative, flowering and fruiting stages. A fortnightly record of relative humidity and temperature was maintained throughout the year.

Roots were gently shaken to remove superfluous soil. They were then transferred along with adhering soil particles into a flask containing sterilized distilled water. After thorough shaking of the flask the roots were removed and suitable dilutions were made from the suspension containing rhizosphere soil.

Soil dilution and plate count method of Timonin (1940) was used for estimating population of *Azotobacter* in the rhizosphere of different crops. Ten ml of the suspension containing rhizosphere soil was transferred to a flask containing 90ml of sterilized distilled water. The diluted suspension was further diluted a number of times in the same way so as to get the final dilution. The final dilution used for planting was selected on the basis of counts obtained in the preliminary experiments and varied form 1/100 to 1/1000. Usually the dilutions which gave counts between 20-30 colonies per petriplate were employed .

0.5 ml aliquots from the flasks containing final dilution were poured in sterilezed petripaltes containing 10 ml of jensen's agar medium (Sucrose , 20g; KH_2HPO_4, 1.0g; $MgSO_4\,7H_2O$, 0.5g ; NaCl, 0.5g; $FeSO_4$, 0.1g; Na_2MoO_4, 0.005g; $CaCo_3$, 2g; Agar, 15g; Distilled water 1000ml). Five replicates were taken in each case. The dishes were rotated by hand in broad, swirling motion to distribute the suspension over by hand in broad, swiriling motion to distribute the suspension over the medium and incubated at 20 ± 2^0C, usually for 24 to 48 hours . The colonies of *Azotobacter* showing light brown to black pigment were marked out and counted.

For the quantitative estimation of the population of *Azotobacter*, weight of the soil present in the final dilution was determined. For this, a know volume of diluted suspension was evaporated on a water bath. The soil residue was dried to a constant weight in an oven at 80^0C and weighed. The population of *Azotobacter/g* oven dry rhizosphere soil was computed on the basis of the soil in the diluted suspension and the number of colonies appeared on the petriplates.

For determining the population of *Azotobacter* in the non-rhizosphere soil, suspension was prepared and plated on petriplates containing Jensen's agar medium. The working sample of non-rhizosphere soil was transferred into a flask containing 100ml of sterilized distilled water. After thorough shaking of the flask, suitable dilutions were made from the suspension containing non-rhizosphere soil. 0.5ml aliquots of the final dilution selected on the basis of preliminary experiments were planted on petriplates containing Jensen's agar medium. The population of Azotobacter/g oven dry non-rhizosphere soil was computed on the basis of weight of the soil and number of colonies appeared on the petriplates following the procedure described for the rhizosphere soil.

The data on population of Azotobacter in rhizosphere and non-rhizosphere soil were statistically analysed following method outlined by Panse and Sukhatme (1985). The minimum difference required for significance of 5% level was recorded.

Isolation and maintenance of isolates of *A. Chroococum*

All the colonies of Azotobacter which appeared on the petriplates from the suspension of rhizosphere or non-rhizosphere soils were examined carefully. The colonies of *A. chroococcum* were identified and marked out on the basis of specific morphological characteristics (average cell size, 2.4 x 9.5μm, presence of microcysts; peritrichous flagellation, brown to black insoluble pigments). They were inoculated on slants of Jensen's agar medium and re-examined critically. Thirty different isolates were identified on the basis of the morphological characters and designated as $SCSA_{1,}$ $SCSA_{2,}$ $SCSA_{3,.}$ $SCSA_{30.}$ they were maintained for detailed study on their nitrogen fixing potential and effect on the performance of selected crops.

Results

Population of Azotobacters in rhizospher and non-rhizosphere soils

The population of Azotobacter in the rhizosphere of 20 crops including 9 kharif crops, [*Abelmoschus esculentus* (L) *Moench, crotolaria juncea* (L) *Lagenaria siceraria* (Molina) *standley, Oryza sativa* (L) *Pennisetum americanum* (L) *Leek Vigna radiate* (L) *Wilezek, Vigna mungo* (L), *Hepper, Sorghum vulgar* (L) *Vigna sinensis* (L)], 7 Rabi Crops. [*Brassica nigra* (L) *Kock, Helianthus annus* (L), *Linum usitatissimum* L., *Luffa acutangula* (L), *Roxby Lycopersicon esculentum Mill, Sesamum indicum* (L) and *Triticum aestivum*)] and 4 others [*Capsicum melongena* (L) *Carica papaya*(L) *Ricinus communis* (L) and *Solanum melongena* (L)] at seedling; vegetative flowering and fruiting states of growth is presented in Table-01. The population of *Azotobacter* in the rhizosphere as well as non-rhizosphere soils fluctuated with time. However a comparison shows that in general, their average population of 4 samples in the rhizosphere of the crop plant was higher than that in the non-rhizosphere soil. Out of twenty crops, only two belonging to Kharif season (*Oryza sativa* and *Pennisetum americanum*) showed a lower population in their rhizosphere as compared to non-rhizosphere soil. Although all the crops but for these two showed an improved population of *Azotobacter* in their rhizosphere, the extent of improvement varied with the crops. However, amongst Kharif crops *Abelmoschus esculentus, Crotolara juncea, Vegna radiate, Vigna mungo,* amongst Rabi Crops, *Linum usitatissimum, Lycopersicon esculentum* and *Sesamum indicum* and amongst other crops *Capsicum frutescens* and *Solanum melongena* showed comparatively better improvement in the population.

A variety of *Azotobacter* appeared to be present in the non-rhizosphere as well as rhizosphere soils, however, the forms belonging to *A. chroococcum* were isolated and identified on the basis of specific morphological characteristics. Thirty different isolates were marked out and designated as $SCSA_{1,}$ $SCSA_{2,}$ $SCSA_{3,}$ $SCSA_{4.,}$ $SCSA_{30}$. They were evaluated for their potentiality to fix nitrogen under in vitro conditions. The potent nitrogen fixers were further evaluated for their efficacy in improving the perform once of two oilseed crops viz. linseed and seasamum.

Table : 01 Population of Azotobacter in the Rhizosphere of different crops at various stages of growth (Population in 1 x 10^4/g oven dry soil)

Crops	Stages of Growth				
	Seedling	Vegetative	Flowering	Fruting	Average
Rabi Crops					
Brassica nigra	5.8	5.1	5.9	5.5	5.5
Helianthus annus	3.9	4.6	5.9	7.4	5.5
Linum usitatissimum	8.9	12.3	5.8	17.7	11.2
Luffa acutangula	5.9	3.3	5.8	4.7	4.9
Lycopersicon esculentum	13.6	9.5	3.2	8.3	8.7
Sesamum indicum	10.1	16.0	19.2	21.5	16.8
Triticum aestivum	2.2	4.5	5.1	4.5	4.1
Non rhizoshper soil	3.5	3.1	3.1	3.7	3.4
Kharif Crops					
Vigna mumgo	13.1	15.7	13.1	12.1	13.5
Vigna Sinesis	3.1	3.1	4.1	6.3	4.1
Sorghum vulgare	7.2	9.5	7.5	6.9	7.7
Abelmoschus esculentus	15.7	11.5	10.4	8.8	11.6
Crotolaria juncea	12.1	10.1	28.0	12.3	15.6
Lagenaria siceraria	4.8	7.2	11.9	14.2	9.5
Oryza sativa	2.8	2.7	3.1	3.4	3.0
Pennisetum americanum	1.9	2.5	2.2	1.0	2.1
Non-rhizoshper soil	2.5	4.1	3.6	3.5	3.4
others					
Ricinus communis	5.3	8.3	6.4	8.2	7.1
Solanum melongena	8.4	15.6	11.5	10.6	11.5
Carica papaya	2.0	4.2	5.8	19.6	7.9
Capsicum frutescens	6.8	12.1	19.9	19.7	14.6
Non-rhizosphere soil	2.3	3.0	2.8	3.2	2.8

Minimum difference required for significance (C.D.) at 5% level:

seedling	0.304	Flowering	0.177
Vegetative	0.171	Fruiting	0.358

Discussion and Conclusion

In 1904, Hiltner coined the term rhizosphere to denote the region of the soil subject to the influence of plant roots and characterized by an intense microbial activity. Many workers have subsequently shown that the micro flora of the rhizoshpere differs both qualitatively and quantitatively from that in the soil beyond the influence of the roots (*Buxto, 1957, Rangaswami, 1968, Rovira, 1969, Richards, 1974).* The increased microbial activity in the rhizosphere has been attributed to the extra nutrients available in the region especially the root exudates. The microbes of the rhizosphere could influence plant growth in many ways as all plant nutrients pass through the region. Attempts have been made to alter the rhizosphere microflora through soil amendments and foliar sprays for the benefit of plant growth.

In view of its superiority in the improving the yield in both the varieties of linseed and sesamum, isolates $SCSA_6$ was identified as the most suitable microbial inoculants for the oil seed crops included in the present study. This isolate was used as a component in subsequent studies directed to devise as integrated package of biofertilizers for the two crops.

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