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Impact of biovolume inocula of A*zospirillum* spp. on winter wheat, oat and maize in semi-arid region of North Gujarat

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ABSTRACT

Changes in biovolume inocula of *Azospirillum* bacteria were studied in soil under winter wheat, oat and maize crops. The cereal seeds were treated with fungicides and inoculated with *Azospirillum brasilense* actively fixing dinitrogen. Plants were grown without nitrogen fertiliser and with nitrogen application in the amount of 60 and 120 kg N ha-1 for wheat, 50 and 100 kg N ha-1 for oats and 100 and 200 kg N ha-1 for maize. The population number of bacteria was estimated at different developmental stages of plants. Inoculation of cereals with *Azospirillum brasilense* bacteria contributed to the increase of their numbers in soil. No significant influence of fungicidal seed dressings on the numbers of *Azospirillum* bacteria has been noted. The application of mineral nitrogen to the crops was favourable to multiplication of *Azospirillum* bacteria under plants.

KEY-WORDS

Azospirillum brasilense;
Diazotrophs;
Endophyts;
Inoculation;
Oats;
Wheat;
Maize;
Fungicides.

INTRODUCTION

In connection with numerous manifestations of a beneficial action of *Azospirillum* bacteria on plants, since the beginning of studies on bacterial species of this genus attempts have been made to use them practically in agriculture through inoculation of crops with these bacteria. The effect of inoculation with *Azospirillum* strains on the yield of cultivated plants was demonstrated many times, particularly under conditions of tropical climate. The effect of inoculation to a large degree dependent on the ability of microorganisms to survive in the soil. Their survival is determined by such factors, as chemical and granulometric composition of soil, temperature, pH, presence of pesticides, plants and other microorganisms.

The two species – *Azospirillum brasilense* and *Azospirillum lipoferum* are found in soils of the temperate zone[9,15] and even in the zone of the cold climate of Finland[14]. *Azospirillum brasilense* is attributed to have affiliation with plants with photosynthesis of type C3 (wheat), while *Azospirillum lipoferum* is considered to have affiliation with plants of type C4 (maize).

The aim of the present paper was to study the effect of inoculation of winter wheat, oats and maize with *Azospirillum* strain on the numbers of bacteria from the

genus *Azospirillum* at different developmental stages of plants on the background of varying nitrogen application and plant protection with fungicidal seed dressings.

MATERIALS AND METHODS

Study area and soil

Two study areas were selected in Patan District of Gujarat; (a) Dharmoda in Chanasama taluka, (b) Sabadalpura in Santalpur taluka. Nature of soils is mainly semi-arid to dry; which was characterized by lack of moisture.

Bacterial cultures

Azotobacter brasiliense and *Azospirillum lipoferum* was isolated from soil of same district, Vanasan in Sidhdhapur taluka. The mixed culture prepared by rhizosphere soil of field grown forage grass, in 500 ml Nitrogen-free sugar media. Sucrose media was incubated for 24 h at room temperature ($28^0 \pm 2^0$C) on a rotary shaker with speed of 150 rpm.

Preparation of inocula

The cultures of *Aztobacter brasiliense* and *Azospirillum lipoferum* were multiplied in Nitrogen-free malate and sucrose broth media respectively, containing 100 mg yeast extract per liter. After inoculation, the media incubated at 33^0C for the three days on an incubator-cum-rotary shaker. Liquid inoculum was prepared by thoroughly mixing in unchlorinated tap water and using with every 2nd watering in field with showing and after thinning the plant 20-25 days after sowing per meter 100-105 ml culture suspension was added at raw length.

Soil analysis

Soil physico-chemical analysis including (Misra, 1986) pH by Orion pH meter, Organic Carbon (Walkey Black method) and Total Nitrogen (By Micro-Kjeldahal method).

Inoculation was performed just before sowing

Treated and untreated seeds of cereals were mixed with a mixture of bacteria and sprayed over the field (soil in rows was mixed with coulters of a grain drill). Additionally, the field was sprayed with the suspension of *Azospirillum brasilense* bacteria after the emergences, whereas in the case of maize the suspension was applied individually under each plant as a strong stream in the vicinity of roots at the stage of 2-3 leaves. The number of *Azospirillum* cells in the suspension amounted to 108 - 109 (c.f.u.). per 1 ml. Such methods were used many times by other authors in their field experiments[1,11,24,28,29,32].

The applied levels of nitrogen fertlizer are: Nl – no fertilization (control), N2 - half the full dose, N3 – full dose, which in terms of a pure ingredient was 120 kg N ha-1 for wheat, 100 kg N ha-1 for oats and 200 kg N ha-1 Nitrogen was applied in the form of ammonium nitrate.

The applied seed dressings were: Baytan Universal 19.5 DS (active ingredients: 2% fuberidazole, 2.5% imazalil, 15% triadimenol) for wheat, Oxafun T (active ingredients: 37.5% carboxine, 37.5% thiuram) for oats, Vitavax 200FS (active ingredients: 20.0% carboxine, 20.0% thiuram) for corn. The cereal crops were grown on the soils developed from loamy sand glacial material with a medium content of potassium, phosphorus and magnesium.

The numbers of *Azospirillum* bacteria under wheat and oat crops in the vegetation season were estimated five times at the tillering, shooting, heading, flowering and milk-dough stage. In soil under corn these analyses were carried out four times - at the stages of: 3-4 leaves, 7-9 leaves, tasselling and flowering. The obtained mean number of bacteria was counted per 1 g of dry weight of soil. To estimate the population of *Azospirillum* the method of subsequent dilutions, with five replications was used. In each soil sample bacterial counts were estimated as index for bacterial growth by MPN method using tubes containing 5 cm3 semisolid nitrogen-free medium[30] The most probable number of microorganisms was obtained from McCrady's tables.

RESULTS AND DISCUSSION

The number of *Azospirillum* bacteria was analysed from the point of view of the action of seed dressing, nitrogen fertilizer and developmental plant stage on these bacteria in crops inoculated and non-inoculated with *Azospirillum brasilense.* Results of the studies concerning this question are presented in TABLE 1 and Figures 1-3.

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TABLE 1 : The numbers *of Azospirillum* bacteria under cereals in inoculated and non-inoculated combinations.

Plant	Azosprillium in 1 g dry wt		
	Inoculated	Noninoculated	Difference
YEAR-I			
Wheat	63	75	12
Oats	189	106	83
Maize	186	52	134
YEAR-II			
Wheat	485	190	295
Oats	340	210	130
Maize	90	61	29

As follows from our studies, inoculation of crops with *Azospirillum* contributed to soil enrichment in these bacteria. The number of *Azospirillum* was nearly always higher in cereal crops inoculated with these bacteria (TABLE 1). Species and developmental stage of a cultivated plant had their influence on multiplication of bacteria. A factor determining *Azospirillum* numbers may also be weather conditions, which is indicated by varying numbers of bacteria under the same crops in different years of the experiment. Second year was considerably more favourable to the development of *Azospirillum* under wheat and oats. The mean number of these bacteria was several times higher than in first year.

TABLE 2 : Soil analysis of cultivars of uninoculated sites

Site	Plot size (hector)	Year	pH	Total N (%gm)		Organic C (%gm)	
				0-10	20-30	0-10	20-30
I	0.691	I	7.6	0.00181±0.00014	0.00265±0.00035	0.490±0.0007	0.500±0.0000
		II	7.0	0.00134±0.00000	0.00257±0.00000	0.630±0.0001	0.870±0.0021
II	1.38	I	7.7	0.00232±0.00002	0.00229±0.00000	0.530±0.0077	0.370±0.0007
		II	7.2	0.00131±0.00019	0.00142±0.00001	0.140±0.0014	0.210±0.0001

TABLE 3 : Soil analysis of cultivars of inoculated sites

Site	Plot size (hector)	Season	pH	Total N (%gm)		Organic C (%gm)	
				0-10	20-30	0-10	20-30
I	0.691	Dry	7.9	0.00274±0.00001	0.00397±0.00001	0.540±0.0007	0.800±0.0000
		Rainy	7.1	0.00173±0.00015	0.00221±0.00000	0.640±0.0000	0.800±0.0000
II	1.38	Dry	7.8	0.00274±0.00002	0.00291±0.00000	0.560±0.0070	0.450±0.0010
		Rainy	7.2	0.00246±0.00001	0.00274±0.00001	1.360±0.0014	1.140±0.0014

TABLE 4 : Grain yield tones / hector

Site	Plot size (hector)	Year	Uninoculated Tones ha^{-1}	Inoculated Tones ha^{-1}
I	0.691	I	1.80	2.02
		II	2.08	3.61
II	1.38	I	0.72	1.72
		II	1.65	3.02

In wheat, during the vegetation season of first year, no significant differences were found in the numbers of *Azospirillum* bacteria between inoculated and non-inoculated combinations. However, in second year, inoculation contributed to a highly significant degree to the population increase *of Azospirillum* bacteria under wheat. At all dates of analyses a higher cell number of these microorganisms was found on the inoculated plots (TABLE 1). In soil under oats the mean numbers of *Azospirillum* in the inoculated combinations were significantly higher in both years of the studies (TABLE 1).

Under maize a markedly larger (over threefold) cell number per 1 g dry weight of soil was found in the inoculated plots. However, a statistical analysis proved the significance of these differences.

The numbers of *Azospirillum* bacteria found in the arid zone of the studied crops were high compared with literature data, being on the level of 104 - 105 cells per 1 g dry weight of soil. Okon[27] reports that bacteria from the genus *Azospirillum* constitute 1-10% of the population of all rhizosphere microorganisms, though some authors consider this number too high, especially under conditions of a temperate climate[26]. A record number of these bacteria was found in the rhizosphere of spring wheat in Brazil (108 cells/g of soil)[3]. Usually, however, the detected numbers of bacteria were smaller and in the case of wheat they were on the level of 103 - 106 cells/g of soil[2,5,25]. The largest divergences con-

cern soils of temperate or winter climate. Under these conditions from 0 to 107 *Azospirillum* cells were found per 1 g dry weight of soil[9,15,19,22,31].

The applied seed dressings (Baytan, Vitavax, Oxafun) did not largerly effect *Azospirillum* bacterium population under wheat, oat and maize crops (Figure 1). The lack of significant differences between treated and untreated combinations is also confirmed by statistic analysis ($\alpha = 0.05$) (not attached here for that reason).

Only in 1997, in the case of both inoculated and noninoculated wheat crops as well as inoculated maize a markedly larger, but statistically also an insignificant number of *Azospirillum* bacteria was observed under treated plants (Figure 1).

In the case of wheat in second year, the cause of decline in the number of *Azospirillum* in combinations with full nitrogen fertilizer was most probably a heavy plant infestation with fungal diseases, and in this connection - a lower plant vigour and as a consequence - a smaller amount of root exudates. As known from literature, dinitrogen fixing bacteria develop successfully in the presence of mineral nitrogen, if they have an ensured source of carbon. Sawicka[30] obtained results similar to ours from the present experiment concerning *Azotobacter* bacteria, the number of which increased with an increase of mineral nitrogen concentration in the soil. It follows from the studies of many authors[16-18,23] that application of high rates of mineral nitrogen decreases the number of diazotrophs or eliminates them from the radical zone of plants, but according to the Kalininskaja T.A.Kalininskaja[17] the most sensitive appeared to be *Azotobacter* and *Azospirillum* bacteria These relationships are not supported by results of the experimental works, in which cereal inoculation with *Azospirillum* bacteria and, therefore, their presence, contributed to a significant increase of plant yield even at the application of nitrogen fertilizer at 80 kg N ha-1[28] and at a higher rate[29].

Results of the conducted studies concerning the influence of developmental plant stages on the numbers of *Azospirillum* bacteria are presented. In first year, no relationships were found between the developmental stage of cereals and occurrence of *Azospirillum* bacteria in soil under these plants. However, the number of *Azospirillum*in soil under wheat and oats grown in second year increased with plant growth and development achieving its maximum value at the moment of flowering, after which it decreased with seed setting and maturation.

That is probably related with the development of root system and photosynthetic activity. This relationship was supported by an earlier decline in the numbers *of Azospirillum* bacteria in untreated combinations, which is caused by their faster drying induced by a stronger infestation with fungal diseases. The dependence of bacterium number on plant developmental stage was also recorded by Sawicka[30]. The numbers of *Azospirillum* bacteria under corn developed in a different way.

In the case of that plant no expected increase in the numbers of *Azospirillum* bacteria was recorded as they passed to subsequent developmental stages, whereas a decline in *Azospirillum* numbers was recorded at the flowering stage. A similar relationship with regard to oats and spring barley was reported[19,21]. Maize is a species favourable to the occurrence and development of *Azospirillum* and other microorganisms on account of a large amount of radical exudatives, characteristic of plants with a photosynthesis cycle. These bacteria numerously colonise root surfaces and intercellular spaces, finding there very suitable association conditions[10]. In the present experimental work the numbers of *Azospirillum* bacteria were estimated in soil near plant roots, but not directly on roots, and may be for that reason the influence of plant developmental stage on the number of studied bacteria has not been perceived, particularly because with plant growth, the main root mass and rhizosphere microorganisms together with it, move into the depth of soil profile.

CONCLUSIONS

Based on the results of this work the following conclusions could be given:

1. Inoculation of soil and cereal seed material with *Azospirillum brasilense* strain can contribute to an in crease in the numbers of these bacteria in soils of temperate climate.
2. Species and developmental stage of a cultivated plant are factors, which determine, to a sufficient degree, the number of bacteria from the genus

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Azospirillum occurring in a crop.

3. The presence of mineral nitrogen in soil can favour multiplication of *Azospirillum* bacteria under cereal crops.

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