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Isolation and Evaluation of *Azotobacter* spp. from Different Crop Rhizosphere

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A B S T R A C T

In this study, eleven soil samples were collected from Terai zone of West Bengal. The *Azotobacter* were isolated by serial dilution method from each sample in specific *Azotobacter* agar (Mannitol) and incubated at 28±2 °C for 48 hour than kept in refrigerator. The best strains of *Azotobacter* were evaluated by *in-vitro* and *in-vivo* screening. The highest population and nitrogen fixation ability was recorded in rhizosphere soil of Mathura tea garden designated as UBAZ-5 ($7.3x10^9$). UBAZ-5 was also found significantly superior in physical characteristics of chilli plant. The shoot length (12.03 cm), root length (14.33 cm), shoot fresh weight (1.26 g) and root fresh weight (0.55 g), shoot dry weight (0.30 g) and root dry weight (0.21 g) of UBAZ-5 bioinoculated chilli plants were found to be superior to un-inoculated check. Hence, use of bioinoculant will increase the physical characteristics and further increases crop productivity.

Introduction

In recent years there is high negative impact due to the usage of agro-chemical on both human and environment, challenging the agriculturalists demanding for an alternative approach to combat it. Biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture which is more eco-friendly and natural. They contain useful microorganisms which could colonize the rhizosphere and promote plant growth through increasing the supply or availability of essential nutrients to the plants (Vessey, 2003). Nitrogen is an important component which provided as chemical fertilizer and possesses hazards to soil health. *Azotobacter* is free living symbiotic N_2 fixing biofertilizer, which fix atmospheric N_2 to its available forms *i.e.* nitrate form. Besides N_2 fixation, *Azotobacter* synthesizes and secretes considerable amounts of biologically active substances like B vitamins, nicotinic acid, pantothenic acid, biotin, heteroxins, gibberelins etc. which enhance root growth of plants (Rao, 1986). Another important characteristic of *Azotobacter* association with crop improvement is secretion of ammonia in the rhizosphere in the presence of root exudates, which helps in modification of nutrient uptake by the plants (Narula and Gupta, 1986). Therefore this study was designed to evaluate the influence of *Azotobacter* on

growth and phenology of chilli plants in the Terai zone of West Bengal.

Materials and Methods

Collection of soil

11 soil samples collected from different districts like Coochbehar, Jalpaiguri and Alipuduar of North Bengal from different crop rhizosphere. The soil sample were collected in pored polythene bags and stored at room temperature.

Isolation of *Azotobacter spp.*

The *Azotobacter* spp. was isolated by serial dilution pour plate technique given by Subba Rao, 1986. 1 gm of soil was mixed with 9 ml sterile distilled water properly then from this 1 ml of soil solution was transferred to another 9 ml test-tube. Likewise 10^{-6} dilution was made and transferred to *Azotobacter* specific medium. The *Azotobacter* colonies were large ovoid pleomorphic in shape and this was purified on *Azotobacter* specific medium slant to preserve the culture.

Assessment of viable population

11 isolates of *Azotobacter* spp. (Table 1) viable population were estimated by 3 tube most probable number (MPN) method by Alexander (Cochran, 1950).

Screening of potential *Azotobacter* spp. isolates based on N_2 fixation in broth culture

Total nitrogen fixation by the *Azotobacter* isolates in the growing medium is quantified by the method (Johan Kjeldahl 1883).

$$\text{Total N} = \frac{(T-B) \times 0.05 \times 14 \times \text{Volume of broth}}{\text{Volume taken}}$$

Screening of *Azotobacter* strains in field condition

The nitrogen fixing ability of the isolated *Azotobacter* spp. was determined in garden soil by pot cultivation method by assessed the growth of chilli. The chilli seed were treated with different isolates of *Azotobacter* strains and sown in pro-trays. The control is devoid of inoculums. The pots were watered regularly and the effect of bacterial inoculum on seed germination on 8^{th} day was checked and then the growth of plant root and shoot length was measured at transplanting stage.

The experiment was conducted with chilli variety NS-238 using 11 isolates of *Azotobacter* species, treated seeds was sown in pro-trays under polyhouse condition. According to recommended dose of chilli single super phosphate @ 0.0166 gm/kg of soil, muriate of potash @ 0.0025gm/kg of soil and FYM @ 1:3 ratios mixed with soil. *Azotobacter* isolates were inoculated through seed dressing and sown in pro-trays and non-inoculated seeds were sown as control. Watering to plants was done according to needs. Plants were allowed to grow for 40 days *i.e.* up to transplanting stage. Plant height, root-shoot fresh weight and dry weight were recorded after harvest.

Results and Discussion

The highest population was recorded in rhizospheric soil of UBAZ-5 ($7.3x10^9$) followed by UBAZ-7 ($7.3x\ 10^8$) and UBAZ-1 ($2.3x10^8$) and lowest population was observed in UBAZ-6 ($0.9x10^5$). *Azotobacter* population depends on the factor such as soil temperature and organic matter which degrade plant residues and help in proliferation of micro-organisms in soil (Iswaran and Marwaha, 1981). *Azotobacter* plays an important role in nitrogen fixation. Therefore, *Azotobacter* isolates were evaluated for their nitrogen

fixation ability in broth culture. The highest nitrogen fixation was found in UBAZ-5 followed by UBAZ-3 and UBAZ-10 (37.8, 33.6 and 32.9 mg/100 ml broth culture respectively, after 8 days of inoculation. The rate of increase in nitrogen fixation was also high in UBAZ- 5, UBAZ-3 and UBAZ-10 (Table 2 and Figs. 1 and 2). The soil of Mathura tea garden was found more of organic matter which makes the soil friable and permeates the air and water for growth of micro-organism (Haris, 1981).

The chilli seeds (Variety- NS-238) treated with different isolates of *Azotobacter* spp. were sown in pro-trays to examine the influence of the isolates on seedling growth at transplanting stage. Various physical characteristics like shoot length, root length, root and shoot fresh weight, root and shoot dry weight were measured and the results have been presented in table 3. The shoot lengths of the inoculated transplants *viz.* UBAZ-3, UBAZ-2, UBAZ-1, UBAZ-5, and UBAZ-4 were found significantly higher (14.20cm, 13.30cm, 13.10cm, 13.00cm and 12.03cm, respectively) compared to uninoculated check (9.60 cm). The isolates UBAZ-6, UBAZ-7, UBAZ-10 and UBAZ-11 were found significantly at par. The root length of inoculated transplants UBAZ-10 was significantly high (17.03 cm) followed by UBAZ-11, UBAZ-9 and UBAZ-5 (15.69cm, 14.67cm and 14.33cm, respectively). The effects of other isolates were significantly at par.

The fresh shoot weight was found significantly high in inoculated transplant UBAZ-5 (1.26 g) followed by UBAZ-10, UBAZ-6, UBAZ-7, UBAZ-3 and UBAZ-11(1.16g, 1.15g, 1.15g, 1.14g and 1.00g respectively). The fresh shoot weight of inoculated transplants UBAZ-1, UBAZ-8 and UBAZ-9 were significantly at par. The dry shoot weight of biofortified transplants were found significantly high in UBAZ-5 treated seedlings (0.30gm) followed by UBAZ-8 (0.23g), and UBAZ-3 (0.19g), while in uninoculated transplants the shoot dry weight was 0.14gm. The root fresh weight was found highest in UBAZ-6 (0.79gm) followed by UBAZ-7, UBAZ-10 and UBAZ-5 (0.55gm) which differed significantly from uninoculated control.

Table.1 Detail of *Azotobacter* isolates isolated from different sources, location and MPN value

Isolates	Source	Location
UBAZ-1	*Camelia sinensis*	Cooch Behar tea garden
UBAZ-2	*Beta vulgaris*	Kalimpong
UBAZ-3	*Musa paradisiacal*	Kalimpong
UBAZ-4	Repository of Plant Pathology laboratory	UBKV
UBAZ-5	*Camelia sinensis*	Mathura tea garden
UBAZ-6	*Brassica oleracea*	Kalimpong
UBAZ-7	*Saccharum spontaneum*	Kalimpong
UBAZ-8	*Bambusa vulgaris*	Kalimpong
UBAZ-9	*Camelia sinensis*	Nagarakata tea garden
UBAZ-10	*Camelia sinensis*	Vijaynagar tea garden
UBAZ-11	*Camelia sinensis*	Dalgaon tea garden

Table.2 Variation in nitrogen fixation ability of different *Azotobacter* isolates

Isolates	N2 fixation (mg/100 ml culture broth)		
	3 days after inoculation	5 days after inoculation	8 days after inoculation
UBAZ-1	26.22	27.30	28.00
UBAZ-2	21.56	24.50	25.90
UBAZ-3	26.60	28.70	33.60
UBAZ-4	23.10	23.10	24.50
UBAZ-5	22.40	25.20	37.80
UBAZ-6	22.40	22.40	24.50
UBAZ-7	24.50	21.70	24.50
UBAZ-8	21.00	21.84	25.20
UBAZ-9	19.60	22.40	23.80
UBAZ-10	25.20	25.20	32.90
UBAZ-11	21.70	25.90	30.10
Control	14.00	15.05	14.70
SEm±	0.843	1.071	1.041
CD (P=0.05)	2.487	3.160	3.071

Table.3 Variation in physical attributes of *Azotobacter* treated chilli plants at seedling stage

Isolates	Shoot length (cm)	Root length (cm)	Shoot weight (gm)		Root weight (gm)	
			Fresh	Dry	Fresh	Dry
UBAZ-1	13.10	14.00	0.91	0.17	0.38	0.14
UBAZ-2	13.30	13.33	0.89	0.14	0.32	0.12
UBAZ-3	14.20	14.00	1.14	0.19	0.50	0.21
UBAZ-4	12.03	12.83	1.10	0.16	0.48	0.15
UBAZ-5	13.00	14.33	1.26	0.30	0.55	0.21
UBAZ-6	9.73	12.27	1.15	0.15	0.79	0.14
UBAZ-7	10.10	13.87	1.15	0.17	0.66	0.10
UBAZ-8	8.40	13.63	0.70	0.23	0.39	0.08
UBAZ-9	9.43	14.67	0.87	0.14	0.51	0.08
UBAZ-10	10.37	17.03	1.16	0.16	0.56	0.09
UBAZ-11	9.63	15.67	1.00	0.14	0.49	0.08
Control	9.60	11.67	0.84	0.14	0.37	0.07
SE(m)±	0.591	0.830	0.031	0.012	0.021	0.006
CD at 95%	1.744	2.449	0.090	0.035	0.061	0.019

Fig.1 Population dynamics (MPN) of different *Azotobacter* isolates in rhizosphere soil

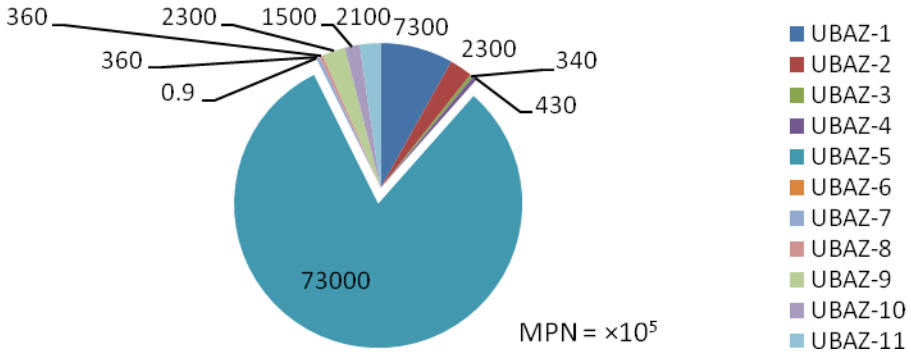
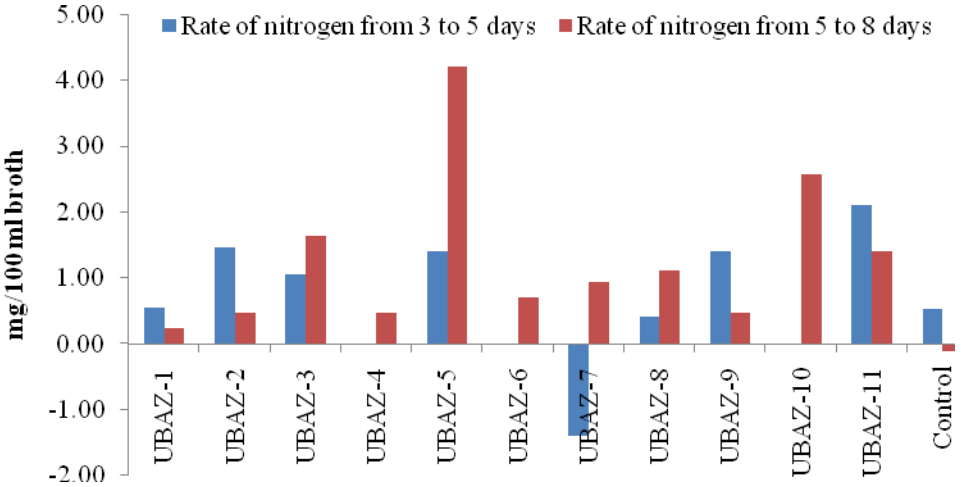


Fig.2 Potential of nitrogen fixation by different *Azotobacter* isolates



The root dry weight was found significantly high in UBAZ-5 and UBAZ-3 (0.21gm) treated transplants followed by UBAZ-4 and UBAZ-1 whereas, the other isolates were found significantly at par in their potential of root growth promotion. Sachin *et al.*, (2004) reported that the inoculation of *Azotobacter chroococcum* had positive effect on the growth parameters of bamboo and maize in pot experiment. Similar result obtained by Kanchana *et al.*, (2014) observed that the plant dry weight of chilli was significantly increased in *Azotobacter* treated plants.

The highest *Azotobacter* population was recorded in rhizospheric soil of Mathura tea garden designated as UBAZ-5 followed by UBAZ-7 and UBAZ-1 of sugarcane and tea rhizosphere from Cooch Behar and Kalimpong, respectively. This variation could be due to differences in the availability of oxygen and/or the availability of essential elements in the soils (Line and Loutit, 1973). *Azotobacter* population depends on factor such as soil temperature and organic matter which degrade plant residues and help in proliferation of the micro-organisms in soil (Iswaran and Marwaha, 1981). The nitrogen fixation and its rate were found highest in UBAZ-5 followed by UBAZ-3 and UBAZ-10 at 8th day of inoculation. The soils of tea gardens have more of organic matter which makes the soil friable and permeate the air and water for better growth of microorganism (Haris, 1981). Difference in capacity of N-fixation by *Azotobacter* spp. had been shown

by Kizilkaya (2009). Sachin *et al*., (2004) reported that the inoculation of *Azotobacter chroococcum* had positive effect on the growth parameters of bamboo and maize in pot experiment. It has been concluded that best *Azotobacter* spp. should be isolated from rhizospheric soil where more of organic matter and rhizosphere were not disturbed which increased the yield potencial in chilli.

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