

Effectiveness of *Azotobacter* bio-inoculant for wheat grown under dryland condition

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Abstract

Judicious use of chemicals along with biofertilizers and organic resources are advocated for sustaining crop productivity and soil health, and meeting a part of fertilizer requirement for different crops. In the present study, different combinations of nitrogen (urea and/or farmyard manure) doses with *Azotobacter* strain (Azo-8) were experimented for a dryland wheat variety GW-273. It was observed that the combination of *Azotobacter* strain (Azo-8) along with urea (60KgN ha⁻¹) and farm yard manure (40KgN ha⁻¹) gave the best response. It resulted in more than 23% and 36% increase in shoot fresh weight and dry weight, 26% and 38% increase in root fresh weight and dry weight, 39% increase in test weight of seeds and 27% increase in yield over control. The results of present experiments can be utilized in integrated nutrient management for wheat cultivation in dryland areas to provide sustainability to the agricultural productivity.

Key words

Azotobacter, Bacterization, Bio-inoculant, Dryland, Wheat

Introduction

Wheat is the most important cereal crop grown in different parts of the world. It is the staple food for over 35% of the global population and provides more calories and proteins in the diet (Laegreid *et al.*, 1999). Most of Indian agricultural lands are deprived of some of the essential nutrients for growth and development of crop plants and one of them is nitrogen. The importance of nitrogen in plants nutrition was first established by the classical work of Boussingault as early as 1838. Nitrogen is required in large quantities by plants for proper growth and yield and is one of the basic constituent of proteins and nucleic acids (Lawlor, 2002). Nitrogen is provided to the cereal crops mainly in the form of synthetic chemical fertilizer, urea (Ladha *et al.*, 2005). Excessive synthetic chemical fertilizers pose a health hazard and adversely affect soil microflora besides being quite expensive and raise the production cost of the marginal farmers (Stefan *et al.*, 2008).

Biofertilizers are inputs containing microorganism

which are capable of mobilizing nutritive elements from complex and non usable form to simple and usable form through biological processes (Cakmakc *et al.*, 2007). *Azotobacter* is a gram negative, aerobic, free-living, heterotrophic, nitrogen-fixing plant growth promoting rhizobacteria (PGPR) which survive in soil for longer period forming cyst and are known to stimulate plant growth either by facilitating the plant's uptake of certain nutrients from the environment or by production of phytohormones (auxins, gibberellins, cytokinins) (Joseph *et al.*, 2007) or enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase (Shaharoona *et al.*, 2006). Moreover, PGPR protect the plants against soil borne phytopathogens by production of antimicrobial metabolites including siderophores *e.g.* production of azotobactin by *Azotobacter vinelandii* (Husen, 2003).

The immediate response to soil inoculation with PGPR varies considerably depending on the bacterium, plant species, soil type, inoculant density and environmental conditions (Bent *et al.*, 2001). Isolation and characterization

of native strains adapted to the local environment may contribute to the formulation of an effective bio-inoculant because; indigenous strains of rhizobacteria generally possess more competitive ability to survive and influence the growth of inoculated plants (Khalid *et al.*, 2004). *Azotobacter* inoculation has been earlier reported to influence seed germination, seedling growth, and increase in yield of cereals upto 30% (Gholami *et al.*, 2009). Therefore, in the present study native strains of *Azotobacter* isolated from the rhizosphere of wheat in order to evaluate their ability to promote wheat growth under dryland condition under different doses of urea and farm yard manure (FYM).

Materials and Methods

Azotobacter bio-inoculant : *Azotobacter* strain (Azo-8), a local isolate from the wheat field of S.D. Agricultural University, Gujarat, India was grown in 100 ml of autoclaved Jensen's broth medium in 500 ml flasks on a rotary shaker at $28 \pm 2^\circ\text{C}$ for 7 days (Kundu and Gaur, 1980). The viable cells in batches of cultures ranged from 0.6×10^9 – 1.0×10^9 cells ml^{-1} . These cultures were then used for preparation of *Azotobacter* bio-inoculant with autoclaved powdered lignite as carrier (Jahuri, 1988).

Field trials and study of plant growth parameters : North Gujarat falls under the semi-arid region and receives an average annual rainfall of about 550 mm. The soil of the experimental plot is of sandy loam type and contains about 83.9% sand, 5.55% silt and 9.83% clay. The field experiments were conducted during the two Rabi seasons of 2008 and 2009 at the agronomy instructional farm of S.D. Agricultural University. The control plot (T_1) was not supplemented with FYM/urea, and the seeds sown in these plots were not treated with bio-inoculant; whereas, the seeds in treatment T_2 were only treated with the bio-inoculant and other fertilizers were not applied. Experimental plots T_6 and T_7 were manured with basal application of FYM (0.5% N) during final land preparation @ of 4.0 and 8.0 t ha^{-1} , respectively. Hence, the various treatments were T_1 (control, N0P0K0), T_2 (Azo-8 only), T_3 (Azo-8 + 20kg N ha^{-1} , urea), T_4 (Azo-8 + 40kg N ha^{-1} , urea), T_5 (Azo-8 + 60kg N ha^{-1} , urea), T_6 (Azo-8 + 60kg N ha^{-1} , urea + 20kg N ha^{-1} , FYM), T_7 (Azo-8 + 60kg N ha^{-1} , urea + 40kg N ha^{-1} , FYM), and T_8 (120kg N ha^{-1} , urea). The nitrogen levels applied through urea/FYM were 0, 20, 40, 60, 80, 100 and 120kg ha^{-1} . Half dose of urea (as per treatments) and entire P (@60 Kg P_2O_5 ha^{-1}) were applied at sowing, while remaining half of urea was applied at 28 days after sowing. The seeds of wheat (*Triticum aestivum*) cv. GW-273 (seed rate 100 kg ha^{-1}) were treated with the prepared *Azotobacter* bio-inoculant (@25 g kg^{-1} of seeds), dried in shade and sown in respective plots as per treatment. The experiment was conducted in a randomised block design with three replications (Panse and Sukhatme, 1957).

The root fresh weight, root dry weight, shoot fresh weight, shoot dry weight at 4 weeks, number of productive tillers m^{-2} at maturity, seed test weight, yields of the wheat crops from the various treatment plots and IAA production by *Azotobacter* isolate was observed. Statistical analysis was done by the method of analysis of variance (Fisher, 1958) and critical difference (CD) was calculated at 5% level of significance using M-STATC software. Rhizospheric soil sample were taken at three weeks intervals from each plot and bacterial population was determined by dilution plating method after 3rd, 6th, 9th, and 12th weeks of sowing. The numbers of *Azotobacter* in suitable dilutions were counted on Jensen's nitrogen free medium.

IAA production : Bacteria were cultured overnight in Luria-Bertani broth in the dark at 30°C . The bacterial cells were removed from the culture medium by centrifugation at 8,000Xg for 10 min. 1 ml of supernatant was mixed vigorously with 2 ml of Salkowski's reagent (4.5 g of FeCl_3 per liter in 10.8 M H_2SO_4) and incubated at room temperature in the dark for 30 min. The absorbance was measured at 535 nm (Benizri *et al.*, 1998). The concentration of IAA was determined by comparison with standard curve and the amount of IAA produced was expressed as ppm.

Results and Discussion

The native strain of *Azotobacter* (Azo-8) was isolated, cultured and used as a bio-inoculant. The number of productive tillers m^{-2} was recorded highest in the treatment T_8 where N was applied only through urea @120 kg N ha^{-1} . However, the yield obtained per hectare was highest in the treatment Azo-8+ 60kg N ha^{-1} (Urea) + 40 kg N ha^{-1} (FYM) (Table 1). The application of nitrogen through FYM and *Azotobacter* (Azo-8) strain played synergistic role along with the nitrogenous fertilizer in increasing wheat yield. With increase in dose of nitrogen application increase in grain yield was observed from treatments T_1 to T_7 . Several studies have revealed the beneficial effects of these bacteria in the improvement of crop growth and yield.

The native strain isolated from a particular region is generally considered better competitor in comparison to non-native strains (Khalid *et al.*, 2004). An important factor to be considered while screening for new isolates is their activity in the environments where they are expected to be used (Ross *et al.*, 2000). *Azotobacter* and graded doses of nitrogen increase phosphorus and potassium uptake by plants significantly (Agrawal *et al.*, 2004). They further concluded that inoculation of *Azotobacter* could save about 20 kg fertilizer nitrogen in wheat crop.

The *Azotobacter* population was recorded in different experimental plots at three weeks interval. Least

Table 1 : Effect of *Azotobacter* bio-inoculant on formation of productive tillers m⁻² and yield (t ha⁻¹) in wheat grown under dryland condition

Treatments		2008		2009	
		No. of productive (tillers m ⁻²)	Yield (t ha ⁻¹)	No. of productive (tillers m ⁻²)	Yield (t ha ⁻¹)
T ₁	Control (N0P0K0)	64.76	2.82	66.94	2.89
T ₂	Azo-8	69.82	3.09	67.92	2.94
T ₃	Azo-8+ 20kg N ha ⁻¹ (Urea)	70.84	3.22	71.66	3.27
T ₄	Azo-8+ 40kg N ha ⁻¹ (Urea)	73.85	3.36	75.21	3.47
T ₅	Azo-8+ 60kg N ha ⁻¹ (Urea)	78.56	3.53	80.14	3.66
T ₆	Azo-8+ 60kg N ha ⁻¹ (Urea) + 20kg N ha ⁻¹ (FYM)	85.75	3.69	86.63	3.77
T ₇	Azo-8+ 60kg N ha ⁻¹ (Urea) + 40kg N ha ⁻¹ (FYM)	91.87	3.91	93.59	4.05
T ₈	120 kg N ha ⁻¹ (Urea)	97.24	3.87	96.16	3.82
	SEM	0.82	0.02	0.38	0.03
	CD @ 5%	2.493	0.074	1.150	0.106
	C.V.	1.800	1.223	0.823	1.737

population count of the bacterium was observed in the control whereas; the highest population was recorded in the treatment with Azo8+60KgN ha⁻¹ (urea) + 40kg N ha⁻¹ (FYM) (Table 2). This may be because *Azotobacter* being a heterotrophic bacteria and thus require organic carbon sources which was abundant in the field supplied with FYM (Narayan and Kehri, 2011). The population of *Azotobacter* in all the treatments increased and reached maximum after 6 weeks of sowing, followed by a decline. Although yield obtained with T₈ was comparable or higher than most of the treatments except T₇ whereas; the *Azotobacter* count in this case was poor than the best treatment. Heavy doses of nitrogenous fertilizers not only adversely affect the activity of the diazotrophs but also their population (Mahajan *et al.*, 2007; Mandic *et al.*, 2005). Higher *Azotobacter* counts have been recorded in the fields treated with mineral fertilizers along with FYM and lower counts in the fields treated with heavy application of mineral fertilizers (Mahajan *et al.*, 2007; Narayan and Kehri, 2011).

Table 2 : Rhizospheric *Azotobacter* population at intervals of three weeks

Treatments	Azotobacter population (x 10 ⁴ cfu g ⁻¹ of rhizospheric soil)			
	3 weeks	6 weeks	9 weeks	12 weeks
T ₁	32	115	85	34
T ₂	125	230	170	73
T ₃	131	243	195	91
T ₄	174	269	201	127
T ₅	240	317	225	180
T ₆	285	347	245	183
T ₇	649	743	465	250
T ₈	143	239	184	124

Note : The values are mean of the bacterial populations recorded for the year 2008 and 2009

In the present experiment, shoot fresh and dry weight increased steadily with increase in nitrogen dose under various treatments. However, this increase stopped at T₇ and a slight reduction in both shoot fresh and dry weight was observed in the treatment with N-application exclusively through urea @120 kg ha⁻¹ (T₈). A more or less similar trend was observed in the case of root fresh and dry weight of the wheat plants. The shoot fresh weight in T₇ observed was 273.8mg and 276.2mg; shoot dry weight 39.2mg and 40.8mg; root fresh weight 124.4mg 129.6mg; and root dry weight 11.9mg and 12.1mg for the two years (Table 3). Fischer *et al.* (2007) reported an increase of 45% in root dry weight and 23% in shoot dry weight with *Azotobacter* (isolate SF4c) bio-inoculant in comparison to that of control. The effects of plant growth promoting bacteria on shoot fresh and dry weight and root fresh and dry weight of wheat plants has also been studied by other workers (Bellis and Ercolani, 2001; Fischer *et al.*, 2007).

The amount of indole acetic acid (IAA) produced by the experimental strain (Azo-8) in the present study was 20.24 ppm. This amount of IAA production closely corresponded to that of many strains of *Azotobacter* (Paul and Verma, 2005). IAA-producing PGPR strains lead to vigorous root growth resulting in greater root surface area and thus enabling the plant to access more nutrients from the soil (Vessey, 2003). The presence of IAA and related compounds could be demonstrated for many diazotrophs like, *Acetobacter diazotrophicus*, *Azospirillum spp.*, *Azotobacter*, and *Paenibacillus polymyxa* (Dobbelaere *et al.*, 2003). Native strains promoted the wheat growth probably because they possessed some PGPR traits such as siderophore or IAA production or solubilization of phosphate (Richardson, 2001).

Test weight (1000 grain weight) observed was highest (42.07g) in the treatment with the bio-inoculant (Azo-8) plus

Table 3 : Effect of *Azotobacter* bio-inoculant on different growth parameters of wheat grown under dryland condition

Treatments	Shoot				Root				1000 grain weight (g)	
	Fresh weight		Dry weight		Fresh weight		Dry weight			
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
T ₁	207.40	212.60	24.20	25.80	86.30	95.70	6.60	7.40	25.22	25.54
T ₂	223.50	218.50	26.90	26.10	99.80	96.20	8.10	7.90	29.83	29.25
T ₃	224.00	232.00	26.20	29.80	101.50	108.50	8.20	8.80	32.15	32.81
T ₄	225.80	238.20	28.30	31.70	104.60	109.40	8.60	9.40	34.42	34.82
T ₅	235.60	244.40	32.50	35.50	107.80	114.20	9.70	10.30	36.78	36.94
T ₆	258.00	266.00	36.60	37.40	112.70	117.30	10.60	11.40	39.53	39.71
T ₇	273.8	276.20	39.20	40.80	124.40	129.60	11.90	12.10	41.96	42.18
T ₈	273.70	268.30	38.90	36.10	122.80	115.20	11.80	11.20	39.32	39.16
SEM	3.10	2.29	0.94	0.67	1.59	2.14	0.13	0.13	0.14	0.09
CD @ 5%	9.408	6.949	2.865	2.041	4.821	6.497	0.395	0.405	0.415	0.273
C.V.	2.236	1.623	5.178	3.543	2.561	3.350	2.390	2.359	0.680	0.446

Note : (1) The values of shoot and root fresh weight and dry weight are in terms of milligram (2) Shoot and root fresh weight and dry weight values show an average of twenty random samples for each replication and treatment (n=3)

60 kg N ha⁻¹ (urea) and 40 kg N ha⁻¹ (FYM); whereas, the least test weight was observed in control (25.38g) (Table 3). Singh and Agarwal (2001) reported that the application of FYM @ 20 t ha⁻¹ recorded significantly higher test weight and seed yield compared to that of control in wheat. Datta *et al.* (2009) also got higher test weight and seed yield with reduced chemical fertilizer dose along with biofertilizer (Azophos) and organic manure (compost). PGPR are known to influence plant growth and development by the production of phytohormones such as auxins, gibberellins, and cytokinins. The rhizobacteria capable of producing growth regulators have stimulatory effect on the plant growth by influencing the increased uptake of N, P, K, Ca and Mg by plants from the soil (Farzana and Radizah, 2005). Enhancement in the number of productive tillers, dry-matter, and grain yield occurs in response to application of *Azotobacter* bio-inoculant (Shaharoon *et al.*, 2006; Yasari and Patwardhan, 2007). However, such growth responses are variable depending on the fertility status of soil and the variety of the crop planted (Subba Rao *et al.*, 1980).

FYM serves to improve the physico-chemical properties of soil and serves to support a higher number of microbial populations which in turn mineralizes the unavailable form of nutrients and thus make them available to the plants. Such beneficial microorganisms produce several growth factors and phytohormones favourable for plant health and yield (Dobbela *et al.*, 2003). FYM and microbial culture improve the microclimate of the rhizospheric region. FYM support a wide variety of microorganisms which compete and suppress many of the harmful soil borne plant pathogens. The increased N-uptake and better utilization of added manures and fertilizers by plants due to these bacteria might be one of the causes for

increase in shoot and root dry weight and seed test weight of wheat crop (Yasari and Patwardhan, 2007).

The results revealed that seed inoculation with *Azotobacter* significantly increased the growth parameters viz. tillers, dry-matter accumulation and grain yield of wheat. Seed bacterization with Azo-8 plus 60 kg N ha⁻¹ (urea) and @ 40 kg N ha⁻¹ (FYM) was found to be the most responsive treatment. The result clearly indicated that there is a saving of 20 kg N ha⁻¹, if *Azotobacter* (strain Azo-8) culture was used (seed treatment) along with 60Kg N ha⁻¹ through urea and 40 kg N ha⁻¹ through FYM. 75 % nutrient supply through chemical fertilizers and 25 % through organic sources resulted in equal yield as obtained by application of cent per cent nitrogen, phosphorus and potassium in Maize-wheat cropping system (Pathak *et al.*, 2002). An improvement in crop performance might be attributed to the N₂-fixing and phosphate solubilising capacity of *Azotobacter* as well as the ability of these microorganisms to produce growth promoting substances (Salantur *et al.*, 2006).

The existence of favourable nutritional environment under synergistic influence of biofertilizers, FYM and inorganic fertilizers possibly have favourable impact on the vegetative and reproductive growth, which ultimately lead to the realization of higher yield (Pathak *et al.*, 2002). PGPR traits are supposed to be commonly distributed among many different species and genera of microorganisms, many of which are native members of the soil microbial community; and most frequently the action of such native strains are multidimensional (Martinez-Viveros *et al.*, 2010). The current observation suggest that inoculation of *Azotobacter* could save about 20 kg fertilizer nitrogen in wheat crop and still result in better plant performance and higher *Azotobacter*

population in soil. Hence, it is concluded that the bacterial fertilizers act as a supplement to the chemical fertilizers and farmyard manure for better plant performance. This study further reflect the importance of simultaneous screening of indigenous rhizobacterial strains for growth and yield promotion under pot and field experiment as a tool to select efficient PGPR for bio-fertilizer development strategy. This may be helpful in reducing the cost of cultivation and simultaneously contribute to save the agroecosystems from getting polluted.

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