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Response of winter wheat (*Triticum aestivum* L.) to inoculation with *Azospirillum* under different nitrogen levels

Hossein Soleimanzadeh¹, Tooraj Mir-Mahmoodi^{2*}

¹Department of Agronomy and Plant Breeding, Pars Abad Moghan Branch, Islamic Azad University, Pars Abad Moghan, Iran

²Department of Agronomy and Plant Breeding, Mahabad Branch, Islamic Azad University, Mahabad, Iran

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Abstract

In order to evaluate the effects of inoculants and chemical fertilizer on yield and yield components, a bread wheat cultivar treated with *Azospirillum* and nitrogen chemical fertilizer by using factorial on the basis of randomized complete block design with three replications in Research Farm of College of Agriculture, Islamic Azad University, Pars Abad Moghan Branch during 2010 growing season. Four nitrogen fertilizer levels of 25%, 50% 75% and 100% N recommended with two levels of *Azospirillum*: with and without *Azospirillum* (control) were assigned in a factorial combination. Results showed that plant height, ear length, grain number per ear, 1000-grain weight, grain yield and biological yield were significantly higher in inoculated plants than in non-inoculated plants. These traits increased with increasing N level above 75% N recommended in non-inoculated plants, whereas no significant difference was observed between 75% and 100% N recommended. The positive effect of *Azospirillum* inoculation decreased with increasing N levels. According to the results of this experiment, application of *Azospirillum* in present of 50% N recommended had an appropriate performance and could increase grain yield to an acceptable level, so it could be considered as a suitable substitute for chemical nitrogen fertilizer in organic agricultural systems.

*Corresponding Author: Tooraj Mir-Mahmoodi ✉ toraj73@yahoo.com

Introduction

Modern agriculture system is completely dependent upon the supply of chemical fertilizers, though they are becoming scarcer and more costly. These are major agents for pollution of water and air. The high cost of chemical nitrogenous fertilizers and the low purchasing power of most of the farmers restricts its use in proper amounts, hampering crop production. Besides, a substantial amount of the urea-N is lost through different mechanisms including ammonia volatilization, denitrification and leaching losses, causing environmental pollution problems (De Datta and Buresh, 1989; Choudhury and Kennedy, 2005). Hence, the efficiency of added urea-N is very low, often only 30-40% and, in some cases, even lower (De Datta, 1978; Choudhury and Khanif 2001, 2004). The application of Plant growth promoting rhizobacteria (PGPR) can decrease the use of urea-N, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent (Jeyabal and Kuppuswamy, 2001; Choudhury and Kennedy, 2004; Kennedy *et al.*, 2004).

Plant growth promoting rhizobacteria (PGPR) are free living soil-borne bacteria that colonize the rhizosphere and when applied to seed or crops, enhance the growth of plants (Kloepper *et al.*, 1980). They have been reported to increase the percentage seed germination, emergence, shoot growth, root growth, total biomass of the plants, induce early flowering and increase the grain yield (Ramamoorthy, 2001). Among them are strains from genera such as *Azospirillum* (Rodriguez and Fraga, 1999). These improvements in growth attributes of plants caused by PGPR are brought about due to their potential of nitrogen fixation and production of phytohormones like auxin, gibberellins, cytokinin, and phosphate solubilization, resulting in the availability of nutrients to plants and increase in roots permeability.

Bashan *et al.*, (2004) and Cakmak *et al.*, (2006) reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in total plant biomass, nutrient uptake, plant height, leaf size, leaf area index

and root length of cereals (Bashan *et al.*, 2004). Dilduza, (2007) suggested that inoculation of corn seeds with *Azospirillum brasilense* increased dry matter accumulation. Omar, (1998) reported a significant increase in the dry matter yield of wheat due to seed priming by PGPR. Zaidi and Khan, (2005) have suggested that seed priming with PGPR increased dry matter accumulation and grain yield of wheat. *Azospirillum* inoculation increased dry matter by 40% in *Triticum aestivum* (Bashan, 1998). Murty and Ladha, (1987) found that inoculation of *A. lipoferum* to rice roots significantly increased shoot fresh and dry weights. Trials with Plant growth-promoting rhizobacteria indicated that yield and dry matter accumulation increase in rice (Sudha *et al.*, 1999), barley (Cakmak *et al.*, 2001 ; Fiahin *et al.*, 2004), wheat (De Freitas., 2000; Cakmak *et al.*, 2007), and sugarcane (Sundara *et al.*, 2002).

At present, the government in Iran is heavily subsidizing mineral fertilizers for wheat and offers guarantee prices to achieve the national policy on self sufficiency for wheat. Besides environmental concerns of the use of high rates of chemical fertilizers, agricultural subsidies put a high burden on Iran's economy. There is now a shift in that policy towards more market-orientation and there are plans to reduce subsidies on fertilizers. The aim of the present study is to learn the influence of *Azospirillum* bacteria and nitrogen fertilizer on yield and yield components of winter wheat in Pars Abad Moghan (north of Iran) conditions.

Materials and methods

Experimental Design and Plant Materials

The experiment was initiated in Research Farm of College of Agriculture, Islamic Azad University, Pars Abad Moghan Branch during 2010 growing season. Pars Abad Moghan is classified among the temperate climatic regions in the country with average rainfall of 389.5 mm per year. The height of the experimental site from sea level was 50m. The mean annual temperature was 15 °C while the mean maximum and minimum temperatures were 31.4 and 1.4°C, respectively. The soil physical and chemical

characteristic of the experimental area is presented in Table 1.

The experimental treatments were arranged as factorial on the basis of a Randomized Complete Block Design with three replications. Treatments were included four levels of nitrogen chemical fertilizer (urea) consisting of $N_1 = 25\%$ (50 N kg.ha^{-1}), $N_2 = 50\%$ (100 N kg.ha^{-1}), $N_3 = 75\%$ (150 N kg.ha^{-1}) and $N_4 = 100\%$ N recommended (200 N kg.ha^{-1}) and two levels of *Azospirillum* (without and with inoculation by *Azospirillum lipoferum*) on winter wheat (*Triticum aestivum* L. cv. pishtaz).

For *Azospirillum* inoculation, wheat seeds were first treated with traditional jaggery or molasses solution prior to treatment with charcoal based *Azospirillum lipoferum* in a beaker and shaken thoroughly to facilitate uniform coating of seeds with the inoculum using colony forming units (CFU) 10^9 cells/ml. CFU was determined by plate count method. *Azospirillum* treated seeds were kept under shade for about half hour for drying before sowing so that *Azospirillum inoculum* could adhere to seeds nicely. Plots were sown on 21 November 2010 with a cone seeder and were 6 m long and 2 m wide, with 10 rows 20 cm apart.

Grain Yield and yield components measurements

Aboveground dry matter production was measured by making cutting at ground level in 0.5 m^2 quadrants per plot. Immediately prior to harvest, number of spikes per m^2 was determined by averaging three counts of 1-m sections of rows with in each plot. The

number of kernel per spikes was determined from 20 spikes taken at random from a 1 m section of each plot and counted with an electronic seed counter. And average kernel weight was determined by weighing 500 kernels randomly drawn from the bulk grain sample from each plot. The central eight rows (of 10 rows) of each plot were harvested for grain yield and converted to grain yield per hectares. Harvest indexes (HI) were calculated using yield from the square meter samples. Data were analyzed by analysis of variance [31]. When significant differences were found ($P=0.05$) among means, Duncan's multiple range test (DMRT) were applied.

Results and discussion

Plant Height

The analysis of variance (Table 2) showed significant effect of nitrogen fertilizer and *Azospirillum* on plant height. It seems that nitrogen plays an important role in enhancement of plant height. The application of chemical nitrogen fertilizer and *Azospirillum* had significant effect to increase the height. In general, the maximum plant height (94.6 cm) was obtained to seed inoculation with *Azospirillum*, while the least value (91.4 cm) was recorded at without inoculation. Similar results have been reported by Kader *et al.*, (2002). They reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as plant height. Means of comparisons for nitrogen levels indicated the maximum (98.6 cm) plant height was recorded for N_4 and minimum value (86.9 cm) was recorded for N_1 treatment (table 3).

Table 1. Soil physical and chemical properties of experimental area.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture	PH	E.C (dS/m)	Organic Carbon (%)	Total N (%)	Available P (ppm)	Available K (ppm)
0 - 30	40.0	30.0	30.0	Clay loam	7.8	3.4	0.49	0.05	6.7	220
Optimum				loam	6.5 - 7.5	2.0 <	>1.0	1.0 >	10 - 15	200 - 300

The number of grains per ear

Number of grains per ear plays an important role to determining grain yield. The number of grains per ear was significantly affected by *Azospirillum* and nitrogen levels, but no in their interaction effect

(Table 2). Maximum number of grains per ear was recorded to inoculation with *Azospirillum* (41.5) and minimum it was recorded at control treatment (39.3). Plants with *Azospirillum* inoculation had about 6% more number of grains in ear in compared of non-

Azospirillum plants. It means that *Azospirillum* plays an important role in wheat generative growth and therefore to make a significant increase in the number of grains per ear. Our results concur partly with observations made by Gholami *et al* (2009), who reported that the grains number increased with seed priming with *Azospirillum*. These results are also in

agreement with De Freitas (2000) who concluded that grain number per ear in wheat was highest at inoculation with *Azospirillum*. Means comparison indicated that the maximum (41.6) number of grains per ear was recorded for 100% N recommended and minimum value was recorded for 25% N recommended (39.1).

Table 2. Analysis variance of measured parameters.

S.O.V	d.f	MS							
		Plant height	Ear length	Grain per ear	number	1000-seed weight	Grain yield	Biological yield	Harvest Index
Rep	2	2.952 ^{ns}	2.332 ^{ns}	6.255 ^{ns}		0.338 ^{ns}	0.257 ^{ns}	0.215 ^{ns}	8.271 ^{ns}
Az	1	35.339 [*]	4.212 [*]	1133.45 [*]		30.616 ^{**}	0.597 [*]	6.546 ^{**}	18.361 ^{ns}
N	3	34.864 [*]	8.787 ^{**}	471.652 [*]		16.864 ^{**}	0.624 ^{**}	12.950 ^{**}	4.860 ^{ns}
Az×N	3	14.059 ^{ns}	0.294 ^{ns}	114.845 ^{ns}		2.794 ^{ns}	0.072 ^{ns}	0.026 ^{ns}	8.419 ^{ns}
Error	14	12.044	1.273	141.957		2.167	0.093	0.788	16.877
C.V. (%)		3.2	9.5	8.1		3.4	10.2	11.8	10.3

*: Significant at 0.05 level, **: Significant at 0.01 level, ns: No significant difference.

Grain Yield

Azospirillum and nitrogen fertilizer had significantly effects on grain yield, but interaction of theirs had no effect on the grain yield. *Azospirillum* could with symbiosis activity itself, cause to increasing N nutrient in around root plants and addition absorb by roots. At all the levels of nitrogen fertilizer, the *Azospirillum* plants had higher grain yield. In fact, at 25% level of N fertilizer, the *Azospirillum* plants had 12% higher grain yield compared to non-*Azospirillum* plants, while at 50%, 75% and 100% N recommended, *Azospirillum* association resulted in higher grain yield of 10%, 4% and 2%, respectively. The grain yield in AzON₄ (6.762 t. ha⁻¹) had no significant effect with Az₁N₂ (6.477 t. ha⁻¹). The results of using biological fertilizer treatment

(inoculation by *Azospirillum*) with 50% nitrogen recommended was not significantly different from the high rate of chemical nitrogen application (AzON₄ treatment). According to the results of this experiment, application of *Azospirillum* in present of 50% N recommended had an appropriate performance and could increase grain yield to an acceptable level, so it could be considered as a suitable substitute for chemical nitrogen fertilizer in organic agricultural systems. A similar trend in yield differences across seed priming with PGPR have been reported by Dobbelaere *et al* (2003) and Cakmak (2005 a, b). They have been reported that PGPR can increase yield. Kloepper and Beauchamp (1992) have been shown that wheat yield increased up to 30% in seed priming with PGPR.

Table 3. Mean comparisons of the main effects.

Treatment	Plant Height (cm)	Ear length (cm)	Grain number per ear	1000-seed Weight (gr)	Grain yield (ton. ha ⁻¹)	Biological Yield (t. ha ⁻¹)	Harvest Index (%)
Az levels							
Az ₀	91.4 ^b	10.9 ^b	39.3 ^b	38.9 ^a	6.157 ^b	17.624 ^b	34.9 ^a
Az ₁	94.6 ^a	11.8 ^a	41.5 ^a	38.7 ^a	6.584 ^a	18.780 ^a	35.0 ^a
N levels							
N ₁ (25%)	86.9 ^c	9.7 ^c	39.1 ^b	37.4 ^c	5.859 ^c	16.219 ^c	36.1 ^a
N ₂ (50%)	91.2 ^b	10.9 ^{bc}	39.4 ^{ab}	39.2 ^{ab}	6.175 ^{bc}	17.713 ^{bc}	34.8 ^a
N ₃ (75%)	95.4 ^{ab}	12.1 ^{ab}	41.7 ^a	38.8 ^b	6.625 ^{ab}	19.150 ^{ab}	34.6 ^a
N ₄ (100%)	98.6 ^a	12.9 ^a	41.6 ^a	40.2 ^a	6.823 ^a	19.746 ^a	34.5 ^a

Means which have at least one common letter are not significantly different at the 5% level using (DMRT).

Biological Yield

Biological yield was significantly affected by *Azospirillum* and nitrogen fertilizer (Table 2). Maximum biological yield was recorded to inoculation with *Azospirillum* (18.780 t. ha⁻¹) and minimum it was recorded at control treatment (17.624 t. ha⁻¹). Bashan *et al.* (2004) and Cakmaki *et al.* (2006) reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in

biological yield. Zaidi and Khan (2005) have suggested that seed priming with PGPR increased biological yield. Perveen *et al.*, (2002); Wani *et al.*, (2007) have been reported increase in biological yield. Rokhzadi *et al.*, (2008) reported that seed priming with plant growth promoting rhizobacteria increased biological yield and yield of chickpea under field conditions. Similar results have been reported by Pal (1998) in maize.

Table 4. Mean comparisons of the interaction effect.

Treatment	Plant Height (cm)	Ear length (cm)	Grain number per ear	1000-grain Weight (gr)	Grain yield (ton. ha ⁻¹)	Biological Yield (t. ha ⁻¹)	Harvest index (%)
Az ₀ N ₁	84.3 ^c	9.4 ^c	29.5 ^b	36.9 ^c	5.485 ^c	15.170 ^d	36.1 ^a
Az ₁ N ₁	86.6 ^{bc}	9.9 ^c	32.5 ^{ab}	37.9 ^{bc}	6.233 ^{bc}	17.269 ^{bcd}	36.0 ^a
Az ₀ N ₂	89.4 ^{bc}	10.4 ^{bc}	29.5 ^b	39.6 ^{ab}	5.873 ^{bc}	16.970 ^{cd}	34.6 ^a
Az ₁ N ₂	93.1 ^{ab}	11.4 ^{abc}	33.3 ^a	38.5 ^{bc}	6.477 ^{ab}	18.455 ^{abc}	35.0 ^a
Az ₀ N ₃	94.2 ^{ab}	11.1 ^{abc}	33.2 ^a	38.7 ^{abc}	6.509 ^{ab}	18.774 ^{abc}	34.6 ^a
Az ₁ N ₃	96.6 ^a	12.8 ^{ab}	34.1 ^a	38.9 ^{ab}	6.742 ^a	19.528 ^{ab}	34.5 ^a
Az ₀ N ₄	97.8 ^a	12.6 ^{ab}	33.0 ^a	40.5 ^a	6.762 ^a	19.581 ^{ab}	34.5 ^a
Az ₁ N ₄	99.4 ^a	13.2 ^a	34.2 ^a	39.8 ^{ab}	6.883 ^a	19.913 ^a	34.6 ^a

Means which have at least one common letter are not significantly different at the 5% level using (DMRT).

The maximum biological yield of 19.746 t. ha⁻¹ obtained in N₄ (100% N recommended) which was not significantly different from N₃ treatment, and the minimum biological yield of 16.219 t.ha⁻¹ was obtained in N₁ (25% N recommended). On the basis of this study, it seems that *Azospirillum* and nitrogen fertilizer have much positive effects on biological yield. It can be also concluded that the initial soil nitrogen content already was not adequate without nitrogen fertilizer application.

Conclusions

results from the present study indicated that plant height, ear length, grain number per ear, 1000-grain weight, grain yield and biological yield have been affect significantly by inoculation with *Azospirillum*, because this biofertilizer can fix and enhance absorb of nitrogen by plant. According to calculated, inoculation wheat seeds in planting date with *Azospirillum* to cause increase seed yield about 7 percentages and, also reduce using of about 50 percentage chemical nitrogen.

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