



RESEARCH PAPER

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Seed yield, some yield components and morphological traits of wheat as affected by *Azotobacter* and *Pseudomonas* bacteria inoculation

E. Ansari Nia

Young Researchers and Elite Club, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran

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Abstract

This study investigated the effects of *Azotobacter chroococcum* strain 5, *Pseudomonas fluorescens* 187, and their interactions on wheat performance. The experimental design was split plot factorial with a complete randomized block design. The treatments included four chemical fertilizers (0, 50, 75 and 100% dose fertilizers) and four levels of plant growth promoting rhizobacteria (*Azotobacter chroococcum* strain 5, *Pseudomonas fluorescens* 187, mixture of these bacteria, and control). At time of physiological maturity, number of spikes per unit area, number of spikelet and grain number per spike, thousand grain weight, grain yield, harvest index, biological yield, plant height, stem diameter and protein content were measured. Results indicated that the combined application of *Azotobacter* and *Pseudomonas* increased grain yield, harvest index, biological yield and protein content by 34.3, 7.7, 12.5 and 13.6%, respectively compared to the controls. *Azotobacter* and *Pseudomonas* inoculation plus fertilization reduced chemical fertilizers application (25-50 %) in the field. Results of this study suggest that farmer can obtain the same wheat yield if they apply half of conventional consumption of chemical fertilizers along with *Azotobacter* and *Pseudomonas*.

*Corresponding Author: E. Ansari nia ✉ aazad_1369@yahoo.com

Introduction

One way to increase crop yield is using the beneficial microorganisms. Plant growth promoting rhizobacteria (PGPR) are some of bacteria that can grow in the root environment and be effective on plant growth (Vessy, 2003; Yolcu *et al.*, 2012). Mechanisms that can promote plant growth include production of phytohormones, biological nitrogen fixation and increased solubility of insoluble elements in soil (Rovera *et al.*, 2008; Rosas *et al.*, 2005). Studies showed that the inclusion of wheat plant with PGPR increased the growth characteristics of wheat; bacteria studied were included Azospirillum (Bashand and Levanony, 1990), Azotobacter (Rai and Gaure, 1988), Basillus (Freitas, 2000), Pseudomonas (Zaidi and Khan, 2005), Clostridium (Gasoni *et al.*, 2001), and Herbaspirillum (Baldani *et al.*, 2000).

Application of plant growth promoting rhizobacteria and phosphate solubilizing bacteria (PSB) combination resulted in a positive effect on plant growth (Rudresh *et al.*, 2005; Zaidi *et al.*, 2003). Mirzaei *et al.*, (2010) applied Azotobacteria and Azospirillum bacteria in different levels of nitrogen for sunflower plant. Their results showed that combined application of these two types of bacteria increased plant growth characteristics and reduce nitrogen fertilizer application by 50%.

Environmental pollution caused by chemical fertilizers is one of the problems of human societies today. Use of biological fertilizers, can reduce chemical fertilizers used. Arafa *et al.*, (2009) and Carrier *et al.*, (2008) showed that the application of Azotobacter can reduce nitrogen fertilizers consumption. The purpose of this study was to investigate the effects of inoculation of Azotobacter and Pseudomonas bacteria and their interactions on wheat yield under field condition.

Materials and methods

Study side

The study was carried out in Khosf Region, Iran (Long. 59°13' E., Lat. 32°53' N., Alt. 1480 m), during 2011-2012 growing season.

Experimental design and treatments

The experimental treatments were arranged in split plot factorial based on a complete randomized block design including four phosphorus fertilizer levels (0, 50, 75 and 100 % of fertilizer requirements), four levels of plant growth promoting rhizobacteria including Azotobacter chroococcum strain 5, Pseudomonas fluorescens 187, mixture of these bacteria, and control. The experiment was replicated three times; total numbers of treatments were 48. Each plot consisted of five lines with 5 meter length, 25cm row and 10 cm plant spacing. Nitrogen fertilizer of urea at rate of 200 kg ha⁻¹ was added to each pot. Nitrogen fertilizer was top dressed in three portions, one third at the time of planting, one third before flowering and the remaining at the time of grain filling. Bacteria were inoculated using seed inoculation method.

Plant analysis

Two square meters was selected from each pot. Number of spikes per unit area, number of spikelet and grain number per spike, thousand grains weigh, grain yield, harvest index, biological yield, plant height, stem diameter and protein content were measured.

Statistical analysis

Two factor analyses of variance (ANOVA) and Duncan multiple range tests (test at 1 and 5% level of probability) were used to partition the variance into the main effects and the interaction between chemical and biological fertilizers. Statistical analysis was performed using SPSS statistical package 20.

Results and discussion

The effects of chemical and biological fertilizers on growth characteristics of wheat (except stem diameter and number of spikelet) were significant ($P \leq 0.01$). The interactive effects of chemical and biological fertilizers on grain yield, harvest index, biological yield and plant height was significant ($P \leq 0.01$) (Table 1).

Application of chemicals fertilizer provided better

nourishment condition to for Azotobacter and Pseudomonas performance, because these bacteria need these elements to grow and development. This result was in agreement by those reported by Mirzaei *et al.*, (2010). Idris (2003) confirmed positive effect of Azotobacter on thousand seed weight. Bouthaina *et al.*, (2010) indicated that the plant height (cm), root length, shoot and root fresh and dry weights, leaf area, chlorophyll content, number of tiller and leave/plant increased significantly with bio-fertilizer treatments. Fig. 1a shows that the highest grain yield was in chemical fertilizers 100% and the combined of Azotobacter and Pseudomonas treatment (5654 kg ha⁻¹), chemical fertilizers 75% and combined of Azotobacter and Pseudomonas treatment (5480 kg ha⁻¹) and chemical fertilizers 100% and Pseudomonas treatment (5400 kg ha⁻¹). Also the lowest grain yield was obtained with no chemical fertilizers with and without inoculation treatments (3120-3400 kg ha⁻¹). A significant difference in the harvest index in chemical fertilizers treatments with biological treatments was observed. The highest harvest index was obtained in chemical fertilizers of 100% application with Azotobacter (44.5) and the least was

obtained in control (35.2) (Fig1b). The application of Azotobacter and Pseudomonas with chemical fertilizers increased biological yield. Chemical fertilizers of 100% treatment with the combined Azotobacter and Pseudomonas treatment increased biological yield by 12.9 % compared to chemical fertilizers 100% treatment without inoculation (Fig 1c). Singh *et al.*, (2004) indicated that inoculation of wheat with Azotobacter under normal condition resulted in the maximum production rates of different wheat cultivars. The results showed biological fertilizers not only increased yield but also reduced the consumption of chemical fertilizers. Carlier *et al.*, (2008) applied different levels of fertilizer applications and inoculation with PGPR and concluded that the level of 50% fertilizer with inoculated bacteria increased significantly the seed weight and seed number per spike. Garcia-Gonzalez *et al.*, (2005) found that treatment with Azospirillum lipoferum, A. beijerinckii, or a combination of the two, plus a 50% dose of urea, had an effect equivalent to treatment with 100% urea without inoculation, in regard to wheat leaf length. Similar results were reported by Mirzaei *et al.*, (2010).

Table 1. Analysis of variance of measured parameters of crop performance.

Variable	df	Spike number	Spikelet number	Grain spike number	Thousand grain weight	Grain yield	Harvest index	Biological yield	Plant height	Stem diameter	Protein content
R	2	2556.2 ^{ns}	75.0 ^{ns}	70.0 ^{ns}	51.2 ^{ns}	33526.0 ^{ns}	5.7 ^{ns}	644354.0 ^{ns}	55.4 ^{ns}	0.3 ^{ns}	1.1 ^{ns}
F	3	4227.6 ^{**}	49.0 ^{ns}	155.4 ^{**}	190.8 ^{**}	7152866.0 ^{**}	154.2 ^{**}	23879559.0 ^{**}	125.4 ^{**}	0.1 ^{ns}	5.5 ^{**}
BF	3	3485.9 ^{**}	62.0 ^{ns}	146.5 ^{**}	175.3 ^{**}	3411065.0 ^{**}	21.2 ^{**}	11308599.0 ^{**}	308.5 ^{**}	0.2 ^{ns}	4.4 ^{**}
F × BF	9	920.5 ^{ns}	18.0 ^{ns}	10.0 ^{ns}	19.6 ^{ns}	263536.0 ^{**}	25.8 ^{**}	1103936.0 ^{**}	126.4 ^{**}	0.4 ^{ns}	1.3 ^{ns}
Error	30	635.3 ^{ns}	17.0 ^{ns}	7.8 ^{ns}	7.8 ^{ns}	28694.0 ^{ns}	5.5 ^{ns}	126115.0 ^{ns}	17.8 ^{ns}	0.2 ^{ns}	0.9 ^{ns}
CV	-	13.5	6.5	8.0	5.5	12.6	8.9	11.2	13.3	4.2	3.3

** Significant at $P \leq 0.01$, ns: Not significant, R: Replication. F: Fertilizers. BF: Biological Fertilizers.

Table 2. Mean comparisons of the main effects on wheat growth properties.

Treatment	Spike number (m ²)	Spikelet number (per spike)	Grain number (per spike)	1000-grain weight(g)	Grain yield (kg ha ⁻¹)	Harvest index (%)	Biological yield (kg ha ⁻¹)	Plant height (cm)	Stem diameter (cm)	Protein content (%)
Fertilizers level										
F0	482.1b	20.9a	19.8b	33.6b	3270.0d	37.4c	9120.0c	75.2b	3.4a	11.1b
F50%	525.4ab	23.2a	22.1ab	44.0a	4250.0c	39.0b	10889.0b	79.4a	3.5a	12.2a
F75%	545.8a	26.3a	24.4a	44.2a	4720.0b	44.4a	10975.0b	82.6a	3.4a	12.3a
F100%	549.7a	24.5a	24.9a	45.0a	5110.0a	45.3a	11680.0a	81.3a	3.5a	12.3a
Biological fertilizer										
No inoculation	497.0b	23.3a	27.3c	38.5b	3620.0d	39.0c	9352.0d	72.7a	3.5a	11.0b
Azoto	527.2ab	23.2a	31.4b	42.1a	4251.8c	40.0b	10524.0c	78.0a	3.4a	12.0a
Pseudo	529.5ab	24.5a	33.5ab	42.9a	4525.2b	40.8ab	11100.0b	82.2a	3.6a	12.1a
Azoto+Pseudo	540.0a	26.7a	36.4a	43.6a	4862.0a	41.1a	11710.0a	84.5a	3.6a	12.5a

Means with different superscript letter(s) are significantly different at $P \leq 0.01$ according to Duncan test

F₀, F_{50%}, F_{75%} and F_{100%} = 0, 50, 75 and 100 % dose of fertilizers, respectively

Azoto: Azotobacter chroococcum, Pseudo: Pseudomonas fluorescens.

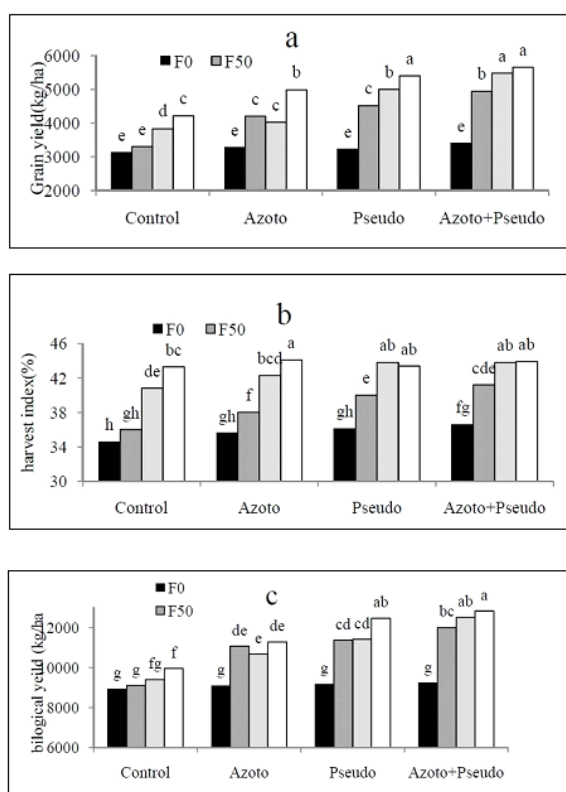


Fig. 1. Interactive effects of chemical fertilizers with biological fertilizer on grain yield (a), harvest index (b), and biological yield (c) P. (Azoto: Azotobacter chroococcum, Pseudo: Pseudomonas fluorescens). F₀, F₅₀, F₇₅ and F₁₀₀ = 0, 50, 75 and 100 % dose of fertilizers, respectively.

Application of chemical fertilizers increased the average number of spikes. However there was no significant difference between 75 and 100 % of fertilizer application rates. Biological fertilizers increased the number of spikes compared to control. The Azotobacter and Pseudomonas and their combination enhanced the number of spikes by 6.0, 6.4 and 7.7 % respectively compared to non-inoculated treatment (Table 2). The Azotobacter and Pseudomonas and their combination also increased the number of grains per spike and thousand seed weight compared to control treatment. The highest number of grains per spike (35.3) and thousand grain weights (43.8 g) obtained using the combined of Azotobacter and Pseudomonas treatment. Also application of chemical fertilizers increased the number of grains (21.2-23.8 per spike) and thousand seed weight (42.1-44.1 g).

Conclusions

The results of this study clearly revealed that: (i) inoculation with Azotobacter and Pseudomonas bacteria improved growth and yield of wheat and also the combined application of Azotobacter and Pseudomonas had more effect in improving the wheat performance, (ii) our results suggested that the application dosages of chemical fertilizer application for commercial wheat production can be significantly reduced by application of Azotobacter and Pseudomonas inoculation plus fertilization.

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