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Response of Winter Barely to Co-Inoculation with *Azotobacter* and Mycorrhiza Fungi Influenced by Plant Growth Promoting Rhizobacteria

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ABSTRACT

This study was conducted in order to investigation the reaction of barley root characteristic influenced by using both mycorrhizae and PGPR in Research field of Islamic Azad University of Arak during winter of 2010-2011. Experiment was conducted in a randomized complete block design with three replications. The factors were 1. Azotobacter (two levels included using and not using bacteria), 2. Pseudomonas (three levels included using P. putida, P. fluorescens and not using bacteria) 3. Mycorrhizae (two levels included using and not using fungi). The results indicated that PGPR had a significant effect on Auxin, Cytokenin, GA, protein and phosphor compared to control. The interaction of Mycorrhizae and Pseudomonas had a significant ($p<0.01$) increase on colonization, biological grain and consecration Zn and Fe, but interaction of Mycorrhizae and Azotobacter were most effective on Zn, Fe concentrations. The results proved the positive effects of microorganism symbiosis with barley root and increase water and nutrition absorption.

Keywords: *Azotobacter*, Barely, Quality parameters, Mycorrhizae, PGPR.

INTRODUCTION

Nitrogen and phosphorus are known to be essential nutrients for plant growth and development. Intensive farming practices that achieve high yield require chemical fertilizers which are not only costly but may also create environmental problems The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health consequently there has recently been a growing level of interest in environmental friendly sustainable agricultural practices. Bio-fertilizer is defined as a substance which contains living organisms which, when applied to seed, plant surface, or soil, colonize the rhizosphere or the interior of plant the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant [11]. Biofertilizers are well recognized as an important component of integrated plant nutrient management for sustainable agriculture and hold a great promise to improve crop yield [16]. Researchers reported through an experiment that the *Pseudomonas* is the most abundant auxin producer micro-organism growth regulator especially IAA (Indole-3-Acetic Acid), often effects the root systematic features such as root primary growth side-root formation and root hairs [14]. PGPR also produce include indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production.

Arbuscular Mycorrhizae Fungi (AMF) can be integrated in soil management to achieve low-cost sustainable agricultural systems [10]. Mycorrhizae fungi occur in most of the soils and colonize roots of many plant species. Mycorrhizae are the structures resulting from the symbiosis between these fungi and plant roots, and are directly involved in plant mineral nutrition The symbiotic root-fungal association increases the uptake of less mobile

nutrients [7], essentially phosphorus but also of micronutrients like zinc (Zn) and copper (Cu), Fe the symbiosis has also been reported as influencing water uptake AMF can also benefit plants by stimulating the production of growth regulating substances, increasing photosynthesis improving osmotic adjustment under drought and salinity stresses and increasing resistance to pests and soil borne diseases [6]. These benefits are mainly attributed to improved phosphorous nutrition [3] and also research on AM has shown that cytokinin (CK) accumulation is specifically enhanced by symbiosis throughout the plant. Additional and proposed involvement of other phytohormones is described too.

At present, the government in Iran is heavily subsidizing mineral fertilizers for wheat and offers guarantee prices to achieve in 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. Hence, any technology that could at least partly substitute fertilizer applications would be both helpful for farmers and Iran's economy. This experiment was designed to evaluate the effect of co-inoculation of *Azotobacter* and Mycorrhizae and also effectiveness of various plant growths promoting rhizobacteria on yield, yield components and quality characters of winter wheat.

MATERIALS AND METHODS

This experiment was conducted in experimental field of Islamic Azad University, Arak Branch at (34.º 3´ N, 49º 48´ E Long,2192 m height from sea level) in Markazi province in winter of 2010- 2011. The soil texture was loam. The experimental design was used a factorial arrangement in a randomized complete block with three replications. Treatments were include three agent: *Azotobacter chrococum* (with and without inoculation) with population 10^8 number per each ml, Mycorrhizae *(Glomus intradices)*, (with and without inoculation) with population 250 - 300 of fungus active organs for each seed planted and *Pseudomonas* (without inoculation, with inoculation *Pseudomonas putida* and with inoculation *P. florescence*). The microorganisms were provided by the biology department of Tehran Water and Soil Institute. The seeds were sown with inoculation with biofertilizers in 14 Oct. 2011. The sowing pattern was based on 300 plants/m^2. The plots had 4 stacks, each 6m long. Field was irrigated due to environment condition and soil moisture. In order to measure plant growth promoting at the flowering stage, 3 samples were taken from flag leaf in each plot. To determine the concentration of hormones, HPLC machine was used and separation was performed by isocratic method.

Twelve weeks after inoculation, plant roots stained for observation of fungi structures and Mycorrhizae colonization [9]. Mycorrhizae fungi colonization was also measured by cutting root samples into 1 cm segments, put them in 10% KOH for 2 days at room temperatures followed by rising them several times with tap water and staining with ink (black ink, Schaeffer) as well as household vinegar (equal to 5% acetic acid) solution 4 min. Then, colonization percent determined using modified intersection method proposed by McGonigle et al. [20]. Percent of root colonization was calculated as follows:

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

Weeding was done by hand and the field was harvested 23 June 2011. Statistically of the result was done by using SAS program. Means were compared using the Duncan's Multiple Range Test (DMRT) at 5% level of probability. Correlation was calculated between oil yield and other plant characters.

RESULTS AND DISCUSSION

The percent of root colonization was effected by fertilizer treatment so single effect of the colonization (p<%1) and concurrent use of Mycorrhizae and *Pseudomonas* effected colonization in the level p<%5 (Table.1) and the maximum colonization obtained by use of combination of Mycorrhiza and *Pseudomonas* (Table 2). Rajendran and Devaraj [13] reported that mycorrhiza population may be increased in the root system in presence of Phosphate solubilizing bacteria. Perhaps it was the result of lack of Mycorrhizae fungus in the soil and requires the use of appropriate doses in combination with other microorganisms to improve plant growth. The rate of auxin affected by Mycorrhizae and *Pseudomonas* individually (Table 1) and highest rate of auxin obtained from application of combinations of auxin and Mycorrhizae (Table 2). Although there is no significant difference between treatments but it is seem that Mycorrhizae has effect in auxin rates by Availability of phosphorus in plant. Probability, the Phosphorus element has an effect on absorption of other elements such as Nitrogen and it can cause an increase in plant auxin [15]. Bare et al. [12] also reported increase of concentration of plant hormones and chlorophyll content is such benefit of Mycorrhizae.

The amount of cytokinin effected by different treatments include Mycorrhiza, *Azotobacter* and *pseudomonas* (p<%1).

Salamone et al. [8] also reported production of cytokinin by *Pseudomonas*. The synthesis of hormones (e.g. auxin and cytokinin) by different strains of *Azotobacter* have been found. The rate of Gibberllin effected by experimental single factors (p<%1).

Raja et al. [17] also observed changes of hormones because of application of *Pseudomonas fluoresces* in paddy seedling. The nitrogen fixing bacteria such as alone *Azotobacter* are capable of nitrogen fixation as well as the ability to free up the same phytohormones and indoleacetic acid which simulates plant growth and nutrient uptake and increase plant growth rate finally [19].

Phosphorus. According to Table 1, phosphorus of seed also affected by single treatment (p<%1) and these factors increased phosphorus content of seed. Contamination of root with arbuscular vesicular fungi can increase phosphorus uptake through increasing the root surface and transmission of element in host plant [21].

Totally, increase in Phosphorus content can be due to plant growth regulators caused by the bacteria. It can improve the absorb water and nutrients in plant [4] The results in Table 1 indicated that grain protein levels in plant effected by the single effects of experimental treatments. The research showed that Arbuscular Mycorrhizae increase the absorption of nitrogen directly through its mycelium. On the other hand, arbuscular Mycorrhizae with increase of absorption of water and nutrients get ready plant physiologically so it cause more nitrogen fixation resulting in greater nitrogen fixation. Increase in water and nutrient absorption, trehalose of root and allocation of carbon to root are due to increased photosynthesis as well as nitrogen fixation in plants having Mycorrhizae [5]. Wagar et al. [1] believe that increase of nitrogen is due to reduce level of ethylene in plant inoculated with Acc bacteria. The results in Table 1 show that rate of concentration of Zn in plant effected by fertilizer treatments in statistic level (p<%1) and as it is indicated in Table 2 , highest rate of Zn concentration is due to application of combination of mycorrhiza and *Azotobacter*.

Behl et al. [18] reported positive effects of use of Mycorrhizae and *Azotobacter* on wheat. They believe the reasons are effects of *Azotobacter* in hair root growth therefore more longitudinal growth of the Mycelium fungi and their penetration into the deep layers of soils and plant nutrient violable increase. The use of Mycorrhizae fungi can increase the efficiency of *Azotobacter* due to having mycelium hyphae and its effect on increases regions of nutrient absorption in root systems [14]. Al-Karaki and Clark [6] also reported increase in absorption of copper and zinc by Mycorrhizae plants. The rate of Iron concentrations were also influenced by fertilizer treatments (p<%1) therefore the highest concentration of iron resulted in application of combination of Mycorrhizae and *Azotobacter*.

Glick et al. [2] reported that there are evidences of increase of availability of plant nutrition in rhizosphere due to activity of growth promoting rhizosphere bacteria. Mycorrhizae fungi receive energetic carbon sources from plants then transmit mineral nutrients like phosphorus, copper, Zinc and iron in complete absorption format to roots.

These bacteria produce a variety of growth promoting hormones, amino acids, vitamins and particular siderophere which increase solubility and absorption of nutrients like iron, Zinc and phosphorus and also help to prevention of plants against disease. Dry matter yield was also influenced by fertilizer treatments and it effected by combination use of Mycorrhizae *Azotobacter* and *Pseudomonas* in statistic level of (p<%5).

The highest dry matter is related to integrated treatment of Mycorrhizae and *Pseudomonas* (Table 2) it seems that inoculation of seeds with growth promoting bacteria by increasing root growth, increased water and nutrient availability and increased plant vegetative and reproductive growth. It causes higher dry matter production per area unit and therefore higher yield. It also seems that phosphate solubilizing microorganisms increased the amount of nitrogen fixation by solubilizing insoluble phosphate and increase the amount of available phosphorus. Its result increase of plants growth, especially in shoot, will happen. Overall, the results of this experiment showed that growth promoting bacteria increased growth and rates of nutrient elements in grains. This increase is mainly due to production plant growth promoters by bacteria and their effect on root growth, which improves absorption of water and nutrients.

It seems that the increase in the rate of nutrients uptake by plants can lead to increase accumulation of dry matter and mineral in stem and leaf.

Table 1. Analysis of variance for the measured traits.

SOV	df	Mean Squares								
		Zn concentration	Fe concentration	phosphor	Protein	Auxin	Cytokinin	Gibberllin	colonization	Dry matter
Rep	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mycorrhiza (M)	1	**	**	**	**	**	**	**	**	**
Azotobacter (A)	1	**	**	**	**	ns	**	**	**	**
Pseudomonas (S)	2	**	**	**	**	**	**	**	**	**
M×A	1	**	**	ns	ns	ns	ns	ns	ns	*
M×S	2	**	**	ns	ns	ns	ns	ns	*	*
A×S	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
M×A×S	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
♀Erorr	22	.048	0.040	135/70	0.0178	2014/48	82/50	363/11	1/05	67752/9
C.V	-	.047	0.059	5/18	4/20	27/3	14/48	18/43	3/22	1/89

*ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.*

Table 2. Effect of the *Mycorrhiza* (M), *Azotobacter* (A) and *Pseudomonas* (S) on the measured traits.

Treatments	Colonization (%)	Auxin (ng.g/fw)	Giberlin (ng.g/fw)	Cytokinin (ng.g/fw)	Phosphorus (ppm)	Protein (%)	Fe (ppm)	Zn (ppm)	Dry mater (Kg/ha)
M1	26.07b	116.4b	87.02b	36.64b	193.4b	8.32b	32.09b	45.16b	2783.5b
M2	37.54a	212.3a	134.66a	88.78a	255.8a	11.76a	35.18a	47.51a	4775.9a
A1	30.40b	149.4b	93.0b	56.7b	212.2b	9.56b	32.96b	45.90b	3259.8b
A2	33.21a	179.3a	113.7a	68.8a	237.0a	10.51a	34.30a	46.77a	4299.7a
S1	28.78c	134.5b	85.0b	50.34b	200.3b	9.11b	32.59b	45.56b	12877.8c
S2	32.83b	170.2ab	108.4a	67.62a	231.9a	10.38a	34.08a	46.71a	13794.3b
S3	33.82a	188.5a	116.7a	70.34a	241.7a	10.64a	34.23a	46.74a	14667.2a
M1A1	24.70d	105.3b	64.18c	30.59d	179.2d	7.79d	31.41d	44.61d	12186.7d
M1A2	27.43c	127.6b	80.03c	42.68c	207.6c	8.87c	32.77c	45.71c	13380.3c
M2A1	36.11b	193.6a	121.92b	82.72b	245.2b	11.34b	34.51b	47.19b	14332.9b
M2A2	38.99a	231.0a	147.39a	94.83a	266.5a	12.17a	35.84a	47.83a	15219.0a
M1S1	23.77d	96.85d	56.94d	24.85d	165.9d	7.44d	31.06d	44.10d	11793.5e
M1S2	26.63c	122.80cd	77.48cd	40.80c	203.03c	8.65c	32.55c	45.68c	12900.7d
M1S3	27.79c	129.70cd	81.90c	44.26c	211.2c	8.90c	32.65c	45.70c	13656.3c
M2S1	33.78b	172.10bc	113.09b	75.47b	234.7b	10.78b	34.12b	47.02b	13962.0c
M2S2	39.03a	217.53ab	139.37a	94.44a	260.7a	12.10a	35.60a	47.73a	14687.8b
M2S3	39.83a	247.28a	151.52a	96.42a	272.1a	12.38a	35.82a	47.78a	15678.0a

Means in a column followed by the same letter are not significantly different at P≤0.05.

CONCLUSION

The results of this study showed that individual consumption of biofertilizers increased phosphorus, protein of grain and concentration levels of auxin, cytokinin and gibberellin hormones. The colonization percentage and dry matter yield also increased by combination of Mycorrhizae and *Pseudomonas* application. The application of Mycorrhizae and *Azotobacter* had the greatest impact on concentration rates of iron and zinc too.

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