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Research Article

EFFECT OF SEEDS INOCULATION BY PLANT GROWTH PROMOTING BACTERIA ON SEEDLING GROWTH OF FIVE WHEAT CULTIVARS UNDER GREENHOUSE EXPERIMENT

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ABSTRACT
In order to study effect of seed inoculation with plant growth promoting bacteria on seedling growth of five wheat cultivars a greenhouse experiment was conducted in the agricultural research institute, university of zabol, IR Iran in year 2014. A completely randomized design based on factorial arrangement with three replications was used. The first factor consisted of five cultivars of wheat (Hamoon, Croos bolani, Hirmand, Bam, Kalak afghan) and the second one was bio-fertilizer types (Phosphate solubilizing bacteria, Bio-phosphor, Nitroxin, Nitro-kara, Mixed and distilled water as control). In this experiment traits measured including, root length, shoot length, shoot fresh weight, root fresh weight, number of leaf, root dry weight, shoot dry weight and root to shoot ratio. The croos bolani cultivar recorded higher shoot length (174.38 mm) followed by kalak afghan cultivar (139.58 mm) and the least was in hamoon cultivar (96.41 mm). In interaction between cultivar and bio-fertilizer were obtained the highest shoot fresh weight was related to kalak afghan and phosphate solubilizing bacteria (C5B1) treatment with mean (0.21 gr). The highest (0.027) and lowest (0.022) levels of shoot dry weight were obtained in bio-phosphor (B2) and control (B6) treatment, respectively. In interaction between cultivar and bio-fertilizer were obtained the highest root to shoot ratio was related to hirmand cultivar and nitroxin (C3B3) treatment with mean (1.4).

Keywords: *Bio-Fertilizers, Inoculation, Germination, Nitroxin, Wheat*

INTRODUCTION
Wheat (*Triticum* spp.) is a cereal grain, originally from the Levant region of the Near East but now cultivated worldwide. In 2010, world production of wheat was 651 million tons, making it the third most-produced cereal after maize (844 million tons) and rice (672 million tons) (World Wheat Crop, 2013). Wheat was the second most-produced cereal in 2009; world production in that year was 682 million tons, after maize (817 million tons), and with rice as a close third (679 million tons) (World Wheat). Bread wheat is the main food of people in many countries and about 70% calories and 80% protein of human diet is supplied from its consumption (Taregh *et al.*, 2011). The seed is one of the most important inputs for higher grain production and the necessity of quality seed is not to be eluded. Quality seed is required for rapid and synchronous seedling emergence, a pre-requisite for successful stand, establishment and uniform plant growth and development. Vigorous seedling growth is important for the successful establishment of wheat and other crops. Investigations in several countries have shown that growth of rice seedling was enhanced by inoculation with plant growth promoting microorganisms which led to increased grain and straw yield and increased the efficiency of fertilizer-N. The capacity of microorganisms to stimulate germination and improve development of plants has been adapted for in vitro and in vivo conditions of some agricultural (Ayyadurai *et al.*, 2006; Tsavkelova *et al.*, 2007). 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 rhizsphere or the interior of plant the plant and promotes growth by increasing the

supply or availability of primary nutrients to the host plant (Vessey, 2003). 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 (Narula *et al.*, 2005; Wu *et al.*, 2005). Background Inoculation of plants to enhance yield of crops and performance of other plants is a century old, proven technology for rhizobia and a newer venue for plant growth-promoting bacteria and other plant symbionts. The two main aspects dominating the success of inoculation are the effectiveness of the bacterial isolate and the proper application technology. Plant growth stimulators are applied to improve seed germination, seedling vigor and plant development. Investigations in several countries have shown that rice seedling growth was enhanced by following inoculation with plant growth promoting microorganisms, leading to increased grain, straw yield and enhancement of the efficiency of fertilizer-N. The inoculation of seeds or seedlings with microorganisms has been adopted as a method of modifying microbial populations around crop plants to promote both development and yield. The stimulation of seedling development by bacteria has been attributed to the production of biologically active compounds. Enhanced seed emergence and consequently vigor seedlings are also influenced by PGPR inoculation. Inoculated PGPR produced IAA in the presence of seed exudates that might have triggered faster germination. Significant increases in crop yields following application of PGPR have been documented under diverse field conditions (Bashan, 1998; Mia *et al.*, 2005). Bio-fertilizers 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). Hence, the present study was undertaken to observe the effect of seeds inoculation by plant growth promoting bacteria on seedling growth of five wheat cultivars under greenhouse experiment.

MATERIALS AND METHODS

In order to study effect of seed inoculation with plant growth promoting bacteria on seedling growth of five wheat cultivars a greenhouse experiment was conducted in the agricultural research institute, university of zabol, IR Iran in year 2014. A completely randomized design based on factorial arrangement with three replications was used. The first factor consisted of five cultivars of wheat (Hamoon, Croos bolani, Hirmand, Bam, Kalak afghan) and the second one was bio-fertilizer types included Phosphate solubilizing bacteria (B1), Bio-phosphor (B2), Nitroxin (B3), Nitro-kara (B4), Mixed (B1+B2+B3+B4) (B5) and distilled water as control (B6). The seeds were surface sterilized with 5% Naocl (sodium hypo chloride) for 5 min to avoid fungal invasion, followed by washing with distilled water. The bio-fertilizer solution was prepared by mixing the bio-fertilizers in water at 1: 5 ratio. Seeds were soaked in the bio-fertilizer solution for 4 hours and sown on next one hours (Gliessman, 2007). Germination tests were conducted using 6 seeds in Plastic Plant Trays. In each, cell (50 ml) one seed were sown. In this experiment traits measured including, root length, shoot length, shoot fresh weight, root fresh weight, number of leaf, root dry weight, shoot dry weight and root to shoot ratio. The statistical analysis was carried on the obtained data according, where the means compared using L.S.D. test at 0.05 level of significance. Analysis of variance by the software SAS and graphs were done with Excel software.

RESULTS AND DISCUSSION

Analysis of variance showed that root length was not significantly affected by cultivars, bio-fertilizers and C×B treatments (Table 1). According to results of analysis of variance of germination, shoot length, shoot fresh weight, root fresh weight and number of leaf were significantly affected by cultivars at 1%

probability level and shoot dry weight at 5% probability level (Table 1). Also the effects of bio-fertilizers treatment was significant on the shoot and root fresh weight at 1% probability level and root to shoot ratio at 1% probability level respectively (Table 1). Interaction of cultivars and bio-fertilizers treatments on shoot and root fresh weight and root to shoot ratio was significant at 1% probability level. Significant differences were not noticed in root length due to bio-fertilizers (Table 1). The root length did not vary due to the interaction between cultivars and bio-fertilizers. However, croos bolani (287.86 mm) recorded higher root length and the least was in bam (245.51 mm). Researchers such Narula *et al.*, (2000), Yasmin and colleagues (2004) Ahmad *et al.*, (2005), Kapulnik *et al.*, (1985), Bashan (1990) and Ravikumar *et al.*, (2004) showed that inoculation with Azotobacter on the Positive roots of wheat and it produces hormones Growth was attributed by Azotobacter.

There were significant differences in shoot length due to different wheat cultivars. The croos bolani cultivar recorded higher shoot length (174.38 mm) followed by kalak afghan cultivar (139.58 mm) and the least was in hamoon cultivar (96.41 mm) (Table 2). The treatment bio-phosphor (B2) had recorded higher shoot length (140.05 mm) and least was in nitro-kara (B4) (114 mm). The improvement of shoot length due to combined bio-fertilizer inoculation in wheat is in agreement with the results of the present study (Yazdani *et al.*, 2010).

Table 1: Analysis of variance of germination indices and seedling growth of wheat

S.O.V	root length	shoot length	shoot fresh weight	root fresh weight	number of leaf	shoot dry weight	root dry weight	root to shoot ratio
Cultivars	5971.1^{ns}	20963.1**	0.0064**	0.035**	0.94**	0.00017*	0.000038 ns	0.052^{ns}
Bio-fertilizers	5042.9 ns	1351.01 ns	0.0026**	0.028**	0.17^{ns}	0.000059 ns	0.00098 ns	0.300*
C×B	4096.2 ns	427.26^{ns}	0.0028**	0.050**	0.13^{ns}	0.000028 ns	0.000085 ns	0.561**
Error	2958.49	623.36	0.0002	0.0002	0.069	0.00003	0.00124	0.0558
CV	20.7	20.4	10.4	7.5	9.3	25.2	25.1	21.4

Higher values of shoot fresh weight were noticed in croos bolani (C2) cultivar (0.15 gr) than the other treatments. Higher shoot fresh weight was observed in the application of phosphate solubilizing bacteria (B1) treatment (0.152 gr) (Table 2). In interaction between cultivar and bio-fertilizer were obtained the highest shoot fresh weight was related to kalak afghan cultivar and phosphate solubilizing bacteria (C5B1) treatment with mean (0.21 gr) (Table 3, figure 1).

According to table (1) there are significant differences among the bio-fertilizers, cultivars and C×B in the mean comparisons root fresh weight at 1% probability level. According to table (2) is expressed such that the maximum root fresh weight equivalent to 0.23 (gr) was obtained to Bam cultivar. As well as in the between bio-fertilizers, control treatment (B6) recorded higher root fresh weight (0.28) compared to other treatments and the least was recorded in nitro-kara (B4) (0.17). In interaction between cultivar and bio-fertilizer were obtained the highest root fresh weight was related to hamoon cultivar and control (C1B6) treatment with mean (0.35 gr) (Table 3, figure 2).

The results showed that just number of leaf was significantly affected by cultivars at 1% probability level (Table 1). Also results showed that the highest number of leaf (3.1) was obtained from hirmand cultivar (C3) and in different levels of bio-fertilizer was related to phosphate solubilizing bacteria (B1) treatment with mean (2.9) number of leaf (Table 2). Kapulnik *et al.*, (1985) reported that seeds inoculation with azospirillum bacteria on corn increases the number of leaves of this plant compared to control.

Cultivars had significant effect on shoot dry weight at 5% probability level. Also the effect of bio-fertilizers treatment and its interaction with cultivars on shoot dry weight was not significant (Table 1).

Table 2: Influence of bio-fertilizers on seedling quality parameters in five cultivars

Variables	root length	shoot length	shoot fresh weight	root fresh weight	number of leaf	shoot dry weight	root dry weight	root to shoot ratio
	mm	mm	gr	gr	-	gr	gr	-
	Cultivars							
Hamoon (C1)	248.67 AB	96.41 C	0.11 C	0.22 B	2.87 B	0.019 C	0.020 C	1.07 AB
Croos bolani (C2)	287.86 A	174.38 A	0.15 A	0.18 C	2.85 B	0.028 A	0.024 BC	0.90 C
Hirmand (C3)	254.19AB	97.98 C	0.13 B	0.23 AB	3.10 A	0.024 AB	0.029 A	1.20 A
Bam (C4)	245.51 B	102.66 C	0.15 A	0.23 A	2.81 B	0.023 BC	0.027 AB	1.17 A
Kalak afghan (C5)	274.14 AB	139.58 B	0.13 B	0.12 D	2.46 C	0.022 BC	0.021 C	0.97 BC
	Bio-fertilizers							
Phosphate solubilizing bacteria (B1)	245.65 A	123.79 AB	0.152 A	0.185 BC	2.911 A	0.023 AB	0.026 A	1.10 A
Bio-phosphor (B2)	252.10 A	140.05 A	0.150 A	0.194 B	2.866 A	0.027 A	0.025 A	0.95 A
Nitroxin (B3)	278.76 A	119.71 B	0.135 B	0.176 DC	2.800 AB	0.022 B	0.023 A	1.05 A
Nitro-kara (B4)	242.76 A	114 B	0.121 C	0.171 D	2.611 B	0.021 B	0.023 A	1.012 A
Mixed (B5) (B1+B2+B3+B4)	287.52 A	114.86 B	0.142 AB	0.191 B	2.866 A	0.023 AB	0.024 A	1.06 A
Control (B6)	265.66 A	120.81 B	0.123 C	0.287 A	2.866 A	0.022 AB	0.024 A	1.09 A

Means followed by similar letters in each column are not significantly different at p=5%, Duncan Multiple Range test

The highest shoot dry weight (0.028 gr) was obtained from croos bolani (C2) cultivar and lowest (0.19) levels of shoot dry weight were obtained in hamoon (C1) treatment (Table 2). In between bio-fertilizers levels, highest (0.027) and lowest (0.022) levels of shoot dry weight were obtained in bio-phosphor (B2) and control (B6) treatment, respectively (Table 2). (Table 1) shows the effects of cultivars and bio-fertilizers on root dry weight.

Cultivars, bio-fertilizers and interaction B×C had no significant effects on root dry weight (Table 1). Amooaghaie *et al.,* (2003) reported that inoculation with azospirillum on seedling growth of wheat increased in compared with the control root and shoot dry weight, %18.3 and %12.3 respectively. They also were reported increased the ratio of root to shoot dry weight of inoculated plants compared to control plants.

Analysis of variance showed that the root to shoot ratio was significantly affected by bio-fertilizers at 5% probability level, interaction B×C at 1% probability level and cultivars treatment had no significant effect on root to shoot ratio (Table 1). In interaction between cultivar and bio-fertilizer were obtained the highest root to shoot ratio was related to hirmand cultivar and nitroxin (C3B3) treatment with mean (1.4) (Table 3, figure 3).

Table 3: Interaction between cultivars and bio-fertilizers on root and shoot fresh weight and root to shoot ratio

Cultivars	Bio-fertilizers	root to shoot ratio		root fresh weight		shoot fresh weight	
C1	B1	1.19	abcd	0.10	L	0.08	Jk
	B2	0.90	Cd	0.13	Jk	0.14	Efg
	B3	1.00	Cd	0.07	M	0.08	Jk
	B4	1.03	Cd	0.15	J	0.14	Efg
	B5	1.18	abcd	0.14	Jk	0.11	Hi
	B6	1.16	abcd	0.35	A	0.10	Ij
C2	B1	0.97	Cd	0.18	I	0.14	Efgh
	B2	0.86	Cd	0.22	Gh	0.18	Bcd
	B3	0.82	D	0.19	I	0.15	Def
	B4	1.09	abcd	0.14	Jk	0.16	Def
	B5	0.80	D	0.14	Jk	0.14	Efg
	B6	0.87	Cd	0.27	Cde	0.19	Ab
C3	B1	1.17	abcd	0.26	Cde	0.14	Efg
	B2	1.26	abc	0.25	Def	0.14	Efg
	B3	1.48	A	0.27	Cd	0.14	Fgh
	B4	1.05	abcd	0.13	Jk	0.10	Ij
	B5	1.10	abcd	0.28	Bc	0.15	Def
	B6	1.18	abcd	0.19	Hi	0.12	Ghi
C4	B1	1.17	abcd	0.26	Cde	0.18	Bc
	B2	0.95	Cd	0.24	Efg	0.16	Def
	B3	1.08	abcd	0.22	Fg	0.16	Cdef
	B4	1.47	Ab	0.31	B	0.14	Efgh
	B5	1.19	abcd	0.27	Cde	0.16	Cde
	B6	1.16	abcd	0.12	Kl	0.11	I
C5	B1	1.03	bcd	0.13	Jk	0.21	A
	B2	0.79	D	0.14	Jk	0.14	Efgh
	B3	0.89	Cd	0.13	Jk	0.14	Efg
	B4	0.96	Cd	0.13	jkl	0.07	K
	B5	1.05	abcd	0.13	jkl	0.14	Efg
	B6	1.12	abcd	0.11	Kl	0.10	Ij

Means followed by similar letters in each column are not significantly different at p=5%, Duncan Multiple Range test

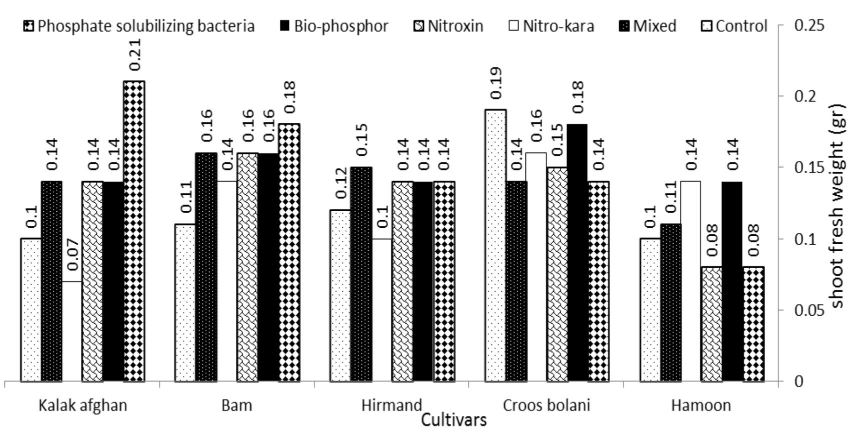

Figure 1: Interaction between cultivar and bio-fertilizer on shoot fresh weight

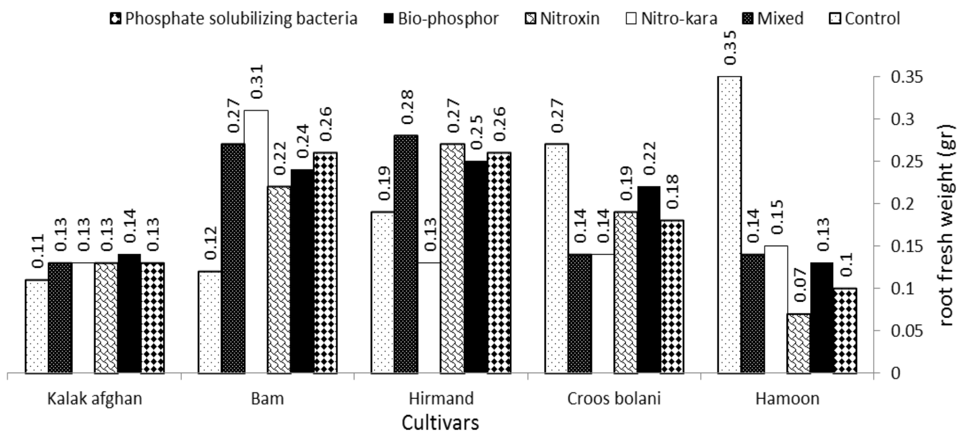

Figure 2: Interaction between cultivar and bio-fertilizer on root fresh weight

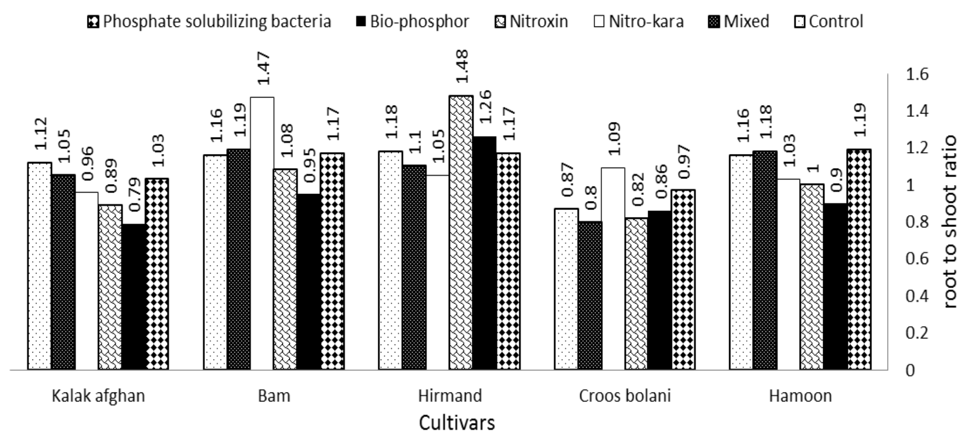

Figure 3: Interaction between cultivar and bio-fertilizer on root to shoot ratio

REFERENCES

Ahmad F, Ahmah I and Khan MS (2005). Indole acetic acid production by the indigenous isolated of Fluorescent pseudomonas in the presence and absence of Tryptophan. *Turkish Journal of Biology* **29** 29-34.

Amooaghaie R, Tenants L and Points G (2003). Effect of Azospirillum bacteria on growth and yield of wheat cultivars. *Journal of Science and Technology of Agriculture and Natural Resources* **14** 127-139. (In Persian with English Summary).

Ayyadurai N, Ravindra Naik P, Sreehari RM, Sunish Kumar R, Samrat SK, Manohar M and Sakthivel N (2006). Isolation and characterization of a novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micropropagation of banana. *Journal of Applied Microbiology* **100** 926-937.

Bashan Y (1998). Inoculants of plant growth promoting rhizobacteria for use in agriculture. *Biotechnology Advances* **16** 729-770.

Bashan Y, Harison SK and Whitmoyer RE (1990). Enhanced growth of wheat and soybean plants inoculated with Azospirillum brasilense is not necessarily due to general enhancement of mineral uptake. *Applied and Environmental Microbiology* **56** 769 -775.

Cakmakc R, Donmez MF and Erdogan U (2007). The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turkish Journal of Agriculture and Forestry* **31** 189-199.

Gliessman SR (2007). *Field and Laboratory Investigations in Agroeclogy* (CRC Press, Taylor and Francis Group).

Husen E (2003). Screening of soil bacteria for plant growth promotion activities in vitro. *The Indian Journal of Agricultural Sciences* **4** 27-31.

Joseph B, Patra RR and Lawrence R (2007). Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicer arietinum L). *International Journal of Plant Production* **1** 141-152.

Kapulnik Y, Gafny R and Okon Y (1985). Effect of Azospirillum spp. Inoculation on root development and NO3 uptake in wheat in hydroponic systems. *Canadian Journal of Botany* **63** 627-631.

Mia MAB, Shamsuddin ZH, Zakaria W and Marziah M (2005). High-yielding and quality banana production through plant growth promoting rhizobacterial inoculation. *Fruits* **60** 179-185.

Narula N, Kumar V, Behl RK, Deubel A, Gransee A and Merbach W (2000). Effect of P-Solubilizing Azotobacter chroococcum on N, P and K uptake in P-responsive wheat genotypes grown under greenhouse conditions. *Journal of Plant Nutrition and Soil Science* **163** 393-398.

Narula N, Kumar V, Singh B, Bhatia R and Lakshminarayana K (2005). Impact of biofertilizers on grain yield inspring wheat under varying fertility conditions and wheat-cotton rotation. *Archives of Agronomy and Soil Science* **51** 79-89.

Ravikumar S, Kathiresan K, Ignatiammal STM, Selvam MB and Shanthy S (2004). Nitrogen-fixation Azotobacters from mangrove habitat and their utility as marine biofertilizers. *Journal of Experimental Marine Biology and Ecology* **15** 157-160.

Shaharoona B, Arshad M, Zahir ZA and Khalid A (2006). Performance of Pseudomonas spp. containing ACC-deaminase for improving growth and yield of maize (Zea mays L.) in the presence of nitrogenous fertilizer. *Soil Biology & Biochemistry* **38** 2971-2975.

Taregh G, Valizadeh M and Hossein S (2011). Effect of drought on germination indices and seedling growth of 12 bread wheat genotypes. *Advances in Environmental Biology* **5**(6) 1034-1039.

Tsavkelova EA, Cherdyntseva TA, Botina SG and Netrusov AI (2007). Bacteria associated with orchid roots and microbial production of auxin. *Microbiological Research* **162** 69-76.

Vessey JK (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **255** 571-586.

World Wheat Crop To Be Third Largest Ever. Farmers Weekly, 152.13. 2010. 134. Academic Search Premier. Web. 13 March 2013.

World Wheat, Corn and Rice. Oklahoma State University, FAO Stat.

Wu SC, Cao ZH, Li ZG, Cheung KC and Wong MH (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* **125** 155-166.

Yasmin S, Bakar MAR, Malik KA and Hafeez F (2004). Isolation characterization and benefical effects of rice associated plant growth promoting bacteria from Zanzibar soils. *Journal of Basic Microbiology* **44** 241-252.

Yazdani Bavaicki R, Rezvani Moghaddam P, Koochaki A, Behzad Amiri M, Fallahi J and Deyhim Fard R (2010). Effects of seed nourished by different levels of nitrogen, biofertilizers and drought stress on germination indices and seedling growth of wheat (Tritium aestivum) cv. Sayonz. *Journal of Agroecology* **2**(2) 266- 276 (In Persian with English Summary).