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EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON GROWTH ANDYIELD OF WHEAT PLANTS CULTIVATED IN SANDY AND CLAY SOILS

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ABSTRACT: Three efficient indigenous isolates of plant growth promoting rhizobacteria [*Azospirillum* sp. (ASH21) *Azotobacter* sp. (ZH21) and *Rhizobium leguminosarum bv. viciae*(RZ11)], were used as biofertilizers, either in single or mixed cultures under different levels of inorganic and organic N-fertilizers for wheat (*Triticum aestivum* L.) plants cultivated in sandy or clay soil. The results concluded that the treatment inoculated with tri-mixture inoculants of ASH21, ZH21 and RZ11combined with 1/2 the recommended dose of inorganic N-fertilizer or the recommended dose of compost increased plant dry weight, nitrogen, phosphorus,and potassium contents, as well as biomass yield , and protein percentage in grains of wheat plants. Gibberellins (GA_3) and indole acetic acid (IAA) production and nitrogenase and dehydrogenase activities were enhanced in wheat plants rhizosphere in both types of soils. These results revealed the importance of PGPR as biofertilizers for wheat plants to obtain the best performance in growth and yield.

Key words: Wheat plants, PGPR, gibberellins and IAA, bio-fertilizers.

INTRODUCTION

Cereals, such as rice, wheat and maize, are the major food source for human being. Wheat growth in temperate climates is the stable food for 35% of the world's population. It provides more calories and proteins in the diet than any other crops (Laegreid *et al*., 1999).

Climate conditions and modern agriculture are severely modifying and polluting the natural environment. Rhizobacteria that exert beneficial effects on plants, are called "Plant Growth Promoting Rhizobacteria" (PGPR), and considered to be an alternative to the use of chemicals. PGPR may benefit the host by promoting plant growth or through biological disease control. PGPR activity has bean reported in strains belonging to several genera, such as *Azotobacter, Azospirillum, Pseudomonas, Acetobacter, Burkholderia and Bacillus* (Kloepper, 1993; Glick and Bashan ,1997).

A wide range of bacteria in the rhizosphere can promote plant growth, orchestrated by rhizosphere bacteria that communicate with the plant using complex chemical signals. Gibberellins, glycolipids and cytokinins are now beginning to be fully appreciated in terms of their biotechnological potential. A critical process that occurs on the surface of the plant in general and of the root in particular is the associative nitrogen fixation in which the nitrogen fixing microorganisms inhabit the surface of the plant root, the rhizoplane, as well as in the rhizosphere. This process is carried out by representatives of the genera *Azotobacter* and *Azospirillum*. Recent evidence suggests that their major contribution may not be due to nitrogen fixation but to the production of growth-promoting hormones that increase root hair development and thus greater ability of the plant to take up nutrients. This is an area of research that is particularly important in tropical agricultural areas.

Inoculation with indigenous *Azospirillum* strains is important procedure when studying their inherent capacity to benefit crops. In some cases, indigenous can perform better than introduced strains in promoting the growth of crops due to their superior adaptability to the environment (Gunarto *et al.* 1999). On the other hand, application of organic fertilizers was shown to enhance the incidence and activities of plant promoting rhizobacteria (Khamis and Metwally, 1998).

For all reasons, there is a widespread interest in the use of combination of mineral fertilizers and biofertilizers as an alternative and cheap source instead of chemical fertilizers alone.

The objective of this work is to study the effect of selected efficient indigenous rhizobacteria alone or combined with organic or inorganic N-fertilizers on growth and yield. of wheat plants cultivated in two soil textures.

MATERIALS AND METHODS

Three experiments were conducted in the Laboratory and Greenhouse of Agric. Microbiology Dept. at the Faculty of Agriculture, Zagazig University, Egypt, to examine the effect of indigenous selected biofertilizers at different levels of inorganic and organic N-fertilizers on the growth and phytohormones production of wheat (*Triticum aestivum* L.) plants cultivated in clay and sandy soil of Sharkia Governorate.

Inocula Preparation

Among 64 isolates, three efficient indigenous isolates of *Azospirillum* sp. (ASH21) *Azotobacter* sp. (ZH21) and *Rhizobium leguminosarum bv. viciae*(RZ11), were chosen as the best indigenous isolates from Sharkia Governorate soils (Salem *et al.,* 2006) to be used in this investigation.

Azospirillum isolates were cultured in NFM Semisolid Malate Medium (Day and Döbereiner, 1976), and incubated in the dark at 28 ± 2°C in rotary shaker 120 rpm for 2-5 days. *Azotobacter* and *Rhizobium* strains were inoculated in Modified Ashby's Medium (Abd El-Malek and Ishac, 1968) and Yeast Extract Mannitol (YEM) Broth (Allen, 1959) respectively, and incubated in the dark at 28°C±2°C in rotary shaker 120 rpm for3-7 days.

Soil Types and Analysis

Two different soil textures were used in these greenhouse experiments, namely clay and sandy soils obtained from Zagazig and Salhia, located in Sharkia Governorate, Egypt. The mechanical and physicochemical analyses of both soils were carried out according to Jackson (1970) and Harrigan and McCance (1966) and listed in Table 1. The soil was collected from top layer (0-30 cm depth) then distributed in plastic pots (25 cm diameter and 20 cm depth) at the rate of 6 kg per pot.

Inorganic Fertilizers

The recommended doses for wheat are, 500 kg fed^{-1}, 375 kg fed^{-1} ammonium sulphate (20.5 % N) 100 kg N and 75 kg N fed^{-1}, for sandy and clay soil, respectively). While, calcium superphosphate is 100 kg fed^{-1} (15.5 P_2O_5 kg fed^{-1}) and potassium sulphate is 100kg

fed^{-1}(48 K_2O fed^{-1}) for both types of soil. Calcium superphosphate and potassium sulphate were added just before sowing in both soils for all treatments, while ammonium sulphate was added in three equal doses after 21, 60 and 90 days of plantation in sandy soil. In clay soil, ammonium sulphate was added in two equal doses after 21 and 60 days.

Organic Fertilizer

Garbage compost was provided from the Organic Fertilizers Company, Cairo, Egypt. It has an organic carbon of 30 %, total nitrogen 1.0 %, moisture, 35 % and pH 7.5 ± 0.2. Garbage compost was thoroughly mixed with soil 20 days before cultivation in both sandy and clay soil [recommended dose is 5 ton fed^{-1} (0.5 %) in sandy soil and 2 ton fed^{-1} (0.2 %) in clay soil].

Effect of Selected Biofertilizers on Growth of Wheat Plants at Different Levels of N-Application

Pots (25 cm) were filled with 6 kg non-sterile sandy or clay soils. The wheat grains (Giza 168 cultivar), were obtained from Wheat Research Section, Field Crops Research Institute, ARC, Giza, Egypt.

Table 1. Physical and chemical analyses of the soil samples

Type of soil analysis	Properties	Salhia soil	Zagazig soil
Physical analysis	pH	8.12	7.6
	EC mmhos.cm^{-1}	3.10	2.67
Mechanical analysis	Fine sand %	24.45	27.1
	Coarse sand %	71.15	7.12
	Silt %	2.00	12.97
	Clay %	2.40	52.82
	Type of soil	Sandy	Clay
Chemical analysis	Cationes		
	Ca^{++}	0.19	0.54
	Mg^{++}	0.30	0.66
	Na^{+}	0.79	1.49
	K^{+}	0.06	0.07
	Aniones		
	HCO_3^{-}	0.31	0.48
	Cl^{-}	0.43	1.28
	SO_4^{--}	0.61	1.52
	CO_3^{--}	Traces	Traces
Total nitrogen %		0.015	0.14

Wheat grains were surface sterilized according to Vincent, (1970). The surface sterilized grains were soaked in *Azospirillum* suspension (1×10^9 cfu) for two hours. The seeds were sown and then 5 ml of cell suspension was applied over the grains in each treated pot and covered with soil.

The first greenhouse experiment was carried out to examine the effect of selected bio-fertilizers on growth of wheat plants. The experiment included the following treatments: uninoculated control, inoculation with isolate ASH21, ASH21+ ZH21, ASH21 + RZ11, or inoculated with ASH21 + ZH21 + RZ11. The experimental design was completely randomized with 5 treatments and 3 replicates.

The second greenhouse experiment was carried out to examine the combined effect of using bio-fertilizers with or without inorganic fertilizers on growth of wheat plants in two soil textures. Pots were amended with the following treatments: uninoculated control without inorganic N-fertilizer, uninoculated + recommended field dose of inorganic N-fertilizer, inoculated with isolate ASH21, inoculated with isolate ASH21+ the recommended field dose of inorganic N-fertilizer, inoculated with isolate ASH21+ 1/4 the recommended dose of inorganic N-fertilizer, inoculated with isolate ASH21+1/2 the recommended dose of inorganic N-fertilizer, inoculated with isolate ASH21+ 3/4 the recommended dose of inorganic N-fertilizer, and inoculated with ASH21, ZH21 and RZ11 + 1/2 the recommended dose of inorganic N-fertilizer. The experimental design was completely randomized with 8 treatments and 3 replicates.

The third greenhouse experiment was carried out to examine the combined effect of selected biofertilizers and organic fertilizers on growth of wheat plants. Pots were amended with the following treatments: control (uninoculated and without organic N-fertilizer), uninoculated + recommended field dose of organic N-fertilizer, inoculated with isolate ASH21, inoculated with isolate ASH21+ recommended field dose of organic fertilizer, inoculated with isolate ASH21+ 1/2 recommended dose, inoculated with isolate ASH21+ double recommended field dose, and inoculated with tri-mixture inoculants of ASH21, ZH21 and RZ11 + recommended dose of organic fertilizer. The experimental design was completely randomized with 7 treatments and 3 replicates.

For all of the above three experiments, plants were thinned to leave 5 plants/pot after ten days and watered daily with tap water. After 60 days from planting, the dry weight of whole wheat plants was determined after drying the plant parts at 70 °C for 24 hours (Somasegaran and Hoben, 1985), the acetylene reduction activity (ARA) of the rhizosphere soil was measured using the method of Hardy *et al.* (1973), dehydrogenase activity was assayed according to Casida *et al.*, (1964), total nitrogen content in whole plant was determined according to the method described by Naguib (1969), total phosphorus content of the whole plant was determined by the hydroquinone method as described by Snell and Snell (1954). Total potassium content in whole plant as described by Brown and Lilleband (1946). At harvesting time: weighing of 1000 grains, total nitrogen content (Naguib 1969), and crude protein in grains were determined.

Extraction and Determination of Phytohormones (IAA, IBA and GA_3) from Root Plants

The extraction from fresh root sample was carried out according to Shindy and Smith (1975). Standard and extracts of hormonal compound were determined according to Frankenberger and Brunner (1983) and Grolamys and Servando (1997).

Finally, the data were subjected to statistical analysis as a complete randomized design according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Response of Wheat Plants to Bio-fertilizers Under Cultivation in Sandy and Clay Soils

Pot experiments were carried out to study the effect of selected bio-fertilizers alone on wheat plants cultivated in either of sandy or clay soils, in terms of plants dry weight, nitrogenase and dehydrogenase activities in rhizosphere, total nitrogen, total phosphorus and total potassium contents and biomass yield of wheat plants.

The results in Table 2 showed that inoculation with the tri – mixture of ASH21, ZH21 and RZ11 resulted in the highest value of whole plant dry weight in clay and sandy soils being 1.43 and 1.19 g/plant respectively, while the lowest value was obtained by

Table 2. Effect of bio-fertilizers on the growth and yields in wheat plants cultivated in sandy and clay soil

Soil textures / Fertilization treatments	Whole plant dry weight g/plant			Nitrogenase activity (µ mole C_2H_4/h/g dry soil)		Dehydrogenase activity (µ g H_2/g dry soil)		Total N/plant (mg)			Total P/plant (mg/plant)			Total K/plant (mg/plant)			Weight of 1000 grains (g)			N% in grains			Protein % in grains		
	Sandy	Clay	Av.	Sandy	Clay	Sandy	Clay	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.
Control	0.23	0.93	0.58	0.45	1.56	3.24	3.25	2.84	11.27	7.06	0.28	1.23	0.76	3.31	11.35	7.33	0.00	29.35	14.68	0.00	1.60	0.80	0.00	9.16	4.58
Inoculation by ASH21 *	1.08	1.24	1.16	5.13	18.73	1.58	3.20	14.39	18.49	16.44	2.54	2.64	2.59	16.57	19.75	18.16	24.85	35.75	30.30	1.59	1.80	1.69	9.12	10.36	9.74
ASH21* + ZH21	1.15	1.33	1.24	11.74	53.62	10.65	8.58	19.18	21.43	20.31	1.95	2.67	2.31	22.18	25.72	23.95	28.35	40.45	34.40	1.77	1.98	1.87	10.18	11.36	10.77
ASH21* + RZ11	0.78	1.22	1.00	5.05	6.55	3.53	5.10	13.05	17.18	15.12	1.51	1.72	1.61	16.32	21.03	18.67	26.70	37.00	31.85	1.68	1.86	1.77	9.64	10.67	10.15
ASH21+ZH21+RZ11**	1.19	1.43	1.31	11.84	63.08	4.84	11.54	25.00	20.25	22.63	5.07	2.67	3.87	28.21	23.77	25.99	29.95	40.70	35.33	1.88	2.02	1.95	10.82	11.59	11.20
Soil texture av.	0.89	1.23						14.89	17.72		2.27	2.18		17.32	20.32		21.97	36.65		1.38	1.85		7.95	10.63	
LSD for	Soil texture	Fertili	S × F					Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili	S × F	Soil texture	Fertili	S × F	Soil texture	Fertili.	S × F
0.05	0.18	0.28	NS					NS	4.84	NS	NS	0.82	1.16	NS	5.72	NS	0.29	0.46	0.65	0.02	0.03	0.04	0.10	0.15	0.21
0.01	0.26	0.42	NS					NS	7.12	NS	NS	1.20	NS	NS	8.42	NS	0.43	0.68	0.96	0.03	0.05	0.07	0.14	0.22	0.32

* Inoculation with *Azospirillum* spp. (ASH21).

** Inoculation with *Azospirillum* spp. (ASH21), *Azotobacter* spp. (ZH21) and *Rhizobium leguminosarum* bv. *viciae* (RZ11).

uninoculated (control) treatment in sandy soil, being 0.23 g/plant and 0.93 g/plant in clay soil. The statistical differences among fertilization treatments and between soil textures were highly significant ($P \leq 0.01$). However, the differences in the interaction between soil textures and fertilization treatments were insignificant.

The results also showed a great variation in the effect of inoculation with different bio-fertilizers on nitrogenase and dehydrogenase activities in rhizosphere of wheat plants grown on both sandy and clay soils. The highest values were obtained by treatment inoculated with tri-mixture inoculant, showing 11.84 and 63.08 μ mole C_2H_4/h/g dry soil in sandy and clay soils, respectively.

The highest values of dehydrogenase activity were also obtained by tri-mixture inoculants of ASH21 + ZH21 and RZ11 in clay soil and with ASH21 in both sandy and clay soils. In the second rank were plants inoculated with ASH21 + ZH21 in both soil textures.

Data also showed that application of bio-fertilizers significantly increased the total nitrogen content in whole plant compared with the uninoculated treatment. The highest average values of total nitrogen content in whole wheat plants were obtained by the treatments inoculated with tri-mixture inoculants in sandy soil and those inoculated with ASH21 + ZH21 without significant differences between them. As for the effect of soil textures, the average total nitrogen content was significantly increased in clay soil (17.72 mg N/plant) as compared to sandy soil (14.89 mg N/plant). The interaction between the two tested factors, concerning total nitrogen content in whole wheat plants was insignificant.

Data concerning the influence of biofertilizers application on the phosphorus content of the wheat plants, showed no significant difference between inoculation treatments in clay soil (2.67 mg P/plant). While, inoculation with tri-mixture in sandy soil induced the highest total phosphorus content, being 5.07 mg P/plant in whole plant.

Also in this respect inoculation with the tri-mixture inoculants induced the highest potassium content reaching 28.21 and 23.77 mg K/plant in whole plant in sandy and clay soil respectively, without significant differences between them and those treated with ASH21 + AH21, in clay soil.

Data in Table 2 also indicated that the bio-fertilization treatments had clear effect on weight of 1000 grains, nitrogen and protein percentage in grains. The highest values being 40.70 g, 2.02 % and 11.59 %, respectively, were recorded by plants inoculated with tri-mixture inoculants of ASH21, ZH21 and RZ11 in clay soil. In the second rank, came plant treated with inoculation of ASH21 + ZH21 followed by those treated with ASH21 + RZ11 and those treated with ASH21 alone. The differences between inoculation treatment were highly significant and so were texture treatments.

However, uninoculated treatment (control) showed the lowest values. Interaction between the two tested factors was also highly significant. In this respect, Panwar (2000) and Sushila and Giri-Gajendra (2000) stated that bio-fertilization by *Azospirillum* and *Azotobacter* enhanced the growth and yield of wheat. Recently, Vivek *et al.*, (2004) studied the relative efficacy of *Azotobacter chroococcum* on tall and dwarf wheat (*Triticum aestivum* L.) in arid soils. They found that grain, straw yield and biomass of root were higher with mutants, which maintained higher survival rate in rhizosphere during growth period of wheat crop.

Fischer *et al.*, (2007) evaluated the effect of isolated PGPR on the growth of wheat plants and they found that one native strain increased the shoot and root biomass by 23% and 45%, respectively

Response of Wheat Plants to Bio-and Inorganic Fertilizers under Cultivation in Sandy and Clay Soils

The plant dry weight, total nitrogen, total phosphorus and total potassium contents as well as grain yield of wheat plants, nitrogenase and dehydrogenase activity in rhizosphere of wheat plants were determined in this experiment.

Data in Table 3 showed that the highest value of whole plant dry weight was obtained by the treatment receiving 1/2 N + inoculation with tri-mixture inoculant of ASH21, ZH21 and RZ11 in sandy soil where the differences between treatments were highly significant. In clay soil, however the differences between fertilization treatments were significant compared to the

Table 3. Effect of bio-fertilizers and inorganic N-fertilizers on the growth and yields in wheat plants cultivated in sandy and clay soil

Fertilization treatments \ Soil textures	Whole plant dry weight g/plant			Nitrogenase activity (µ mole C_2H_4/h/g dry soil)		Dehydrogenase activity (µ g H_2/g dry soil)		Total N/plant (mg)			Total P/plant (mg/plant)			Total K/plant (mg/plant)			Weight of 1000 grains (g)			N% in grains			Protein % in grains		
	Sandy	Clay	Av.	Sandy	Clay	Sandy	Clay	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.
Control	0.23	0.93	0.58	0.450	1.556	3.242	3.252	2.84	11.27	7.06	0.28	1.23	0.75	3.31	11.35	7.33	0.00	29.35	14.68	0.00	1.60	0.80	0.00	9.16	4.58
Inoculation by ASH21*	1.08	1.24	1.16	5.128	18.733	10.62	8.582	14.31	18.49	16.40	2.53	2.64	2.59	16.70	19.77	18.24	24.85	35.75	30.30	1.59	1.80	1.69	9.12	10.36	9.74
Nitrogen dose (N)	1.42	1.68	1.55	0.298	1.989	9.180	5.672	23.52	34.39	28.95	3.33	5.84	4.59	28.12	37.00	32.56	34.95	48.25	41.60	2.03	2.15	2.09	11.46	12.36	11.91
N + ASH21*	1.55	1.56	1.56	7.801	4.172	3.580	7.598	32.68	33.16	32.92	3.86	4.67	4.26	32.35	32.09	32.22	38.60	44.05	41.33	2.18	2.00	2.09	12.54	11.53	12.03
1/4 N + ASH21*	1.37	1.36	1.37	7.569	6.082	9.924	5.199	25.65	26.60	26.13	3.19	3.72	3.46	28.49	31.76	30.13	28.45	39.50	33.98	1.72	1.86	1.79	9.88	10.67	10.27
1/2 N + ASH21*	1.84	1.44	1.64	9.441	7.989	4.058	4.432	34.85	36.16	35.51	4.97	5.94	5.46	39.81	36.51	38.16	34.10	44.25	39.18	2.03	2.02	2.02	11.78	11.62	11.70
3/4 N + ASH21*	1.51	1.52	1.52	8.479	4.512	3.499	9.666	30.90	29.58	30.24	4.04	4.86	4.45	31.89	30.09	30.99	36.90	45.10	41.00	2.13	2.14	2.14	12.12	12.33	12.22
1/2 N+ASH21+ ZH21+RZ11**	2.12	1.61	1.87	9.501	29.645	11.14	9.274	43.99	38.82	41.41	4.84	6.26	5.55	46.85	40.44	43.64	38.30	46.70	42.50	2.19	2.13	2.16	12.60	12.25	12.42
Soil texture av.	1.39	1.42						26.09	28.56		3.38	4.40		28.44	29.88		29.52	41.62		1.73	1.96		9.94	11.28	
LSD for	Soil texture	Fertili.	S × F					Soil texture	Fertili.	S × F	Soil texture	Fertili	S × F	Soil texture	Fertili	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F
0.05	NS	0.28	0.40					NS	6.54	NS	0.62	1.24	NS	NS	6.45	NS	0.37	0.75	1.06	0.02	0.04	0.06	0.14	0.28	0.40
0.01	NS	0.41	NS					NS	9.46	NS	0.90	1.80	NS	NS	9.34	NS	0.54	1.08	1.53	0.03	0.06	0.09	0.20	0.41	0.58

* Inoculation with *Azospirillum* spp. (ASH21).

** Inoculation with *Azospirillum* spp. (ASH21), *Azotobacter* spp. (ZH21) and *Rhizobium leguminosarum bv. viciae* (RZ11).

N: Recommended dose of nitrogen (100 kg N in sandy soil and 75 kg N in clay soil).

uninoculated control or inoculation with ASH21 only, respectively. However statistical analysis showed that the differences between soil textures were not significant, while the differences between fertilization treatments were highly significant ($P \leq 0.01$). The differences in the interaction between soil textures and fertilization treatments were also significant ($P \leq 0.05$).

Regarding the nitrogenase activity, the values ranged from 0.298 to 29.645 μ mole C_2H_4 /h/g dry soil. Treatment receiving 1/2 N + inoculation with tri-mixture inoculant in clay soil, resulted in the highest enzyme activity, being 29.645 μ mole C_2H_4 /h/g dry soil, comparing with the control (1.556 μ mole C_2H_4 /h/g dry soil). In sandy soil, however, the same previous treatment gave also the highest values for N_2-ase in the rhizosphere of wheat plants.

The specific dehydorgenase activities in the rhizosphere of wheat (μ g hydrogen/g dry soil), the values ranged from 3.242 to 11.14 μ g H_2/g dry soil in sandy and uninoculated control. Here also the same treatment receiving 1/2 N + inoculation with tri-mixture. In clay soil the latter treatment resulted in 11.14 and 9.274 μ g H_2/g dry soil.

Data also showed that the application of bio-fertilizers plus inorganic N-fertilizers increased the total nitrogen content (mg N/plant) in the whole parts of wheat plants compared to the uninoculated treatment (control). Plants grown in clay soil resulted in higher values of total nitrogen contents in wheat plants than sandy soil, the average being 28.56 and 26.09 mg N/plant, respectively. The statistical differences among fertilization treatments were highly significant ($P \leq 0.01$), while the differences between soil textures and the interaction between soil textures and fertilization treatments were insignificant.

Concerning the total phosphorus content in whole plant the values ranged from 0.28 to 6.26 mg P/plant. In clay soil, the treatment received 1/2 N + inoculation with tri-mixture inoculants showed the highest value compared to the control, being 6.26 and 1.23 mg P/plant, respectively. In sandy soil, however, treatment received 1/2 N + inoculation with ASH21 only showed the highest value as compared to the control, being 4.97 and 0.28 mg P/plant, respectively. It can be noticed that clay soil resulted in higher values than sandy soil. The differences among fertilization treatments and the differences between soil textures were highly significant ($P \leq 0.01$), but the interaction between soil textures and fertilization treatments were insignificant.

The effect of bio-fertilizers and inorganic N-fertilizers on total potassium contents showed that the treatments inoculated with tri-mixture inoculant combined with 1/2 N dose gave the highest values of total potassium content in whole plant of wheat plants, being 46.85 and 40.44 mg/plant in sandy and clay soils, respectively as compared with other treatments. The statistical differences among fertilization treatments were highly significant ($P \leq 0.01$), but the differences between soil textures and interaction differences between soil textures and fertilization treatments were not significant.

Results from Table 3 indicated that there were highly significant ($P \leq 0.01$) differences among fertilization treatments, soil textures and the interaction between fertilization treatments and soil textures in biomass yield (g/1000grains) of wheat plants grown in sandy and clay soils. The highest value was obtained by treatment receiving N-dose + inoculation with ASH21 in sandy soil reaching 38.60 g/1000 grains and treatments receiving N-dose only without bio-inoculants and 1/2 N dose + inoculants of ASH21 + ZH21 + RZ11 in clay soil, being 48.25 and 46.70 g/1000 grains, respectively, comparing with the lowest value obtained by the uninoculated treatment (control) in sandy and clay soils. In general, results show that the average values of biomass (g/1000 grains) of wheat plants grown in sandy soil were lower than those obtained from wheat plants grown in clay soil, being 29.52 and 41.62 g/1000grains, respectively. In addition, there were highly significant differences ($P \leq 0.01$) among fertilization treatments, soil textures and interaction between soil textures and fertilization treatments in nitrogen (%) and protein (%) of wheat plants grown in both sandy and clay soils. The highest value was obtained by the treatment received 1/2 N + inoculation with tri-mixture inoculant in sandy soil being, 2.19 N% and 12.60 protein % in grains. However, in clay soil treatment received N-dose without bio-fertilization and treatment received 3/4 N dose + inoculation with

ASH21 only resulted in the highest values i.e., 2.15 and 2.14 % N and 12.36 and 12.33 % protein in grains, respectively. The uninoculated treatment (Control) in sandy soil did not form any grains in plant.

These results are in agreement with those reported by Goher *et al.* (1988) who reported that bio-fertilization of wheat plants, using N_2-fixing *Azotobacter* and *Azospirillum* reduced the inorganic nitrogen requirement and increased the biomass yields. Also in the same aspect Mitkees *et al.* (1996) concluded that application of bio-fertilizers (*Azospirillum* spp. + *Bacillus* + *Klebsiella* + *Azotobacter*) for wheat plants could compensate considerable parts of mineral N-fertilizers, since it saves about 50 Kg N fed^{-1} in old land and 40 Kg fed^{-1} in new land. Sarwar *et al.* (1998) reported also that the application of *Azospirillum lipoferum* inoculants and three levels of nitrogen fertilizers (10 Kg/ha, 45 Kg/ha and 90 Kg/ha) to rice plant gave highly significant increase in growth and yield of the crop specially when nitrogen was applied at 45 Kg/ha.

El-Demerdash *et al.,* (1993), Galal and Elghandour (1994) and Galal and Thabet (2002) concluded that inoculation with bio-fertilizers and application of inorganic N-fertilizers to wheat plants gave the best results with regard to yield and N uptake in shoot and grains.

Response of Wheat Plants to Bio-and Organic Fertilizers under Cultivation in Sandy and Clay Soils

Data in Table 4 showed that the whole plant dry weight, generally, ranged between 0.23 and 1.37 g/plant. The differences between soil textures and among fertilization treatments were highly significant ($P \leq 0.01$), but the interaction between soil textures and fertilization treatments, was insignificant. The application of compost combined with bio-fertilization of tri-mixture inoculant increased the whole pant dry weight as compared to the average of all other treatments in both sandy and clay soils.

The stimulatory effect may be due to application of compost as an organic material which can also provide the microorganisms with suitable sources of nutrients, growth factors and energy to survive and persist in the rhizosphere of plants (Molla *et al.,* 2001).

The effect of bio-fertilizers and organic amendments on nitrogenase

Table 4. Effect of bio and organic fertilizers on the growth and yields in wheat plants cultivated in sandy and clay soil

Soil textures / Fertilization treatments	Whole plant dry weight g/plant			Nitrogenase activity (µ mole C_2H_4/h/g dry soil)		Dehydrogenase activity (µ g H_2/g dry soil)		Total N/plant (mg)			Total P/plant (mg/plant)			Total K/plant (mg/plant)			Weight of 1000 grains (g)			N% in grains			Protein % in grains		
	Sandy	Clay	Av.	Sandy	Clay	Sandy	Clay	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.	Sandy	Clay	Av.
Control	0.23	0.93	0.58	0.45	1.56	3.242	3.252	2.84	11.29	7.07	0.28	1.23	0.76	3.31	11.35	7.33	0.00	29.35	14.68	0.00	1.60	0.80	0.00	9.16	4.58
Inoculation by ASH21*	1.08	1.24	1.16	5.13	18.73	10.650	8.582	14.31	18.31	16.31	2.54	2.64	2.59	16.57	19.82	18.19	24.85	35.75	30.30	1.58	1.80	1.69	9.12	10.36	9.74
Recommended comp. dose	0.49	0.96	0.73	1.15	2.05	4.272	4.939	7.67	12.91	10.29	0.81	2.04	1.43	8.20	15.49	11.85	26.60	36.90	31.75	1.68	1.86	1.77	9.64	10.72	10.18
Comp. + ASH21*	1.13	1.28	1.21	6.22	4.24	14.534	5.663	20.60	17.73	19.17	3.00	2.81	2.91	24.25	20.43	22.34	28.95	37.60	33.28	1.80	1.95	1.88	10.36	11.22	10.79
2 Comp. + ASH21*	0.52	1.23	0.88	0.64	8.81	7.183	4.393	8.88	16.76	12.82	0.89	2.70	1.79	10.00	23.95	16.98	26.80	40.15	33.48	1.69	1.87	1.78	9.46	10.73	10.09
1/2 Comp. + ASH21*	0.86	1.14	1.00	3.18	8.07	6.188	3.897	13.73	16.72	15.23	1.74	2.74	2.24	15.72	21.03	18.38	28.10	36.25	32.18	1.86	1.89	1.87	10.67	10.35	10.51
Comp.+ ASH21+ZH21+RZ11**	1.19	1.37	1.28	48.79	161.53	16.713	11.269	23.21	25.95	24.58	3.69	3.41	3.55	25.60	26.70	26.15	30.75	40.95	35.85	1.94	1.93	1.93	11.13	11.08	11.10
Soil texture av.	0.79	1.16						13.04	17.09		1.85	2.51		14.81	19.82		23.72	36.71		1.51	1.84		8.62	10.51	
LSD for	Soil texture	Fertili	S × F					Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F	Soil texture	Fertili.	S × F
0.05	0.14	0.26	NS					2.28	4.27	NS	0.32	0.61	0.86	2.56	4.79	NS	0.35	0.66	0.93	0.02	0.04	0.06	0.12	0.23	0.32
0.01	0.21	0.38	NS					3.32	6.21	NS	0.47	0.88	NS	3.72	6.97	NS	0.51	0.96	1.36	0.03	0.06	0.09	0.18	0.33	0.47

* Inoculation with *Azospirillum* spp. (ASH21).

** Inoculation with *Azospirillum* spp. (ASH21), *Azotobacter* spp. (ZH21) and *Rhizobium leguminosarum bv. viciae*(RZ11).

Comp.: Recommended dose of compost (5 ton/fed. in sandy soil and 2 ton/fed. in clay soil).

and dehydrogenase activities in rhizosphere of wheat plants revealed that the application of bio-fertilizers and different levels of compost increased the nitrogenase activity (μ mole C_2 H_4 /h/g dry soil) in the rhizosphere of wheat plants grown in both sandy and clay soils compared with the control treatment. The highest values of nitrogenase activity in sandy and clay soils, were obtained by the treatment receiving compost plus inoculation with tri-mixture inoculant being 48.79 and 161.53 μmole C_2 H_4 /h/g dry soil, respectively. The control treatment showed the lowest values being, 0.45 and 1.56 μ mole C_2 H_4 /h/g dry soils in sandy and clay soils, respectively.

Concerning the dehydrogenase activity (μg H_2/g dry soil) in rhizosphere of wheat plants grown in sandy soil, the values ranged from 3.242 to 16.713 μ g H_2/g dry soil. The results also behaved the same general trend like those of nitrogenase. The highest values were recorded by treatment received compost plus inoculation with tri-mixture inoculant in sandy and clay soils, being 16.713 and 11.269 μ g H_2/g dry soil, respectively. The respective values for the control treatments were 3.242 and 3.252 μ g H_2/g dry soil.

From Table 4. it can be shown that application of bio-fertilizers and different levels of organic fertilizers increased the total nitrogen content in whole plant in wheat ranged from 2.84 to 25.95 mg/plant. The differences between soil textures and among fertilization treatments were highly significant ($P \leq 0.01$). While, the interaction between soil textures and fertilization treatments was insignificant.

Data in Table 4 showed also that total P content in whole plant, generally ranged between 0.28 and 3.69 mg P in whole plant. The highest P content (3.69 mg P/plant) was obtained by treatment that received compost + tri-mixture inoculant in sandy soil as compared with all other treatments. Concerning the soil texture effect, the data indicated high significance on total P content in whole plant, as such, the highest total P content in whole plant (2.51 mg P/plant) was obtained by clay soil. The interaction between fertilization treatments and soil texture effects on total P content in whole plant was also significant. Meanwhile, the highest values were obtained by using compost + inoculation with tri-mixture inoculant showing 3.69 and 3.41

mg P/plant in both sandy and clay soils, respectively.

The obtained data are in agreement with those reported by Radwan *et al.* (2002) who found that association of multi strains of bio-fertilizers (phosphate dissolving bacteria + *Azotobacter* spp + *Azosperillum* spp + *Pseudomonas* spp) with compost farming manure, led to significant increases in grain, straw, N and P content and biological yield of wheat plants.

Data also showed that application of bio-fertilizers and organic amendment increased the total potassium content in whole plant of wheat grown in both sandy and clay soils. The results showed that the treatment receiving compost plus inoculation with tri-mixture inoculant resulted the highest values of total potassium content (26.15 mg K/plant) in whole plant. As for soil texture effect, clay soil significantly increased total potassium content in whole plant, 19.82 mg K/plant as compared with sandy soil. Interaction between fertilization treatments and soil texture concerning potassium content in shoot, root and whole plants was insignificant.

Results obviously showed that weight of 1000 grains, nitrogen and protein percentage were significantly affected by the studied fertilization treatments. The highest weight of 1000 grains (40.95 g), nitrogen (1.93 %) and protein (11.10 %) in grains were obtained by treatment received compost + tri-mixture inoculant as compared with the other treatments. As for the soil texture, clay soil achieved the significantly highest value of 1000 grain weight, nitrogen and protein percentage in grains as compared to the sandy soil.

Interaction between the two studied factors concerning weight of 1000 grain and nitrogen and protein percentage were significant as such the highest values usually obtained from treatments applied compost + inoculation with tri-mixture inoculant in clay soil.

Compost addition could supply growing plants with essential nutrients as well as improving the physical, chemical and biological properties of soil that consequently reflected on rhizospheric microbial activities and subsequently root colonization. These results coincided with those obtained by Badawi (2003) and Desoki (2004). In addition, the heterotrophic nature of the PGPRs may explain the favorable effects of combining of compost and PGPRs application.

The obtained data are in agreement with those obtained by Singh and Sharma (1999) who found that wheat plants inoculated with *Azotobacter* increased yield from 4.81 to 5.01 ton. The available nutrient status of soil after harvest of the wheat crop was higher where organic fertilization was applied. Ardakani *et al.* (2001) showed that, individually *Azospirillum* and *mycorrhiza* caused significant increases in spike/m^2 and grain weight yield of wheat plants. Moreover, manure increased most of yield components specially when used with *Azospirillum* and mycorrhiza.

Effect of Bio-fertilizers Only or Combined with Inorganic or Organic N-fertilizers on GA_3, IAA and IBA Content in Roots of Wheat Plants

Many actual and putative biofertilizres-PGPR produce phytohormones that are believed to be related to their ability to stimulate plant growth. Data presented in Table 5 showed the effect of application of bio-fertilizers only or combined with inorganic or organic N-fertilizers on GA3, IAA and IBA content in roots of wheat plants cultivated in both sandy and clay soils. The results showed that the highest values of GA3 or IAA content were obtained by treatment inoculated with tri-mixture inoculants of ASH21, ZH21 and RZ11 combined with 1/2 N inorganic fertilizers in both sandy and clay soils. The lowest values were obtained by uninoculated treatment (control) in both sandy and clay soils. IAA-producing PGPR increase root growth and root length, resulting in greater root surface area which enable the plant to access more nutrients from the soil (Vassey,2003). The presence of IAA related compounds could be demonstrated for many diazotrophs (Dobbelaera *et al.*,2003).

Regarding the IBA content in roots of wheat plants grown in both sandy and clay soils the results indicated that the treatments inoculated with ASH21 and combined with 1/2 N inorganic fertilizers showed the highest values in both sandy and clay soils. These results are in harmony with

those obtained by De-Freitas and Gremida (1990), who reported that the beneficial effect of N2-fixing bacteria are also due to production of growth promoting substances which help for increasing crop yield.

In this respect, Dobbelaera *et al.* (2001) found that *Azospirillum* is able to promote plant growth and increase yield in many crops. The production of plant growth promoting substance, led to an improvement in root development and increase in the rate of water and mineral uptake.

It can be concluded that inoculation with indigenous PGPR strains is an important practice when studying their inherent capacity to benefit crops. Indigenous strains can perform better than introduced strains in promoting the growth of the plants due to their superior adaptability to the environment.

Further studies are performed to evaluate the interaction of the PGPR with other plants and identify other native strains isolated from wheat roots.

Table 5. Effect of bio-fertilizers only or combined with inorganic or organic N-fertilizers on GA_3, IAA and IBA in root of wheat plants

Treatment	GA_3 (μ g/100g fresh roots)		IAA (μ g/100g fresh roots)		IBA (μ g/100g fresh roots)	
	Sandy	Clay	Sandy	Clay	Sandy	Clay
Control	10.43	7.86	0.01	0.03	0.00	0.00
Inoculation by ASH21*	12.57	15.17	0.12	0.42	3.44	199.49
Nitrogen dose	10.88	12.16	0.05	0.05	0.00	7.52
1/2 N + ASH21*	11.63	16.25	0.30	0.07	326.42	326.42
1/2 N+ ASH21+ZH21+RZ11**	50.42	50.42	0.78	1.06	174.37	174.37
Comp. + ASH21*	13.48	19.67	0.03	0.08	2.89	2.56
Comp. + ASH21+ZH21+RZ11**	23.79	28.63	0.42	0.43	38.43	252.94

* Inoculation with *Azospirillum* spp. (ASH21).

** Inoculation with *Azospirillum* spp. (ASH21), *Azotobacter* spp. (ZH21) and *Rhizobium leguminosarum bv. viciae*(RZ11).

N: Recommended dose of nitrogen. Comp: Recommended compost dose.

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تأثير ميكروبات منطقة الريزوسفير والمنتجة لمنظمات نمو النبات علي نمو وانتاج نباتات القمح المنزرعة في اراض رملية وطينية

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تم استخدام ثلاث عزلات محلية معزولة من منطقة الريزوسفير لنبات القمح وهي *Rhizobium leguminosarum bv viciae* ، *Azotobacter* spp، *Azospirillum* spp. كمخصبات حيوية إما مفردة أو مختلطة معا في وجود مستويات مختلفة من السماد النيتروجيني المعدني والسماد العضوي والمنزرعة في نوعين من الأراضي بمحافظة الشرقية (رملية وطينية). وقد أوضحت النتائج أن المعاملات الملقحة بالميكروبات الثلاثة المستخدمة في هذه الدراسة معا بالاضافة إلى نصف الجرعة الموصي بها من السماد النيتروجيني المعدني أو الجرعة الموصي بها من السماد العضوي أدت إلى زيادة ملحوظة في محتوى النبات الكلي من النيتروجين والفوسفور والبوتاسيوم وكذلك زيادة محتوي الحبوب من النيتروجين بالإضافة إلى زيادة نشاط إنزيم النيتروجينيز والديهيدروجينيز في منطقة جذور نباتات القمح النامية في كلا نوعي التربة. كما أدت نفس المعاملة السابقة إلى زيادة إنتاج إندول حمض الخليك والجبريلينات المنتجة وهذا يوضح أهمية استخدام الميكروبات المشجعة لإنتاج منظمات النمو في النبات PGPR لزيادة نمو وإنتاجية محصول القمح.