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RESPONSE OF WHEAT PLANTS TO SOME BIOFERTILIZATION TECHNIQUES IN NEWLY RECLAIMED SANDY SOIL

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ABSTRACT: *A field experiment was carried out during two winter seasons (2012 and 2013) at El-Noubaria, El-Behiera Governorate, Egypt, to evaluate the effect of soil inoculation with symbiotic N_2-fixing bacteria (a composite inoculum containing mixed culture "1:1" of Azotobacter chroococcum and Azospirillum lipoferum) on plant growth and yield of wheat crop grown newly reclaimed sandy soil. Foliar application of pink pigment facultative methylotorphic bacteria PPFMs (producing phytohormone) with either level of N-mineral fertilizer (60 and 120 kg N/fed), was also introduced .*

The obtained results showed that inoculation with both N_2-fixers combined with PPFMs bacteria recorded the highest significant increases for all tested parameters of wheat growth, yield and yield components. Bacterial inoculation in combination with N- mineral fertilizers gave increase percentages of 25, 18, 8, 27, 12 and 6% for numbers of tillers (No/m^2), weight of grains (g/plant), 1000 grains weight (g/1000grains), total biological yield (ton/fed.), grain yield (ton/fed.) and crude protein (%) as compared with mineral N- fertilize as such, respectively. Bacterial inoculation did support microbial population (total number of bacteria and count of each of Azotobacter and Azospirillum).

Key words: *PGPR, N_2-fixers, Tritium aestivum, cereal, bacterial co-inoculation.*

INTRODUCTION

Wheat (*Triticum astivum* L.) is the most important cereal crop and it is the major source of food in Egypt. So increasing wheat production is an essential national target to fill the gap between production and consumption.

Production could be increased through cultivation of high yielding cultivars and appropriate agronomic practices (Tawfik *et al.,* 2006). Throughout the growing season, plants are exposed to different stresses, *i.e.* drought, heat, salinity and low soil fertility, causing reductions in crop yield particularly in newly reclaimed area (Ali and Abo-El-wafa 2006). Extensive cultivation of wheat in the newly reclaimed soil in Egypt seemed to be imperative to circumvent the problem of insufficient wheat grain supply. However, intensive chemical fertilizers have introduced undesirable and same times catastrophic consequences by polluting air, soil and aquatic systems. The response of wheat crop to N-fixers reached 120 kg N/fed. (Abdel-warth, 2002). The efforts to decrease N-chemical fertiliation by using biofertilizres might both reduce the high costs and environmental pollution. Therefore, it is necessary to find out a correct and compatible level of nitrogen fertilizer with these biofertilizers. Inoculation with N2-fixers, *i.e. Azotobacter chroococcum* and *Azospirillum lipoferum* have been widely used to inoculate non legume crops. They have proved to increase the yield of field cereal crops by about 15-25 %. Indeed, the N-biofertilizres is a cheap technique to produce plant protein, as well as they an important role in buildup soil fertility (Hegazi *et al*.; 1998., Mahmoud *et al*., 2006 and Abdel-Warth *et al*., 2010). Bacteria belonging to the genus methylobacteriaum, known as pink-pigmented facultative methylotrophic bacteria (PPFMs) are ubiquitous in nature and have been detected in various environments habits (corpe 1985 and Green 2001). PPFMs bacteria are aerobic, Gram-negative bacteria, able to grow on a wide range of multi-carbon substrates, as well as are known to produce auxins (Doronina *et al*., 2002) and cytokinins (Koenig *et al.*; 2002 and Orf *et al*., 2005).

The aim of this study was to investigate the effect of applying N_2-fixing bacteria as biofertilizers and/or (PPFMs) bacteria under N-mineral treatment, on some growth parameters, yield and yield components of wheat plants grown newly reclaimed sandy soil, as well as on the activity of rhizobacteria.

MATERIALS AND METHODS

A field experiment was carried out at El-Noubaria, Beheira Governorate in two winter seasons (2012 and 2013) to study the effect of some effective microorganisms on improving productivity of wheat plants. Some physical and chemical properties of the soil used are illustrated in Table (1) according to Page *et al.* (1982) and Klute (1986).

Compost was ploughed with the surface layer of the soil (0-20 cm) at a rate of 4ton/fed., two weeks before sowing. Calcium super-phosphate (15.5% P_2O_5) was added to all experimental area at a rate of 200 kg/fed. 15 day prior to cultivation. Nitrogen fertilizer (Ammonium nitrate, 33.3% N) was added at two rates of 60 & 120 kg/fed., in two equal parts, after 30 and 60 days from sowing. Potassium sulphate (contains 48% K_2O) was added (50 kg/fed) throughout two equal split doses, after 30 and 60 days from planting.

The most active N2-fixers *Azotobacter chroococcum* and *Azospirillum lipoferum* isolated and identificated in Microbiology lap of the Desert Researches Center Cairo, Egypt. They were added in combination as soil application while the PPFMs *Methylobacterium* genera were isolated and purified in the Agricultural Microbiology Dept., Soil Water and Environmental Research Institute (SWERI), A.R.C., Giza, Egypt. The PPFMs were introduced as foliar application. Liquid cultures (48 hr old) at a rate of 10^7 cfu/ml were applied with the irrigation water directly after sowing, then 21 and 45 days later on. The PPFMs bacteria were splashed at the same times.

Table (1): Some properties of the soil under investigation.

Properties	Analyses	Determinations	Values
Physical	Particle size distribution (%)	Coarse sand	57.18
		Fine sand	30.12
		Silt	9.85
		Clay	2.85
	Soil texture	Texture clay	Sandy
Chemical	Ca Co_3 (%)		2.14
	pH (1:2.5 soil/water suspension)		8.4
	Total soluble salt	EC (dS m^{-1})	0.30
	Soluble cations (meq 1^{-1})	Ca^{++}	1.40
		Mg^{++}	0.65
		Na^{+}	0.50
		K^{+}	0.45
	Soluble anions (meq 1^{-1})	CO^{-}	-
		HCO^{-}	0.10
		Cl^{-}	0.91
		SO_4^{-}	1.99
	Available nutrients (mg kg^{-1})	N	24.00
		P	4.00
		K	54.0

Microbiological analysis of the rhizosphere soil of wheat plants was carried out for total bacterial count, azotobacters and azospirilla densities, by culturing on an Ashby's agar medium (Abdel-Malek and Ishac, 1968) and a Dobereiner's semi solid malate medium (Dobereiner *et al*., 1976), respectively.

Wheat cultivar "Gamaiza 9" grains were sown in plots (3X4 m). Irrigation water used had a EC of 0.546 dSm^{-1} , which is considered as a good quality. At harvest, plants were collected to estimate the traits, *i.e.* plant height, number of grains/spike, 1000-grain weight and biological yield, according to the method reported by A.O.A.C. (2003).

The experimental treatments (T) used can be summarized as follows:

1. T1: without inculcation and amended with a half dose of N (60kg/fed.).
2. T2: without inculcation and amended with a full dose of N (120 kg/fed.).
3. T3: inoculation with *Azotobacter* + *Azospirillum* (soil application) + ½ N (60 kg/fed.).
4. T4: PPFMs (foliar application) + ½ N (60 kg/fed.).
5. T5: N2-fexers+PPFMs+½ N (60 kg/fed.).
6. T6: Inoculation with *Azotobacter* + *Azospirillum* (soil application) + N (120 kg/fed.).
7. T7: PPFMs (foliar application) + N (120 kg/fed.).
8. T8: N2-fixers+ PPFMs + N (120 kg/fed.).

Statistical analysis "LSD at a level of 0.05" was carried out, according to Snedecor and Cochran (1982).

Chemical analysis of wheat grains was performed, after harvest, to determine nitrogen, phosphorus and potassium content, according to Cottenie *et al.* (1982).

RESULTS AND DISCUSSION

Concerning the effect of applying the two biofertilizres types (N-fixers and PPFMs) under both mineral N-fertilizer levels on some growth parameters of wheat plants. The results in Table (2) clearly showed that plants received higher mineral N in presence of the biofertilizres recorded the highest values for plant height, number of tillers and number of spikes, those values were 126.7, 130.8 & 379 and 401, 312 & 342 for plant height, number of tillers and number of spikes on first and second season, respectively. Application of N_2-fixing bacteria alone or in combination with PPFMs as biofertilizres resulted in significance differences among all the tested parameters, compared to the mineral N treatments. On the other hand, tee second season showed higher values for tested parameters than the first one.

Significant effects were found for each of spike length, number of grains, and weight of grains Table (3). The treatments receiving both bacterial inoculations recorded the highest values of spike length (17.1 cm), number of grains (54.8 g) and weight of gains (2.34 g), as compared with the other treatments undertaken.

Data presented in Table (4) show the effect of different bacterial inoculants (N_2-fixers + PPFM) on the total biological yield, grains yield and 100 – grains weight. Such parameters were significantly increased. The treatment which received both bacterial inoculations (N-fixers + PPFM) and fertilized with 120 kg N/fed. possessed the highest values of total biological yield (6.78 ton/fed.), grains yield (2.25 ton /fed.) and 1000 grain weight (57.82 g) compared to the other tested treatments.

Results of the grain contents of nitrogen, phosphorous, potassium, and protein, as well as the IAA content are presented in Table (4). With respect to the effect of inoculation with *Azotobacter* + *Azospirillum* and/ or PPFMs bacteria resulted marked increases in the mentioned parameters as compared with the uninoculated treatments receiving N-mineral fertilizer alone. Significant augmentations were found among the tested treatments, where the highest values were recorded for the treatments which received both bacterial inoculants and fertilized with 120kg N/fed., and these values were 1.94, 0.29, 0.65, 11.54 & 10.52 for nitrogen, phosphorus, potassium, cured protein and IAA, respectively.

Table (2): Wheat plant traits as affected by application of N_2-fixing bacteria and/or PPFMs bacteria under two levels of N-mineral fertilizer.

Parameter / Treatment (T)				Plant height (cm/plant)			Number of tillers (No./m²)			Number of spike (No./m²)		
N-fertilizer (kg/fed.)		inocula										
		N_2 fixers	PPFMs	S_1	S_2	mean	S_1	S_2	Mean	S_1	S_2	mean
T1	60	-	-	91.2	97.3	94.7	196	201	199	187	192	190
T2	120	-	-	119.9	121.3	118.6	310	325	318	280	301	291
T3	60	+	-	111.2	117.2	114.2	226	241	234	193	196	195
T4	60	-	+	98.7	101.3	100.0	276	293	285	198	211	205
T5	60	+	+	105.4	109.6	107.5	317	339	328	210	234	222
T6	120	+	-	120.8	122.6	121.7	338	351	345	287	298	293
T7	120	-	+	118.5	119.7	119.1	356	381	369	293	317	305
T8	120	+	+	126.7	130.8	128.8	379	401	390	312	342	327
mean				111.2	114.9	113.1	300	317	309	245	261	253
L.S.D. 0.05				6.28			36.76			47.52		

Table (3): Spike length, number of grains and weight of grains of wheat crop as affected by application N2-fixing bacteria and/or PPFMs bacteria under two levels of N-mineral fertilizer.

Parameter / Treatment (T)				**Spike length (cm/spike)**			**Number of grains (No. spike)**			**Weight of grains (g/ spike)**		
N-fertilizer kg/fed		**Inocula**										
		N_2 fixers	**PPFMs**	**S_1**	**S_2**	**mean**	**S_1**	**S_2**	**Mean**	**S_1**	**S_2**	**Mean**
T1	60	-	-	9.8	9.9	9.9	23.3	25.1	24.2	1.09	1.13	1.11
T2	120	-	-	13.9	14.3	14.1	43.9	44.7	44.3	1.93	1.98	1.96
T3	60	+	-	16.2	10.6	10.4	29.8	30.2	30.0	1.31	1.37	1.34
T4	60	-	+	10.4	10.8	10.6	31.3	33.7	32.5	1.37	1.47	1.42
T5	60	+	+	11.3	11.7	11.5	33.4	35.8	34.6	1.47	1.58	1.53
T6	120	+	-	14.2	14.9	14.6	47.2	49.1	48.2	2.01	2.13	2.07
T7	120	-	+	15.3	16.2	15.8	49.3	51.6	50.5	2.11	2.24	2.18
T8	120	+	+	16.9	17.2	17.1	537	55.8	54.8	2.25	2.43	2.34
mean				12.8	13.2	13.0	38.9	40.8	39.9	1.69	1.79	1.74
L.S.D. 0.05				1.73			5.30			0.23		

Table (4): Biological yield, grains yield and 1000 grains weight of wheat crop as affected by application of N2-fixing bacteria and/or PPFMs bacteria under two levels of N-mineral fertilizer.

Parameter / Treatment (T)				Biological yield (ton/fed.)			Grain yield (ton/fed.)			Grain weight (g/1000 grain)		
	N-fertilizer kg/fed	inocula										
		N_2 fixers	PPFMs	S_1	S_2	mean	S_1	S_2	mean	S_1	S_2	mean
T1	60	-	-	2.54	2.65	2.60	0.79	0.81	0.80	40.61	43.72	42.17
T2	120	-	-	4.49	4.76	4.63	1.83	1.92	1.89	46.93	49.81	48.37
T3	60	+	-	2.79	2.94	2.87	0.93	0.98	0.96	42.51	44.22	43.37
T4	60	-	+	2.82	2.98	2.90	0.94	0.97	0.96	43.11	45.72	44.42
T5	60	+	+	3.60	3.79	3.70	1.20	1.23	1.22	44.25	47.27	45.76
T6	120	+	-	5.16	5.49	5.33	1.72	1.83	1.78	49.37	53.23	51.30
T7	120	-	+	5.95	6.16	6.06	1.95	2.02	1.99	50.22	55.17	25.70
T8	120	+	+	6.42	6.78	6.60	2.14	2.25	2.20	53.72	57.82	55.77
mean				4.22	4.44	4.33	1.44	1.50	1.47	46.34	49.62	47.98
L.S.D. 0.05				0.54			0.19			1.35		

Application of the bacterial inoculation did support yield production of wheat crop (Table 5), values appearing in Table (6) recorded increases of 25, 7, 18, 8, 27, 12, 6 & 25% for number of tillers, number of spikes, weight of spike grains 1000-grain weight, biological yield, grain yield, crude protein and IAA content. Similar findings were reported by Hussein and Radwan, (2001), that inoculated wheat grains with biofertilizres significantly increased the grain yield/fed, harvest index, 1000-grain weight and spike grain weight by 6.5, 3.4, 2.1 and 8.8%, respectively, rather than the non-biofertilized treatments. Mohmoud and Mohamed (2008) and Badran (2009) concluded that biofertilizres stimulated wheat growth and grain yield. Holland (1997) stated that, the activities of PPFMs could make a biochemically measurable and physiologically meaningful contribution to plant metabolites. Omer (2004) and Orf *et al.* (2005) found that PPFMs produced cytokine and other phytohormones, which stimulated the plant growth and development. In this respect, Shehata *et al.* (2006) and Orf *et al.* (2014) reported that application of local isolates of PPFMs with other N_2-fixing bacteria gave higher records of all plant parameters and increased in productivity of legume crops tested.

Results of the rhizospheric bacteria shown in Tables (7&8) indicated that the initial numbers were 1.23×10^5, 0.12×10^2 and 0.05×10^2 for the total bacteria, *Azotobacter* and *Azospirillm* respectively. Data in Table (7) revealed that application of the bacterial inoculants did support the microbial counts and scored higher values as compared with the uninoculated treatments. Moreover, introduction of PPFMs bacteria had a superior effect compared to the N_2-fixing bacteria alone or in combination with PPFMs. These results are in agreement with those reported by Subba Rao (1988), Abd El-Ghany *et al.* (2010) and Abdel Warth *et al,* (2010), that bacterial inoculation improved soil fertility, increase the number and biological activities of desired microorganisms in root environment.

TABLE 5

TABLE 6

Table (7): Total bacterial cunts of soil planted with wheat plants as affected by application of N2-fixing bacteria and/or PPFMs bacteria under two levels of N-mineral fertilizers.

Parameter / Treatment				Total bacterial counts cfu/g dry soil (- X 10^5)			*Azotobacter* counts cfu/g dry soil (- X 10^5)			*Azospirillum* counts cfu/g dry soil (- X 10^5)		
	N-fertilizer kg/fed	inocula										
		N_2 fixing	PPFMs	S_1	S_2	mean	S_1	S_2	mean	S_1	S_2	mean
T1	60	-	-	10.25	11.31	10.78	5.11	6.50	5.81	1.43	1.48	1.46
T2	120	-	-	12.37	14.51	13.44	15.93	16.53	16.23	4.93	5.14	5.04
T3	60	+	-	23.72	25.77	24.75	12.61	13.73	13.17	7.31	8.11	7.71
T4	60	-	+	24.85	25.93	25.39	9.72	10.13	9.93	5.37	6.52	5.95
T5	60	+	+	27.93	28.11	28.02	12.70	14.53	13.62	9.31	10.22	9.77
T6	120	+	-	26.31	28.93	27.62	16.37	17.91	17.14	11.72	11.89	11.80
T7	120	-	+	28.22	28.75	28.49	16.85	15.22	15.04	10.67	10.72	10.70
T8	120	+	+	29.11	29.88	29.50	17.81	18.22	18.02	12.75	13.21	12.98
mean				22.85	24.15	23.50	13.14	14.10	13.62	7.94	8.41	8.18
Initial count				1.15X10^4			0.12X10^4			0.05X10^4		

Table (8): Increases percent of total number for various (RMO) bacteria at soil planted with inoculated wheat plants.

Parameter / Treatment	Total bacterial counts cfu/g dry soil (- X 10^5)		*Azotobacter* counts cfu/g dry soil (- X 10^5)		*Azospirillum* counts cfu/g dry soil (- X 10^5)	
	Value	%	Value	%	Value	%
Initial count	1.23	-	0.12	-	0.05	-
Mineral fertilizers	12.11	88	11.02	90.8	3.25	64.0
60 kg N + inoculation	26.05	202	12.24	101.0	7.81	155.2
120 kg N + inoculation	28.54	222	16.72	138.3	11.83	235.6

On the other hand, the treatment which received 120 kg N/fed and inoculated with a combination of N_2 fixing and PPFMs bacteria gave the highest bacterial coubts, to show the values 29.50, 18.02 and 12.94 for total, *Azotobacter* and *Azospirillum*, in both seasons, respectively. It is worthy to note that the percentages of increase were due to the applied N-mineral fertilizer and the two bacterial treatments together with 60 kg N/fed. or 120 kg N /fed., referring to the initial counts of each bacterial agent (Table 8). Such percentages of increase were 88, 202 and 222, 90.8, 101.0 and 138.3, 64.0, 155.2 and 235.6 for the numbers of *Azotobacter* and *Azospirillum*, respectively. These results are in harmony with those obtained by Pondy *et al.* (1998), Abotaleb *et al.* (2002), Mahmod *et al.* (2006), Anjum *et al.* (2007) and Abd el-Warth *et al.* (2010) who reported that the bacterial inoculation had an activation effect on the population of both total bacteria and diazotrophic bacteria and increased their numbers more than 50%.

This work leads us to conclude that application of N-fertilizer at 120kg N/fed. combined with a mixed biofertilizers inoculation (N_2 fixers and PPFMs bacteria) to wheat grains improved the crop yield, as well as enhanced soil fertility and increased the number and biological activities of rhizospheric microorganisms.

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إستجابة نباتات القمح لبعض تقنيات التسميد الحيوي في الأراضي الرملية المستصلحة حديثاً

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الملخص العربي

أجريت تجربة حقلية خلال موسمي ٢٠١٢–٢٠١٣ في النوبارية–محافظة البحيرة–مصر، وذلك لدراسة تأثير التلقيح البكتيري للأرض بمثبتات الأزوت الجوي اللاتكافلية (لقاح مختلط من بكتريا الأزوتوبكتر كروكوكم وبكتريا الأزوسبيريللم ليبوفيرم بنسبة ١:١)، في وجود أو عدم وجود البكتريا القرمزية المتغذية علي الميثانول والمنتجة للهرمونات النباتية (وذلك كمعاملة رش) في وجود مستويين من التسميد النيتروجيني المعدني ٦٠، ١٢٠ كج ن/فدان علي النمو والمحصول ومكوناته لنباتات القمح. وأيضاً دراسة تأثير التلقيح البكتيري علي التجمع الميكروبي في أرض التجربة.

وأوضحت النتائج المتحصل عليها أن التلقيح البكتيري قد أعطي أعلي القيم المتحصل عليها لقياسات نمو النباتات والمحصول ومكوناته. حيث سجل التلقيح البكتيري في وجود التسميد المعدني فروقاً معنوية وزيادات بلغت ٢٥–١٨–٨–٢٧–١٢ و٦% وذلك بالنسبة لكل من التفريع (للمتر المربع) ووزن الحبوب (جرام/نبات) ووزن الـ١٠٠٠ حبة والمحصول البيولوجي (طن/فدان) وحصاد الحبوب (طن/فدان) وأيضاً نسبة البروتين علي التوالي وذلك مقارنة باستخدام السماد النيتروجيني المعدني بمفرده.

هذا وقد أدي التلقيح البكتيري إلي دعم التجمع الميكروبي لمنطقة جذور نباتات القمح في الموقع التجريبي حيث سجلت زيادات بلغت ٢٢٢ و١٣٨,٣ و٢٣٥,٦ لكل من العدد البكتيري الكلي وعدد الأزوتوبكتر وعدد الأزوسبيريللم علي التوالي وذلك في وجود ١٢٠كجم ن/فدان مقارنة بالعدد الأولي والمسجل قبل زراعة الموقع التجريبي وبصفة عامة أدي التسميد الحيوي (التلقيح البكتيري) إلي الحصول علي قيم عالية لمحصول القمح ومكوناته هذا بالإضافة إلي تحسين خصوبة التربة وزيادة أعداد التجمع الميكروبي والنشاط الحيوي في منطقة الجذور.