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RESPONSE OF WHEAT CROP TO MICROBIAL INOCULATION AND YEAST STRAIN UNDER DIFFERENT MINERAL PHOSPHORUS FERTILIZER LEVELS AT NORTH EASTERN DELTA OF EGYPT

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ABSTRACT

The effect of inoculation with mycorrhiza, phosphate dissolving bacteria (*Bacillus megaterium*) and yeast strain (*Saccharomyces cervisiae*) on the growth of wheat plants, was studied in two successive field experiments, at El-Serw Agricultural Research Station (Northeastern Delta of Egypt). Three levels of inorganic phosphorus fertilizer were applied, (0, 15, and 30 kg P_2O_5/fed). Split plot with 4 replicates was designed. Dry matter contents per plant, available phosphorus in the rhizosphere and root systems of plants were determined, after 30, 60 and 90 days of sowing, as well as, at harvesting .Grain and straw yields were also determined.

The obtained results showed that, fungal and bacterial inoculation in the presence of inorganic phosphorous fertilizer, stimulated wheat grain and straw yields, and increased available p in the soil around root zone. Noticeable amounts of p were determined with phosphate dissolving bacteria and mycorrhiza, than with yeast inoculation. *B. megaterium* inoculation with 30 kg P_2O_5 /fed gave the highest values of grain and straw yield (23.68 ardab/fed and 3.84 ton/fed, respectively) during the first season. While, in the second season 15 kg P2O5 /fed with PDB inoculation gave the highest values (22.77 ardab/fed and 3.8 ton/fed., respectively).

INTRODUCTION

Chemical problems resulted from unavailability of phosphorus, potassium and most micronutrients because of high concentration of OH ions. The movement of phosphates in soils is very limited, and soils are said to have high 'fixing powers' for phosphates.

In Egypt, the intensive cultivation depletes the soil from such nutrients, that should be compensated by fertilizer application. Also, the high capacity of fixation in alluvial clayey soils, and those of high $CaCO_3$ content aggravated such problem; especially p fixation (El-Aggory Eglal *et al.*, 2001).Phosphorus (p) is one of the major nutrient elements limiting agricultural production in the world. It is added to the soil in the form of phosphate fertilizers, a part of which is utilized by plants, and the rest is rapidly converted into insoluble complexes in the soil (Vassilev and Vassileva, 2003).

Wheat (Triticum *aestivum*) is one of the most important cereals in Egypt, in respect to its value and areas, a Various diastrophic bacteria have been found in association with this plant. The effects of inoculating cereals and grasses with various bacteria are well documented (Kloepper *et al.*, 1988; Omar and Ahmed, 2003).

Among the rhizosphere organisms involved in plant interactions with the soil, the plant growth promoting rhizosphere (PGPR),e.g. phosphate and

potassium dissolvers and free living N_2-fixing bacteria, rhizobia and Arbuscular Mycorrhizal Fungi (AMF).

Yeasts are residents of soils and rhizosphere of various plants, although their numbers are low in comparison with other microorganisms. This group of organisms seems to play an important role in soil biofertility, because of their capability for producing hormones, amino acids and vitamins (Monib *et al.*, 1982). El-kholy and Omar (2000) found that seed of wheat inoculated with nitrogen fixing bacteria, and two strains of yeasts (*Saccharomyces cerevisiiae*) had positive effect of both yield and nitrogen content of plant.

The objective of the present investigation is to evaluate the potentialities of some phosphate dissolving microorganisms and yeast strain, as promoting agent, to increase the productivity of wheat yield.

MATERIALS AND METHODS

Two field experiments on wheat variety Sakha 93, were carried out during the two successive seasons (2002-2003), at El-Serw Agricultural Research Station (Damietta Governorate), Egypt. The split plot design with four replicates was used .The plot size was 10.5m^2 .Main plots were arranged for different doses of mineral phosphorus fertilizers (0,15 and 30 kg P_2O_5 /fed, in form of monosuperphosphate,15.5% P_2O_5).The subplots were devoted for inoculation as follows:

- Uninoculated control
- Inoculation with mycorrhiza (*Glomues spp*.)
- Inoculation with phosphate dissolving bacteria (*Bacillus megaterium*).
- Inoculation with yeast strain (Saccharomyces *cerevisiae*)

Prior planting, soil sample was taken from the surface layer (0-30 cm, depth) and analyzed for physical and chemical properties Table (1) _ as described by Black (1982). Phosphorus fertilizers were added prior planting and during plant bed preparation, and mixed into the soil. Ammonium nitrate (33%N) was used as nitrogen fertilizer (75 kg/fed) in two equal doses with all treatments. The first dose was applied 3 weeks after planting and the second was added at panicle initiation.

Table 1: Soil Physical and chemical properties analysis the experimental sites.

Soil characteristics	**1st season, 2002**	**2nd season, 2003**
Soil texture	Clayey	Clayey
CEC (meq/100 g soil)	48.4	49.2
Soil PH (1:2.5 water susp.)	8.1	8.3
EC (soil paste at 25c^0), dS/m	4.6	5.3
O.M %	1.4	1.3
$CaCO_3$ %	1.48	1.52
Available N ppm	42	39
Available P ppm	8.1	7.6
Available K ppm	400	430

Microbial inoculation was performed using seed coating technique, according to Omar, *et al.*,(1989). Bacterial strains namely, *Bacillus*

megaterium, yeast strain, *Saccharomyces cerevisiae* and fungal spores of mycorrhiza *Glomues spp*. were obtained from soils, Water and Environment Res. Inst., ARC, Giza, Egypt. Each inoculated grain received 10^6 cells on its surface. The rate of Glomues spp. was 150 spores / ml and added to plant at 10 ml/plant at seed sowing. Each of bacterial and yeast strains were replicated three times as liquid culture 5L/fed.after sowing monthly.

Soil rhizosphere samples and whole plants (3plants /treatment), were collected 30, 60, and 90 days after sowing, to determine the available phosphorus around plant roots (Olsen ext), and the dry matter per plants. After harvesting, grain and straw yields were estimated (kg/fed).The dry grain and straw samples from each plot were ground and wet digested with H_2SO_4-$HClO_4$ mixture as described by Peterburgski (1968). Phosphorus was determined according to Black (1982).The statistical analysis was carried out according to Snedecore and Cochran (1989).

RESULTS AND DISCUSSION

Growth and yields of wheat plants:

The obtained results (Tables, 2 and 3) revealed significant increase in plant dry matter weight (shoots and roots), with the three tested doses of mineral phosphate fertilizer. Therefore, dry weight of shoot increased from 15.21 to 32.89 g/plant for season 2002 and 12.72 to 54.34 g/plant for season 2003 when mineral phosphorus fertilizer was increased up to 30kg P_2O_5 /fed .However, significant increase was determined with microbial inoculation and phosphorus fertilizer .

Table 2: The dry matter weight of wheat plants (g/plant) inoculated with PDB, mycorrhiza and yeast (season, 2002).

Treatments (Days after sowing)	0 kg P_2O_5/fed			15 kg P_2O_5/fed			30 kg P_2O_5/fed		
	Shoot g	Root g	Shoot / Root g	Shoot g	Root g	Shoot / Root g	Shoot g	Root g	Shoot / Root g
Uninoculated									
30	1.1	0.08	13.75	1.10	0.16	6.88	1.33	0.26	5.12
60	2.26	0.16	14.13	3.03	0.33	9.18	3.82	0.52	7.35
90	15.21	2.73	5.57	20.28	2.99	6.78	28.73	3.90	7.37
Mycorrhiza									
30	1.37	0.17	8.06	1.47	0.21	7.00	2.18	0.33	6.61
60	3.58	0.35	10.23	4.32	0.47	9.19	5.73	0.74	7.74
90	25.61	4.29	5.97	27.95	4.42	6.32	32.89	4.81	6.84
Yeast									
30	1.21	0.09	13.44	1.21	0.18	6.72	1.46	.027	5.41
60	2.49	0.18	13.83	3.33	0.36	9.25	4.20	0.57	7.37
90	16.21	3.00	5.40	22.31	3.29	6.78	29.38	4.29	6.85
PDB									
30	1.17	0.13	9.00	1.10	0.30	3.67	1.52	0.35	4.34
60	1.50	0.27	5.56	3.76	0.55	6.84	6.01	0.72	8.35
90	15.60	4.03	3.87	23.53	5.33	4.41	31.60	5.20	6.08

LSD	Shoot		Root	
	5%	1%	5%	1%
Inoculation	0.646	0.914	0.208	0.2948
P fertilization	0.646	0.914	0.208	0.2548

Table 3: The dry matter weight of wheat plants (g/plant) inoculated with mycorrhiza, yeast and PDB (season, 2003).

Treatments	0kg P_2O_5			15 kg P_2O_5/fed			30 kg P_2O_5/fed		
(Days after sowing)	Shoot g	Root g	Shoot / Root g	Shoot g	Root g	Shoot / Root g	Shoot g	Root g	Shoot / Root g
Uninoculated									
30	1.01	0.18	5.61	1.20	0.16	7.50	1.50	0.33	4.55
60	2.27	0.30	7.57	2.51	0.35	2.16	2.96	0.55	5.38
90	12.72	1.76	4.61	25.60	3.64	7.03	32.76	3.77	8.69
Mycorrhiza									
30	1.18	0.20	5.90	1.64	0.30	5.47	1.82	0.51	3.57
60	2.20	0.37	5.95	5.73	0.72	7.96	6.70	1.10	6.10
90	20.88	6.60	3.16	30.42	8.58	3.55	49.40	9.62	5.14
Yeast									
30	1.10	0.19	5.79	1.50	0.27	5.56	1.55	0.35	4.43
60	1.74	0.33	5.27	5.25	0.72	7.29	6.10	0.69	8.84
90	14.28	3.96	3.61	29.25	7.15	4.10	38.35	8.71	4.40
PDB									
30	1.30	0.22	5.91	1.80	0.33	5.45	2.00	0.56	3.57
60	2.24	0.41	5.46	6.30	0.79	7.97	7.37	1.21	6.10
90	22.97	7.26	3.16	33.46	9.44	3.54	54.34	10.52	5.17

LSD	Shoot		Root	
	5%	1%	5%	1%
Inoculation	0.53	0.75	0.17	0.24
P fertilization	0.64	0.90	0.20	0.29

The corresponding figures with dry weight of roots were from 2.73 to5.23 g/plant for season 2002, and 1.76 to 10.52 g/plant for season 2003 .It was interesting to observe significant increase in shoots and roots with increasing mineral fertilizer doses (Tables 2 and 3).

Results presented in Table (4) show the effect of bacterial and yeast inoculation on grain and straw yields of wheat plants. The obtained results revealed significant increase in grains and straw yields. Inoculation with phosphate dissolving bacteria or mycorrhiza, resulted in an increase for grain yield over uninoculated treatment, being 23.68 and 22.54 ardab/fed. in the presence of 30kg P_2O_5 /fed for season 2002, while the corresponding figures for season 2003 were 22.37 and 22.29 ardab/fed., respectively. Simillar results of straw yield were observed (Table, 4).

Yeast inoculation stimulated straw yield (3.59 and 3.51 ton/fed for season of 2002 and 2003) in comparison with control, respectively. In treatments inoculated with yeast (Table, 4). Increase in grains yield was accounted under effect of 0 kg P_2O_5/fed. being 20.06 and 17.95 ardab/fed for the season of 2002 and 2003 in comparison with control 19.48 and 17.18 ardab/fed., respectively. These observations confirmed the beneficial stimulation of the bacterial or yeast inoculation on the growth parameter of plants (Omar *et al.*,1989, and El-Kholy and Omar, 2000).

Table 4: Grain and straw yields of wheat (ton/fed) inoculated with mycorrhiza, yeast and PDB (seasons 2002 and 2003).

Treatments	1st season (2002)			2nd season (2003)		
	0kg P_2O_5/ fed	15 kg P_2O_5/ fed	30 kg P_2O_5/ fed	0kg P_2O_5/ fed	15 kg P_2O_5/ fed	30 kg P_2O_5/ fed
Grain yield (ardab/fed.)						
Uninoculated	19.48	20.84	20.90	17.18	19.29	20.23
Mycorrhiza	20.64	22.18	22.54	18.90	21.16	22.29
Yeast	20.06	21.07	21.61	17.95	20.41	20.25
PDB	21.29	23.19	23.68	19.53	22.77	22.37
Straw yield (ton/fed.)						
Uninoculated	3.14	3.35	3.51	2.90	3.20	3.38
Mycorrhiza	3.51	3.55	3.79	3.49	3.58	3.65
Yeast	3.20	3.40	3.59	3.28	3.35	3.51
PDB	3.60	3.75	3.84	3.48	3.80	3.70

LSD, 5%	Grain	Straw	LSD, 5%	Grain	Straw
Inoc.	1.26	0.36	Inoc.	1.34	0.26
P Fert.	0.91	0.24	P Fert	0.63	0.32
Ino*fert.	1.18	0.37	Ino.*fert	0.92	0.32

Data are presented in table (5) indicated that, the effect of phosphate dissolving bacteria, mycorrhiza and yeast strain, on phosphorus uptake in wheat grain and straw yield, and different levels of inorganic phosphorus fertilizer, gave much higher significant increase in phosphorus contents in grain and straw yields by phosphate dissolving bacteria followed by mycorrhiza and yeast strain, respectively. Generally, the interaction between the microbial inoculation and mineral phosphorus fertilizer (30 kg/fed) gave the best results of grain and straw uptake, in both growth seasons.

Table5: Phosphorus uptake by wheat plants (kg/fed) as affected by inoculation and inorganic phosphorus fertilizer.

Treatments	1st season (2002)			2nd season (2003)		
	P0 kg/fed	P15 kg/fed	P30 kg/fed	P0 kg/fed	P15 kg/fed	P30 kg/fed
Grain yield						
Control	2.78	4.26	4.67	3.56	5.10	5.80
Mycorrhiza	4.16	5.04	5.55	5.27	6.10	6.10
Yeast	3.97	4.46	5.18	4.90	5.32	5.71
PDB	4.72	5.75	6.11	5.48	6.57	6.29

LSD	5%	1%	5%	1%
Inoc.	0.52	0.98	0.32	0.52
P fert.	0.32	0.75	0.22	0.38

Straw yield

Control	0.61	1.04	1.25	0.55	1.10	1.36
Mycorrhiza	1.10	1.73	2.49	2.04	3.21	3.26
Yeast	0.55	1.27	1.74	1.10	1.89	2.50
PDB	1.55	2.16	2.73	2.38	3.41	3.60

LSD	5%	1%	5%	1%
	058		0.62	
	0.43	0.78	0.17	0.22

Phosphorus dissolving bacteria and Mycorrhizal fungi are among the most widespread ecologically important plant endosymbionts. Chezhiyan *et al.*, (1999), Das *et al.*, (2001) and Ismail and Hasabo (2000) indicated that, the role of these endosymbionts in improving plant growth, and crop production and merits uses as biofertilizer. PDB and VAM are effective tools for potential phosphate solubilizers, and good root colonization, to improve grain phosphorus content, which was accompanied by a corresponding increase in grain yield, (Harris *et al.*, 2006). In addition, yeast play an important role in soil biofertility, because of its capability for producing hormones, amino acids and vitamin (Monib *et al.*, 1982).

Table 6: Available phosphorus (ppm) in the surface layers of soil during wheat growth in 2002 and 2003 seasons

Treatments	1st season (2002)			2nd season (2003)		
days after sowing	P0 kg/fed	P15 kg/fed	P30 kg/fed	P0 kg/fed	P15 kg/fed	P30 kg/fed
Control						
30	7.98	10.35	10.8	7.83	10.10	11.20
60	7.70	9.35	9.93	7.53	9.90	10.43
90	7.35	8.33	9.80	7.73	8.30	9.33
After harvesting	7.18	7.85	8.13	7.18	7.90	8.10
Mycorrhiza						
30	8.7	11.10	12.48	9.38	10.93	12.43
60	8.30	10.88	12.43	8.63	10.40	11.78
90	7.60	9.68	10.48	8.58	9.48	10.23
After harvesting	7.28	8.23	9.25	7.70	8.65	9.45
Yeast						
30	8.25	10.48	11.15	8.60	9.52	11.33
60	7.95	9.45	10.28	8.18	10.10	10.68
90	7.45	8.45	10.23	7.90	8.55	9.75
After harvesting	7.18	8.10	8.20	7.25	8.10	8.40
PDB						
30	9.85	11.45	12.94	9.93	11.75	12.78
60	8.83	11.85	13.48	9.23	10.83	12.25
90	8.10	10.33	11.75	8.35	10.13	10.10
After harvesting	7.48	8.58	9.40	7.83	9.32	9.03
LSD	5%		1%	5%		1%
Inoc . 30	0.164		0.221	0.272		0.368
60	0.193		0.260	0.145		0.196
90	0.354		0.478	0.377		0.509
After harvesting	0.130		0.175	0.201		0.241
P Fert. 30	0.109		0.165	0.279		0.423
60	0.222		0.336	0.193		0.292
90	0.112		0.169	0.423		0.641
After harvesting	0.113		0.179	0.241		0.365

Regarding with available phosphorus in the surface layers of soil, data in Table (5) revealed that significant increase of available phosphorus (ppm) at all inoculated treatments as compared with control and all the phosphorus mineral fertilizer doses specially after 30 and 60 days from sowing. As the results showed that the high increase in available phosphorus (ppm) for the treatments inoculated with phosphate dissolving bacteria at all different levels of mineral fertilizer specially with 30 and 60 days after sowing being 12.94, 12,78 and 13.48 and 12.25 ppm at the rate of 30 P_2O_5 kg/fed . for season of 2002 and 2003 as compared with control, respectively.

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استجابة محصول القمح للتلقيح الميكروبي المذيب للفوسفات والخميرة تحت مستويات من التسميد الفوسفاتي المعدني في الشمال الشرقي لدلتا مصر

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أجريت تجربتان حقليتان على محصول القمح صنف سخا 93 في محطة بحوث السرو الزراعية بالشمال الشرقي لدلتا مصر في موسميين متتاليين 2002 / 2003 وذلك لدراسة استجابة محصول القمح ومكوناته للتلقيح الميكروبي بفطر الميكورايزا والبكتريا المذيبة للفوسفات (باسيلس ميجاتيريوم) وكذلك سلالة الخميرة سكاروميسس سرفيزيا مع ثلاثة ثلاثة مستويات من التسميد الفوسفاتي المعدني.

صممت تجربة قطع منشقة في أربع مكررات اشتملت على ثلاثة مستويات من التسميد الفوسفاتي (صفر, 15، 30 كجم فو2ا5 /فدان) على صورة سماد سوبرفوسفات أحادي 15 % فو2ا5 (كقطع رئيسية) أما القطع المنشقة فكانت عبارة عن أربع معاملات هى الكنترول (بدون تلقيح) والتلقيح بفطر الميورايزا (جلوميس) والتلقيح الميكروبي المذيب للفوسفات (باسيلس ميجاتيريوم) والتلقيح بسلالة الخميرة سكاروميسس سرفيزيا.

سجلت قيم الوزن الجاف لنباتات القمح (جم/للنبات) وكذلك الفوسفور الميسر في التربة في محيط المجموع الجذري (جزء في المليون) وذلك بعد 30، 60, 90 يوم من الزراعة و بعد الحصلد. كذلك قدرت أوزان محصول القمح من كل من الحبوب, أرداب/فدان والقش بالطن/فدان وأيضا قدرت الكمية الممتصة من الفسفور عن طريق حبوب القمح (كجم/فدان). ويمكن تلخيص أهم النتائج فيما يلي:

- كان للتلقيح الفطري والبكتيري في وجود التسميد الفوسفاتي المعدني تأثير ايجابي معنوي على محصول القمح من الحبوب والقش وكذلك على زيادة الفوسفور الميسر في التربة في محيط المجموع الجذري.
- كانت أعلى كمية ممتصة من عنصر الفسفور في حبوب القمح ناتجا عن التلقيح بالبكتريا المذيبة للفوسفات وفطر الميكورايزا متفوقا عنه في حالة التلقيح بالخميرة.
- حقق التلقيح بالبكتريا الذيبة للفوسفات (باسيلس ميجاتيريوم) اعلى القيم لمحصول القمح من الحبوب والقش (23.68 أرداب/فدان , 3.84 طن/فدان على التوالي) وذلك في وجود التسميد الفوسفاتي المعدني بمعدل 30 كجم فو2أ5 /فدان وذلك في الموسم الأول, بينما حقق التلقيح السابق أعلى القيم لمحصولي الحبوب والقش (22.77 أرداب/فدان, 3.8 طن /فدان) في وجود التسميد الفوسفاتي المعدني بمعدل 15 كجم فو2أ5 /فدان وذلك في الموسم الثاني.