Egypt. J. Agric. Res., 80 (1), 2002.

COMPLEMENTATION OF DIAZOTROPHS AND YEAST AS PLANT GROWTH PROMOTING AGENTS FOR WHEAT PLANTS

OMAR, M.N.A. AND ISMAIL, H.

Soils, Water and Environment Res. Inst., ARC, Giza, Egypt

(Manuscript received 1 August 2001)

Abstract

A field experiment was conducted at El-Khatatba farm (El-Behera Governorate) on wheat variety Sakha 69 to study the influence of some plant growth promoting rhizobacteria (*Rhizobium leguminosarum*, *Bacillus polymyxa*) and a single yeast strain (*Saccharomyces cerevisiae*) as well as mixture of both on the crop yield under two nitrogen fertilizer levels (60 and 120 kg N/fed.) . Nitrogenase and dehydrogenase (DHA) activities were measured during the growth period. Nitrogenase activity of early stages of growth was increased, then gradually decreased with plant age. Changes in the DHAs were significant with raising nitrogen fertilizer level and microbial inoculation. Results indicated that the plant growth promoting rhizobacteria had a positive effect on both vegetative and yield characters (dry weight of plants and panicles). Similar trend was observed for seed yield at full dose of nitrogen fertilizer as well as nitrogen content in seeds of inoculated plants with *Saccharomyces cerevisiae* and *Bacillus polymyxa* compared with the uninoculated control.

Keywords: Diazotrophs, *Saccharomyces cerevisiae*, *Bacillus polymyxa*, Inoculation, Wheat, Nitrogenase, Dehydrogenase.

INTRODUCTION

Gramineous plants such as rice, wheat and maize are major crops for food production. Wheat is one of the most important crops in Egypt in respect to its value and area. Since various diazotrophs have been found in association with Gramineous plants, they are possible candidates for beneficial interactions with cereal crops. *Azospirillum spp.* and other nitrogen-fixing bacteria can secrete iodole acetic acid (Hartmann *et al.*, 1983), gibberellins like substances and cytokine (Tien *et al.*, 1979). Schmidt (1985) mentioned that there was a stimulation of various species of *Rhizobium* strains occurred in the rhizosphere of oats, corn and wheat .The extend of response depended on plant cultivars and *Rhizobium* strains (Pana-Cabriales and Alexander, 1983).

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 (*Bacillus polymyxa, Azospirillum brasilense and Azospirillum lipoferum*) and two strains of yeast (*Saccharomyces cerevisiae and Candida utilis)* simultaneously with nitrogen fertilization at El-Serw province(Damitta Govern orate) had a positive effect on both yield and nitrogen content of plants (El-Kholy and Omar 2000).

The aim of the present investigation is to study the effect of nitrogen application and seed inoculation with individual strains of either *Rhizobium* , *Bacillus* or *Saccharomyces cerevisiae* beside their mixture on growth, yield and nitrogen content of wheat.

MATERIALS AND METHODS

A field trial was carried out using wheat plants (*Triticum aestivum*, var Sakha 69) at El-Khatatba farms. Chemical and physical analyses are present in (Table, 1). Split plot design with 3 replicates was used. Main plots were allocated for nitrogen fertilizers; i.e.60 and 120 kg N/fed. as ammonium sulphate (20.5 % N). The applied treatments were as follows:

Uninoculated plants as control;(seeds inoculated with *Rhizobium leguminosarum* biovar. *trifolii*,; yeast strain (*Saccharomyces cerevisiae*) was used to inoculate the seeds as a promoting agent; *Bacillus polymyxa* was used as a nitrogen fixing bacterium and seeds inoculated with mixture of all. All the microbial strains used in this study were obtained from Department of Agricultural Microbiology, Soils, Water and Environment Res. Inst., ARC, Giza. Inoculation was performed using seed coating technique. Grains were thoroughly mixed with the appropriate amount of each strain (400 g inoculant/fed.). A single inoculated grain harbored ca.10 millions bacteria or yeast on its surface (Omar *et al.*, 1989). Gum Arabic (0.2%) was used as adhesive agent. For combined inoculation the ratio was 1:1:1. Seeds of control treatment were soaked in diazotroph free nitrogen medium. The plot area was $10m^2$ and all plots received the same amount of P_2O_5 as super phosphate (15% P_2O_5) with the dose of 100 kg/fed., as one dose prior to sowing. Two levels of nitrogen fertilizer (60 and 120 kg N/fed.)

were added on soil in two equal doses at sowing and 60 days later. Nitrogenase activity (N_2ase) assayed using GLC, model HP6890 according to the method described by Schollhorn and Burris (1967). Dehydrogenase activity (DHA) of the rhizosphere soil was estimated according to Thalmann (1967). Total nitrogen content of seeds was determined using the standard procedure of Chapman and Pratt (1961). Results were statistically analyzed for LSD according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Nitrogenase activity

Data presented in Table (2) showed that acetylene-reducing activity was 1548.9 nmoles C_2H_4/100g/hr with 60 kg N/fed after 2 months of planting when the treatment was inoculated with *B. polymyxa*. However, the acetylene reducing activities that recorded after 3 months of planting at full dose of nitrogen were 1737.7, 1585.5 and 1041.5 nmoles C_2H_4 /100g/hr when a mixed inoculum, *Saccharomyces cerevisiae* and *B. polymyxa*, were used. At the early stages of growth, nitrogenase activity was high then gradually decreased with plant age. The nitrogenase activity decreased to 32.8 and 37.0 nmoles C_2H_4/100g/hr in the plots that treated with mixture of inocula in presence of half dose and full dose of nitrogen, respectively. So, the nitrogenase activity might be attributed to the effect of exudation of carbon compounds that have special importance to the growth of N_2-fixing microorganisms. Such reports support that these bacteria produce growth regulating compounds mainly indole acetic acid, gibberellins and cytokine –like substance which may improve plant productivity by hormonal stimulation besides N_2-fixation (Tien *et al.*, 1979 and Hirsch *et al.*, 1997).

Dehydrogenase activity

The values presented in Table 2 indicated that changes in the dehydrogenase among the used strains were significant not only with the nitrogen fertilizer but with microbial inoculation as well. Marked decrease in dehydrogenase was recorded at flowering stage with all treatments compared with the earlier stage. This decrease might be due to reduction of the microflora counts. At half dose of nitrogen in first period, *Rhizobium* inoculated treatment was increased by 11% over the uninoculated treatment. While, at full dose of nitrogen, all the treatments were higher in activity of dehydroge-

nase than the control, especially in case of combined inoculation. In the second period of plant growth the mixed culture gave the highest activity. No differences were recorded among all the treatments in the third period. The activity of dehydrogenase decreased gradually. All treatments showed the same activity, except the *Rhizobium* inoculated treatment which was higher in activity at full dose of nitrogen .The number of microorganisms was the highest in the mixed inoculum, this revealed that the biological activity in the rhizosphere area of that treatment was high especially at full dose of nitrogen .The high activity of dehydrogenase enzyme and the released carbon dioxide in the rhizosphere cause the formation of carbonic acids and the decrease of the pH of the medium . This process let to the high rate of the absorption of the nutrient ,that couldn't be available at the high pH .This was very useful for the growth of the plant and increase the yield.

Dry weight of wheat plants

Results of dry weight of wheat plants as affected by inoculation with PGPR and yeast strain are presented in Table 2. Significant differences in wheat biomass were observed with the different inoculation treatments. Inoculation with *Saccharomyces cerevisiae* caused high dry weight (5.11 g) with full dose of nitrogen followed by *Bacillus polymyxa* (5.07 g) in comparison with uninoculated plants (3.64 g). These results are in accordance with those of Abdel Aziz *et al.,* (1989), who found that wheat plants inoculated with PGPR showed higher biomass and better development than uninoculated ones. Dry weight of panicles positively responded to inoculation with increases 6 and 52% over the control. *B. polymyxa* gave increase of 51% over the control at full dose of nitrogen. Mixed inoculant gave increase of 35% and 19% over the control at half and full dose, respectively.

Nitrogen percent of seeds

Data in Table 4 indicated that nitrogen percent of grains was significantly increased by inoculation .An increase ranged between 5 and 37% over the control was recorded. *R. leguminosarum* gave an increase in nitrogen percentage of 37% over the control at full dose of nitrogen. *B. polymyxa* showed respective increases of 11 and 5% at half and full dose of nitrogen, respectively.

Wheat grain yield

Irrespective of introduced strains and dose of mineral nitrogen, the grain yield was increased by 11% and 47% over the control (Table, 4). Yeast gave increases of 14% and 47% at half and full dose of nitrogen, respectively. *R. leguminosarum, B. polymyxa* gave respective increases of 18% and 28%. Mixed inoculant increased grain yield by 31 and 21% at half and full dose of nitrogen, respectively. Dobereiner (1996) reported that contribution of BNF in Brazil and tropical might reach 70% for sugar cane and up to 50% in cereals through the activity of entophytic diazotrophs in non-legumes plants.

One or more of the following reasons could explain the increase of nitrogen content in case of *Rhizobium* inoculation.

The growth of many associative nitrogen fixers on the capsular material surrounding *Rhizobium* and the capability of those microflora to fix nitrogen, nitrogenase activity of *Rhizobium* itself could be induced with wheat plants (Hess and Scholl, 1981) and non leguminous plants excrete some substances such as pentose sugar and succinate which stimulate the growth of *Rhizobium* and other associative diazotrophs and improve their ability. If these substances are found in the rhizosphere, nitrogen fixation may be induced by *Rhizobium* free state (Subba Rao, 1986). The plant response to inoculation with associative nitrogen fixers (*B. polymyxa*) and symbiotic nitrogen fixers (*Rhizobium)* is mainly due to nitrogen fixation and production of growth promoting substances such as indole acetic acid and gibberellins (Berkum and Bohlool, 1980;Ishac, 1988 and Omar, *et al.* 1989).

From the previous results, it could be concluded that inoculation of wheat plants with combined culture of *Saccharomyces cerevisiae* and *B. polymyxa* was compatible with a normal level of nitrogen fertilizer, and yield could be significantly increased at the full dose of nitrogen /fed. through inoculation. In connecting with this point it is worth mentioning that Murty and Ladha (1988) suggested that the increase of mineral uptake by plants could be due to a general increase of the root system area and not to any specific enhancement of the normal ion uptake mechanism. Inoculation of wheat with diazotrophs is expected to supplement the plants with a reasonable amount of their nitrogen requirements provided that there is compatibility between the plant and

bacteria. This point still deserves further investigation to achieve a decisive conclusion about the possible role of diazotrophs in nitrogen feeding of plants under different conditions of nitrogen fertilization.

Table 1. Physiochemical properties of Ismailia sandy soil

(1) Particle size distribution, $CaCO_3$ and organic-matter (OM) (%)

C. Sand	F. Sand	Sill	Clay	$CaCO_3$	OM
75.12	22.56	6.41	0.41	0.21	0.11

(2) Chemical analysis (paste)

		EC	Soluble Ions (meg/L)							
S.P	pH	Mmohs cm^{-1}	Ca	Mg	Na	K	CO_3^{-2}	H CO_3^{-2}	Cl-	SO_4^{-2}
19	8.1	0.65	1.9	1.6	3	0.3	0	2.99	2	1.91

Table 2. Effect of some diazotrophs and *Saccharomyces cerevisiae* on nitrogenase and dehydrogenase activities of wheat plants.

Treatments	N2ase activity Nanomole C2H4/100/hr			DHA activity ug TPF/g/day		
	Periods in months					
	I	II	III	I	II	III
60 kg N/fed. Inoculated by :						
Rhizobium leg.	264.9	662.2	0	103.3	81.3	57
Bacillus polymyxa	619.5	1548.9	17	76.6	75.95	53.6
Saccharomyces cerevisiae	261.8	654.7	5.76	80.1	70.3	50.6
Mixture	309.8	1215.4	32.8	62.9	102.4	66.5
Uninoculated	486.1	1089.7	19.6	93.4	74.4	68.4
120 kg N/fed. Inoculated by:						
Rhizobium leg.	772.6	936.2	0	73.7	75.2	58.9
Bacillus polymyxa	581.1	1041.5	0	81.4	74.2	48.3
Saccharomyces cerevisiae	634.2	1585.5	43.3	73.6	72	46.6
Mixture	762.5	1737.7	37	82.5	95.9	49.5
Uninoculated	581.1	969.5	8.8	60.9	73	70.8

Interaction

Nitrogenase: LSD 1% for Nitrogen 44.8ab Periods 56.17abc Strains 85.07aaabb

Dehydrogenase: LSD 1% 2.03 ab 2.56 3.49abccd

Table 3. Effect of diazotrophs and *Saccharomyces cerevisiae* on dry weight and panicles of wheat plants.

Treatments	dwt of plant/g			dwt of panicles/g		
	Periods in months					
	I	II	III	I	II	III
60 kg N/fed.						
Inoculated by :						
Rhizobium leg.	0.879	3.65	3.09	- -	7.25	9.38
Bacillus polymyxa	0.78	2.5	3.56	- -	10.36	15.5
Saccharomyces cerevisiae	0.99	2.27	5.15	- -	12.16	12.72
Mixture	1.12	3.3	4.28	- -	8.05	19.97
Uninoculated	0.75	2.64	3.6	- -	9.7	14.74
120 kg N/fed.						
Inoculated by:						
Rhizobium leg.	0.77	2.59	3.71	- -	8.4	21
Bacillus polymyxa	1.35	2.89	5.07	- -	11.62	26.96
Saccharomyces cerevisiae	0.98	2.96	5.11	- -	10.79	15.9
Mixture	126	3	2.74	- -	9.39	21.15
Uninoculated	1.05	2.2	3.64	- -	4.67	17.77

LSD 5%		0.061	
LSD 1%:	Nitrogen	0.082	aa
	Dwt of plant Periods0	0.119	abc
	Strain	0.136	abccc

Table 4. Effect of diazotrophs and *Saccharomyces cerevisiae* on yield and nitrogen % of wheat seeds under two levels of nitrogen fertilizer.

Treatments	Yield of seeds Ton/fed.	Total Nitrogen Of wheat seeds %
60 kg N/fed.		
Inoculated by :		
Rhizobium leg.	1.47	3.05
Bacillus polymyxa	1.372	3.4
Saccharomyces cerevisiae	1.503	2.72
Mixture	1.719	3.06
Uninoculated	1.317	2.46
	NS	NS
120 kg N/fed.		
Inoculated by:		
Rhizobium leg.	1.636	4.37
Bacillus polymyxa	1.77	3.33
Saccharomyces cerevisiae	2.031	3.41
Mixture	1.659	3.17
Uninoculated	1.375	2.52
LSD:		
0.05	0.216	0.77
0.01	0.362	1.07

NS : Non Significant

REFERENCES

1. Abdel Aziz, R.A.,Y.Z. Ishac and S.M. Abdel Malek. 1989. Preliminary studies on the effect of inoculation with rhizobacteria on the growth of wheat. Egypt.J.Appl.Sci.,4(1) 1-9.

2. Berkum, P. and B.B. Bohlool. 1980. Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses . Microbiological Reviews,44,491-517.

3. Chapman, H.D. and F.P. Pratt. 1961. Methods of Analysis of Soils. Plant Wasters.Cal.Univ.,175-183.

4. Dobereiner, J. 1996. Biological nitrogen fixation by endotrophic diazotrophs in nonleguminous crops in the tropics.7th International Symposium on nitrogen fixation with non-legumes.Oct.16-21,1996 NIBGE,Faisalabad-Pakistan.

5. El-Kholy, M.H. and M.N.A. Omar. 2000. Grwoth response of wheat as affected by yeast and some diazotrophs inoculation under two levels of nitrogen fertilizer. In : International Symbosium on Nitrogen Fixing and Crop Production, Cairo ,Egypt ,May 11-13 ,1999. FAO Ed.

6. Hartmann, A., A. Fubeder and W. Klingmuller. 1983. Mutants of *Azospirillum* affected in nitrogen fixation and auxin production .In Klingmuller ,W.(ed.) , *Azospirillum* II : Genetics ,Physiology ,Ecology , Birkhauser , Basel ,pp 78-88.

7. Hess, D. and M. School. 1981. Nitrogen fixation in artificial association of nonlegumes and *Rhizobium*. Biological Nitrogen Fixation for Tropical Agric. ,Cali ,Colombia.

8. Hirsch, A.M., Y. Fang, S.A. Asad and Y. Kapulnik. 1997. The role of phytohormones in plant-microbe symbioses. Plant and Soil, 194:171-184.

9. Ishac ,Y.Z. 1988. Inoculation with associative N2 –fixers in Egypt . 4th Inter .Sym. on Nitrogen Fixation with Non-Legumes ,p 241-246.

10. Monib, M., M.K. Zahra and R.R. Armanios. 1982. Occurrence of yeasts in Egyptian and Nigerion Soils ,Zbl. Mikrobiol., 137,369-373.

11. Murty, M .G. and J. K. Ladha. 1988. Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic condition. Plant and Soil 108:281-285.

12. Omar, N., T. Heulin, P. Weinhard, M.N. Alaa El-Din and J. Balandreau. 1989. Field inoculation of rice with *in Vitro* selected plant growth promoting rhizobacteria. Agronomie ,9:803-808.

13. Pana-Cabriales, J.J. and M. Alexander. 1983 . Growth of *Rhizobium* in unamended soil . Soil Sci.Soc. Amer.J.,47,241-245.

14. Schmidt, E.L. 1985. Recent advances in the ecology of *Rhizobium* .6th Inter. Symp. N2- fixer .Corvallis OR. Aug.

15. Schollhorn, R. and R.H. Burris. 1967. Acetylene as a competitive inhibitor of nitrogen fixation .In:Proc.Nat.Acad.Sci.USA.,58:213-216

16. Snedecor, G.W. and W.E. Cochran. 1980. Statistical Methods ,8th Edn. Iowa State University .Press, Ames .USA P 503

17. Subba Rao, N.S. 1986. Soil Microorganisms and Plant Growth .2nd (ed.) Mohan Pvimani for Oxford IBH Pull .

18. Thalmann, A. 1967. Uber die mikrobiello aktivitata under thr benziehung zu fruchtbar he ertsmer kmalene iniger . Acherbeden unter besonderer berksichtigung der dehydrogenase aktivitat.(TTC rediukhion) Diss-Ggiesen ph.D Thesis,Germany

19. Tien ,T.M., M.H. Gaskins and D.H. Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.) Appl.Environ .Microbiol ., 37:1016-1024.

التأثير المتكامل لمثبتات الازوت الجوى و الخميره (سكارومسيس سيرفيزيا) كمنشطات للنمو على القمح

محمد نبيل عبد المجيد عمر وحسن اسماعيل

معهد بحوث الاراضى و المياه و البيئه - الجيزه - مركز البحوث الزراعية

اجريت تجربة حقلية بمزرعة بالخطاطبة (محافظة البحيرة) على نباتات القمح صنف سخا ٦٩ لدراسة تأثير بعض منشطات النمو الريزوبكتيرية

(ريزوبيم ليجومونوزارم ؛ باسلس بوليمكسا ، سكارومسيس سيرفيزيا وخليط من هذه السلالات) تحت مستوين من التسميد المعدنى (٦٠ و ١٢٠كيلو جرام ازوت/ فدان)

وقد تم تقدير النشاط الانزيمى الخاص بعملية تثبيت الازوت الجوى (النتروجينيز) وكذلك انزيم الد يهيدروجينيز الخاص بعملية التنفس اثناء فترات النمو لنباتات القمح واظهرت النتائج زياده نشاط انزيم النيتروجينيز فى مراحل النمو الاولى وبعدها قل النشاط مع زياده عمر النبات.التغيرات فى نشاط انزيم الديهدروجينيز بين السلالات المستخدمه كانت معنويه مع التسميد المعدنى والتلقيح البكتيرى. اضافة منشطات النمو الريزوبكتيرية اظهر نشاط وتأثير معنوى موجب على كلا من الصفات الخضرية والمحصولية وهى: الوزن الجاف للنباتات وعدد السنابل ووزنها الجاف ولوحظ هذا الاتجاة لمحصول الحبوب تحت مستوى تسميد معدنى كامل والمحتوى الازوتى فى الحبوب فى المعاملات الملقحة وخاصة المعاملة بالخميرة والباسلس مقارنة بتلك غير المعامله بأى لقاحات .