A TWO - YEAR FIELD TRIAL ON EVALUATING RHIZOBACTERIA INOCULATION ON GROWTH AND YIELD OF RICE PLANTS

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Abstract

Two field trials on rice plants (*Oryza sativa*) variety Giza 178 cultivated at El-Serw Agricultural Research Station were conducted in two successive seasons (2001 and 2002). Microbial inoculation with two strains of nitrogen fixers, namely *Bacillus polymyxa* and *Azotobacter chroococcum* as well as yeast strain (*Saccharomyces cerevisiae*) was applied. Effect of inoculation time (rice nursery and transplanting stages) was studied. Split plot with 4 replicates was designed. Plant height, number of productive tillers, panicle length and weight were recorded. Grains and straw yields were also estimated. Nitrogen percent of grains and straw were determined. Microbial inoculation of nursery and transplanting stage revealed a significant effect on both yield and plant growth parameters in the presence of half and full recommended dose of nitrogen in comparison with other treatments. Similar response was detected with yeast inoculant except nitrogen percent of grains.

Keywords: PGPR, *Bacillus polymyxa, Azotobacter chroococcum,* Yeast,Rice

INTRODUCTION

Rice is the most important staple food after wheat and a second major export agricultural commodity in Egypt. Rice also provides beneficial means in reclaiming salt affected soils. Bacterial inoculation and nitrogen fixation in the rice rhizosphere have shown that in most cases, yield was increased after inoculation with nitrogen fixing bacteria (Omar *et al.*, 1992). Microaerophilic *azospirilla* were found to enhance yields of both lowland and upland rice. Nitrogen-fixing bacteria, living in association with roots of plants, were investigated to establish their effects on plant growth and yield (Mertens and Hess, 1984). *Azospirillum* is very common in the rice rhizosphere (Omar *et al.*, 1989b) and various strains have also been isolated from maize and wheat roots (Fages and Mulard, 1988).

Many studies indicated the importance of *Azospirillum* for fixing nitrogen in cereals (Omar *et al.*, 1989 b and Zhang *et al.*, 1990). These bacteria proved to be able to produce auxins and other growth substances in the plant rhizosphere (Jain and Patriquin 1985). Sarig *et al.* (1984) found that nitrogen content of plant aerial tissues increased by *Azospirillum* inoculation. Plant growth promoting rhizobacteria (PGPR) have positive effect on the growth and yield of crop plants by various mechanisms including production and secretion of plant growth regulators, by eliciting root metabolic activities. Yeasts play an important role in soil biofertility; because of their capability for producing hormones, amino acids, cytokinin, indole and vitamins (Monib *et al.*, 1982).

The present study aimed to evaluate the beneficial effect of inoculation with nitrogen fixing bacteria and yeast as a plant growth promoting rhizobacterium (PGPR). Different techniques to inoculate rice plant either in nursery and /or transplanting was also considered.

MATERIALS AND METHODS

Two field trials on rice (*Oryza sativa*) variety Giza 178, at El -Serw Agriculture Research Station, were conducted in two successive seasons (2001 and 2002). The split plot design with four replicates was used. The plot size was 10 m^2. The main plots were arranged for different doses of nitrogen fertilizer (0, 20 and 40 Kg N/fed as ammonium sulfate 20.6% N). Sub-plots were devoted to evaluate the inocula of *Azotobacter chroococcum, Bacillus polymyxa* and yeast *Saccharomyces cerevisiae,* provided from the Soils, Water and Environment Res. Inst., ARC, Giza in both nursery beds of rice plants and transplanting soils. The treatments were as follows: -

I_0-Uninoculated control

I_1 –Inoculation in rice nursery

I_2-Inoculation after transplanting

I_3-Inoculation twice in the nursery and after transplanting

Prior to planting and during plant bed preparation, a soil sample was taken from the surface layer (0-30cm depth) and analyzed for some physical and chemical properties (Table, 1) as described by Black (1982). Super-phosphate (15%P_2O_5) was added to all plots at the rate of 15 Kg P_2O_5/fed before transplanting. The mineral nitrogen fertilizer was broadcasted in three equal doses: the first one was immediately prior to transplanting of rice seedlings while the other two doses were applied 30 and 50 days after transplanting , respectively .

Bacterial inoculation in nursery bed was performed using seed coating technique. Each inoculated grain harbored 10^6 cells on its surface. Maximum care was taken to avoid cross-contamination in the field after transplanting , i.e. plots were separated by mud bunds, 50 cm wide inside blocks, and water was prevented from flowing between plots. Bacterial and yeast inoculation was repeated again in both nursery bed and transplanting soil after seed sowing in the nursery bed and after transplanting by using liquid culture (10^9 cells/ml of bacteria or yeast) as a soil application technique at a rate of 5 L/fed. Liquid inoculant was added 3 times during the growth period up to the flowering stage. Before harvesting, plant height, number of productive tillers, panicle length and weight were recorded for all plots. After harvesting, grains and straw yields were estimated. Nitrogen content in grain and straw was determined as described by Black (1982). The statistical analysis was carried out according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The obtained results (Table 2) revealed a significant increase in plant height with the three tested doses of mineral nitrogen fertilizer. Plant height increased from 101.6 to 124.7 cm, when mineral nitrogen fertilizer increased up to 40 kg / fed. However, insignificant increase with number of productive tiller was recorded. With respect to inoculation of nursery, transplanting, or nursery and transplanting area with *Azotobacter chroococcum, B. polymyxa,* the obtained data showed insignificant increase in plant height or number of productive tillers. However, significant increase was obtained with microbial inoculation and mineral nitrogen fertilization. Thus, plant height increased from 121.6 to 136.2 and from 121.5 to 135.2 and from 105.9 to 113.4 cm, when nitrogen fertilizer dose was increased from zero to 40kg/fed in combination with *Azotobacter chroococcum, Bacillus polymyxa* or *S. cerevisiae,* respectively. The corresponding figures for number of productive tillers were from 17.6 to 22.80, from 17.8 to 23.0 and from 16.20 to 21.30 tillers/hill, respectively. Although increasing of plant height and number of productive tillers were accounted with *B. polymyxa* in nursery, transplanting or nursery and transplanting soil; nonsignificant increase was detected. It was interesting to observe a significant increase in panicle length and panicle weight with increasing mineral nitrogen dose (Table 3).

Table 4 shows the effect of diazotrophs and yeast inoculation on grain and straw yields of rice plants. The obtained results revealed significant increases in grain and straw yields. Inoculation of nursery and transplanting soil with *Azotobacter chroococcum* or *B. polymyxa* resulted in increase of grain yield over control treatment being 0.6 and 0.4 ton/fed in the presence of 40kg/fed mineral nitrogen fertilizer. The corresponding values of straw yield were 0.9 and 0.7 ton/fed, respectively. Inoculation of nursery and transplanting soil with *S. cerevisiae* caused an increase in grain yield over control being 0.9 ton/fed. Yeast inoculation stimulated straw yield (6.7 ton/fed) in comparison with control. In treatments inoculated with *Azotobacter chroococcum, B. polymyxa* or *S. cerevisiae* in nursery, transplanting or nursery and transplanting soil (Table 4) a highly significant increase in grain yield was accounted in presence of 40kg/fed mineral nitrogen and bacterial inoculation (*Azotobacter chroococcum, B. polymyxa* or *S. cerevisiae*) with either nursery or transplanting soil. These observation confirmed the beneficial effect of diazotrophs or yeast inoculation on growth parameters of plant whether biofertilizers were added to nursery or transplanting soil with *Azotobacter chroococcum* or *B. polymyxa* (Omar *et al.* 1989 a and El-Kholy and Omar, 2000). Regarding nitrogen percent of rice grains and straw, data in Table 5 revealed insignificant increases of nitrogen of both rice grains and straw, which treated with mineral nitrogen fertilizer beside inoculation by *Azotobacter chroococcum* or *B.polymyxa* either in nursery, transplanting or nursery and transplanting soil.

From the previous results it could be concluded that enhancement of rice production by inoculation of rice plants with the nitrogen-fixing bacteria is comparable with that nitrogen fertilization, and yield significantly increased at 40 kg N/fed. Rice inoculation with N_2-fixing bacteria supplements the plants with a reasonable amount of their nitrogen requirements provided that there is affinity between the plant and bacteria. These data confirmed also that yeasts play an important role in soil biofertility. Inoculation with yeast significantly stimulated plant growth and grain yield. The effect of inoculation time in rice nursery, after transplanting and /or in nursery and after transplanting was evaluated. The data revealed that inoculation of rice nursery bed and transplanting soil gave significant effects on grain yield.

REFERENCES

1. Black, C. A. 1982. Methods of Soil Analysis, Part 2 Chemical and Microbiological properties. Amer. Society of Agronomy Inc., Madison, Wisconsin, USA.

2. El-Kholy, M. H. and M. N. A., Omar 2000. Growth 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.

3. Fages, J. and D. Mulard. 1988. Isolement de bacteries rhizospheric et effet de leur inoculation en pots chez *Zea mays*. Agronomie, 8:309-314.

4. Jain, D. K. and D. G. Patriquin 1985. Characterization of a substance produced by *Azospirillum* which cause branching of wheat root hairs. Can. J. Microbiol. 31, 206-210.

5. Mertens, I. and D. Hess 1984. Yield increases in spring wheat (*Triticum-aestivum* L) inoculated with *Azospirillum lipoferum* under greenhouse and field condition of a temperate region. Plant and Soil. 82, 87-99

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

7. Omar, N., Odile Berge, S. N. Shalaan, J. L. Hubert, T. Heulin and J. Balandreau 1992. Inoculation of rice with *Azospirillum brasilense* in Egypt. Rusults of five different trials between 1985 and 1990. Symbiosis, 13:281-289.

8. Omar, A. M. N., C. Richard, P. Weinhard, and J. Balandreau 1989a. Using the spermosphere model technique to describe the dominant nitrogen fixing microflora associated with wet land rice in two Egyptian soils. Biol. Fertil. Soils. 7:158-163.

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

10. Sarig, S. Y. Kapulink, I. Nur and Y. Okon 1984. Response of nonirrigated *Sorghum bicolor* to *Azospirillum* inoculation . Expl. Agric. 20:59-66.

11. Snedecor, G. W. and W. E., Cochran, 1989. Statistical Methods, 8th edition. Iowa state university press, Ames. USA .

12. Zhang C. and J., Li S. Ping 1990. Response of rice to inoculation with diazotropic bacteria. In "The Associative Nitrogen Fixation in the Rice Rhizosphere ", You Chongbiao (Chief Editor), Agricultural Publishing House, pp.355-361 (in Chinese).

Table 1. Some physical and chemical properties of the soil of the experiment .

Soil characteristics	1st season	2nd season
Soil texture	Clay	Clay
Soil pH (1:2.5 water suspension)	8.4	8.2
EC (soil paste extract at 25^{0}C) dSm^{-1}	5.8	4.2
O.M.%	1.2	1.3
$CaCO_3$%	1.5	1.4
Available-N (K-sulfate ext.) ppm	36.6	38.2
Available-P (Olsen ext. P) ppm	7.8	8.2
Available-K (Amm. acet. ext.) ppm	380	400

Table 2. Plant height and number of tillers of rice plant (at harvest) as affected by mineral nitrogen fertilizer and PGPR inoculation (Combined data of 2001 and 2002 seasons) .

PGPR inoculation	N Fertilization					
	Plant height (cm)			Number of productive tillers (-per hill)		
	0 kg N /fed	20 kg N/fed	40 kg N/fed	0 kg N/fed	20 kg N/fed	40 kg N/fed
	Azotobacter chroococcum					
I_0	101.6	117.0	124.7	13.00	16.6	18.3
I_1	114.5	122.5	129.4	15.5	17.6	20.3
I_2	120.4	126.3	134.6	17.1	19.9	22.1
I_3	121.6	130.1	136.2	17.6	20.1	22.8
LSD (5%)	4.94			N.S		
	Bacillus polymyxa					
I_0	102.3	118.3	126.9	13.5	16.5	18.3
I_1	115.4	122.8	130.6	16.3	18.0	20.1
I_2	120.4	126.1	134.3	17.6	20.1	22.7
I_3	121.5	126.8	135.2	17.8	20.6	23.0
LSD (5%)	N.S			N.S		
	Saccharomyces cerevisiae					
I_0	95.6	102.8	108.3	13.0	16.1	17.9
I_1	103.2	107.2	111.9	14.9	17.3	20.0
I_2	104.9	108.5	112.9	15.8	18.0	20.9
I_3	105.9	109.9	113.4	16.2	18.2	21.3
LSD (5%)	0.4			0.1		

Table 3. Panicle length, panicle weight and 1000-grain weight of rice plants as affected by mineral nitrogen fertilizer and PGPR inoculation (Combined data of 2001 and 2002 seasons).

Saccharomyces cerevisiae

Inoculation	N Fertilization								
	Panicle length (cm)			Panicle weight (g)			1000-grain weight (g)		
	0 kg N/fed	20 kg N/fed	40 Kg N/fed	0 kg N/fed	20 kg N/ fed	40 kg N/fed	0 kg N/ fed	20 kg N/fed	40 kg N/ fed
Azotobacter chroococcum									
I_0	18.8	20.6	21.5	2.5	3.4	3.8	23.0	23.3	23.6
I_1	20.3	21.4	21.5	3.3	3.8	4.1	23.1	23.5	24.0
I_2	21.3	21.7	21.6	3.7	3.9	4.3	23.4	23.6	24.2
I_3	21.5	21.9	21.8	3.9	4.2	4.3	23.6	23.8	24.2
LSD (5%)	0.3			0.1			0.3		
Bacillus polymyxa									
I_0	18.8	20.7	21.5	2.6	3.5	3.8	29.9	23.4	23.7
I_1	20.4	21.5	21.9	3.3	3.8	4.1	23.2	23.5	23.8
I_2	21.3	21.7	22.2	3.7	3.8	4.2	23.4	23.7	24.0
I_3	21.5	21.7	22.2	3.8	3.9	4.2	23.5	23.8	24.0
LSD (5%)	0.3			0.2			0.3		
Saccharomyces cerevisiae									
I_0	18.3	19.4	20.6	2.0	2.2	2.3	21.1	21.3	23.0
I_1	20.3	21.5	21.9	2.2	2.7	2.9	22.1	22.9	23.5
I_2	20.5	22.3	22.3	2.5	3.0	3.1	22.5	23.5	23.6
I_3	20.8	22.4	22.3	2.6	3.2	3.2	22.7	23.5	23.6
LSD (5%)	0.1			0.1			0.1		

Table 4. Grain and straw yields of rice (ton/fed) as affected by mineral fertilizer and PGPR Inoculation (Combined data of 2001 and 2002 seasons)

PGPR inoculation	N Fertilization					
	Grain yield (ton/fed)			Straw yield (ton/fed)		
	0 kg N/ fed	20 kg N/ fed	40 kg N/ fed	0 kg N/ fed	20 kg N/ fed	40 kg N/ fed
	Azotobacter chroococcum					
I_0	1.8	2.4	3.2	2.8	4.2	5.4
I_1	2.2	3.1	3.6	3.8	5.2	5.8
I_2	2.9	3.4	3.6	5.1	5.6	6.1
I_3	3.2	3.7	3.8	5.3	5.8	6.3
	Bacillus polymyxa					
I_0	1.8	2.6	3.3	2.8	4.1	5.3
I_1	2.3	3.1	3.5	3.8	5.0	5.6
I_2	3.0	3.6	3.7	5.0	5.4	6.0
I_3	3.2	3.6	3.7	5.1	5.6	6.0
LSD (5%)	0.2			0.2		
Saccharomyces cerevisiae						
I_0	1.7	2.2	2.6	3.4	3.9	4.3
I_1	2.3	3.0	3.3	4.0	4.8	5.0
I_2	2.6	3.2	3.4	4.5	5.2	6.2
I_3	2.7	3.4	3.5	4.8	5.9	6.7
LSD (5%)	0.04			0.1		

Table 5. Nitrogen percent in rice grains and straw yields as affected by mineral nitrogen fertilizer and PGPR inoculation, Combined data of 2001 and 2002 seasons)

PGPR inoculation	N Fertilization (kg N/fed)					
	N% in grains			N% in straw		
	0 kg N / fed	20 kgN/ fed	40 kg N/ fed	0 kg N/ fed	20 kg N/ fed	40 kg N/ fed
	Azotobacter chroococcum					
I_0	1.10	1.25	1.37	0.464	0.540	0.595
I_1	1.20	1.38	1.50	0.498	0.602	0.623
I_2	1.26	1.42	1.62	0.543	0.632	0.702
I_3	1.33	1.44	1.65	0.583	0.648	0.714
LSD (5%)		0.02			0.01	
	Bacillus polymyxa					
I_0	1.10	1.20	1.35	0.357	0.434	0.462
I_1	1.20	1.36	1.45	0.413	0.514	0.537
I_2	1.23	1.42	1.47	0.418	0.519	0.541
I_3	1.28	1.43	1.48	0.423	0.521	0.546
LSD (5%)		0.08			0.005	
	Saccharomyces cerevisiae					
I_0	1.11	1.25	1.36	0.49	0.56	0.61
I_1	1.23	1.30	1.41	0.53	0.58	0.67
I_2	1.28	1.38	1.44	0.58	0.63	0.68
I_3	1.30	1.35	1.44	0.60	0.63	0.70
LSD (5%)		N.S			N.S	

تقييم تجربتين حقليتين للتلقيح بالريزوبكتريا علي نمو ومحصول نباتات الارز

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أجريت تجربتان حقليتان على نباتات الارز صنف جيزة ١٧٨ في محطة بحوث السرو الزراعية في موسمين متتاليين ٢٠٠١ ، ٢٠٠٢ .

أستخدمت سلالتان من مثبتات الازوت الجوى في التلقيح البكتيري باسلس بوليمكسا والازوتوباكتر كروكوكم واستخدمت كذلك سلاله الخميرة سكاروميسس سرفنيزيا كمنشط حيوي . تم دراسة تاثير ميعاد اضافة اللقاح البكتيرى في مشتل الارز وكذلك الارض المستديمة. صممت تجربة قطع منشقة مع اربع مكررات .سجلت أطوال النباتات ، عدد الافرع ، طول السنبلة ووزنها . قدرت اوزان محصول الحبوب والقش . قدرت نسبة الازوت % في القش والحبوب .أظهرت النتائج أن التلقيح البكتيري في المشتل والارض المستديمة له تأثير معنوى على المحصول و دلائل نمو النبات في وجود نصف الجرعة الكاملة للأزوت المعدني بالمقارنة بالمعاملات الاخرى . واعطت سلالة الخميرة نفس اتجاه النتائج ماعدا نسبة الازوت % في الحبوب .