

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

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### 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 indole 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 extent 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<sup>2</sup> and all plots received the same amount of P<sub>2</sub>O<sub>5</sub> as super phosphate (15% P<sub>2</sub>O<sub>5</sub>) 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 ( $N_2$ ase) 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 led 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,  $\text{CaCO}_3$  and organic-matter (OM) (%)

| C. Sand | F. Sand | Sill | Clay | $\text{CaCO}_3$ | OM   |
|---------|---------|------|------|-----------------|------|
| 75.12   | 22.56   | 6.41 | 0.41 | 0.21            | 0.11 |

(2) Chemical analysis (paste)

| S.P | pH  | EC    |                  | Soluble Ions (meg/L) |     |    |     |                    |      |     |                    |
|-----|-----|-------|------------------|----------------------|-----|----|-----|--------------------|------|-----|--------------------|
|     |     | Mmohs | $\text{cm}^{-1}$ | Ca                   | Mg  | Na | K   | $\text{CO}_3^{-2}$ | H    | Cl- | $\text{SO}_4^{-2}$ |
| 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                      | N <sub>2</sub> ase activity<br>Nanomole C <sub>2</sub> H <sub>4</sub> /100/hr |        |      | DHA activity<br>ug TPF/g/day |       |      |
|---------------------------------|---|--------|------|------------------------------|-------|------|
|                                 | Periods in months   |        |      |                              |       |      |
|                                 | I   | II     | III  | I                            | II    | III  |
| 60 kg N/fed.                    |   |        |      |                              |       |      |
| Inoculated by :                 |   |        |      |                              |       |      |
| <i>Rhizobium leg.</i>           | 264.9   | 662.2  | 0    | 103.3                        | 81.3  | 57   |
| <i>Bacillus polymyxa</i>        | 619.5   | 1548.9 | 17   | 76.6                         | 75.95 | 53.6 |
| <i>Saccharomyces cerevisiae</i> | 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:                  |   |        |      |                              |       |      |
| <i>Rhizobium leg.</i>           | 772.6   | 936.2  | 0    | 73.7                         | 75.2  | 58.9 |
| <i>Bacillus polymyxa</i>        | 581.1   | 1041.5 | 0    | 81.4                         | 74.2  | 48.3 |
| <i>Saccharomyces cerevisiae</i> | 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 :                 |                   |      |      |                   |       |       |
| <i>Rhizobium leg.</i>           | 0.879             | 3.65 | 3.09 | --                | 7.25  | 9.38  |
| <i>Bacillus polymyxa</i>        | 0.78              | 2.5  | 3.56 | --                | 10.36 | 15.5  |
| <i>Saccharomyces cerevisiae</i> | 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:                  |                   |      |      |                   |       |       |
| <i>Rhizobium leg.</i>           | 0.77              | 2.59 | 3.71 | --                | 8.4   | 21    |
| <i>Bacillus polymyxa</i>        | 1.35              | 2.89 | 5.07 | --                | 11.62 | 26.96 |
| <i>Saccharomyces cerevisiae</i> | 0.98              | 2.96 | 5.11 | --                | 10.79 | 15.9  |
| Mixture                         | 1.26              | 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 Periods 0 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<br>Ton/fed. | Total Nitrogen<br>Of wheat seeds % |
|---------------------------------|----------------------------|------------------------------------|
| 60 kg N/fed.                    |                            |                                    |
| Inoculated by :                 |                            |                                    |
| <i>Rhizobium leg.</i>           | 1.47                       | 3.05                               |
| <i>Bacillus polymyxa</i>        | 1.372                      | 3.4                                |
| <i>Saccharomyces cerevisiae</i> | 1.503                      | 2.72                               |
| Mixture                         | 1.719                      | 3.06                               |
| Uninoculated                    | 1.317                      | 2.46                               |
|                                 | NS                         | NS                                 |
| 120 kg N/fed.                   |                            |                                    |
| Inoculated by:                  |                            |                                    |
| <i>Rhizobium leg.</i>           | 1.636                      | 4.37                               |
| <i>Bacillus polymyxa</i>        | 1.77                       | 3.33                               |
| <i>Saccharomyces cerevisiae</i> | 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            |                            |                                    |

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

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

اجريت تجريبه 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 مستويين من التسميد المعدنى (٦٠ و ١٢٠ كيلو جرام ازوت/ فدان) وقد تم تقدير النشاط الانزيمى الخاص بعملية تثبيت الازوت الجوى (النيتروجينيز) وكذلك انزيم الديهيدروجينيز الخاص بعملية التنفس اثناء فترات النمو لنباتات القمح و اظهرت النتائج زياده نشاط انزيم النيتروجينيز فى مراحل النمو الاولى وبعدها قل النشاط مع زياده عمر النبات. التغيرات فى نشاط انزيم الديهيدروجينيز بين السلالات المستخدمه كانت معنويه مع التسميد المعدنى و التلقيح البكتيرى. اضافة منشطات النمو الريزوبكتيرية اظهر نشاط و تأثير معنوى موجب على كلا من الصفات الخضريه و المحصولية و هى: الوزن الجاف للنباتات و عدد السنابل ووزنها الجاف و لوحظ هذا الاتجاه لمحصول الحبوب تحت مستوى تسميد معدنى كامل و المحتوى الازوتى فى الحبوب فى المعاملات الملحقه و خاصة المعاملة بالخميرة و الباسلس مقارنة بتلك غير المعامله باى لقاحات .