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ENHANCEMENT OF GROWTH, NODULATION AND YIELD OF BEAN PLANTS BY SOIL INOCULATION WITH *Saccharomyces cerevisiae*

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ABSTRACT

A study was conducted to examine the possibility of enhancing the effectiveness of three different *Rhizobium* inoculants with two varieties of bean in the presence of *Saccharomyces cerevisiae*. Results showed that symbiotic parameters; nodule number, dry weight of nodule, N_2-ase activity on plant roots as well as dry weight and nitrogen content of shoots were increased due to the addition of *Saccharomyces cerevisiae* to the soil. Straw and seed yields and their N-contents were also increased due to *Saccharomyces cerevisiae* inoculation in Giza 6 and Nebrasca varieties. The highest records of the symbiotic parameters, seed and straw yields were obtained in plants received the recommended doses of NPK fertilizers combined with *Saccharomyces cerevisiae* inoculation, followed by those inoculated with *Rhizobium leguminosarum* bv. *phaseoli* strains (ARC 302 and 3629) together with *Saccharomyces cerevisiae* and received 20 kg N fed^{-1} in cv. Giza 6, and plants inoculated with *Rhizobium leguminosarum* bv. *phaseoli* strains (182 and ARC 301) together with *Saccharomyces cerevisiae* and received 20 kg N fed^{-1} in Nebrasca variety. No obvious significant differences were recorded in the abovementioned results.

Therefore, the results of the present study emphasize that seed inoculation of bean plants with mixed strains of *Rhizobium* and *Saccharomyces cerevisiae* under 20 kg N fed^{-1} was much better than the addition of the recommended doses of NPK fertilizers together with *Saccharomyces cerevisiae*.

Key words: *bean, nitrogen fixation , nodulation ,Phaseolus vulgaris, Saccharomyces cerevisiae ,yeast.*

1. INTRODUCTION

Leguminous plants, especially beans (*Phaseolus vulgaris*) are considered a food with high nutritional value and one of the richest sources of plant protein. Bean protein was found to contain different essential amino acids plus high concentrations of niacin, lysine and folic acid (Hemphill and Jackson, 1982).

In Egypt, one of the major problems facing bean cultivation in most locations is the failure to form effective nodules which has been attributed to the seed diffusates (Fawaz *et al.*, 1970 ; Dadarwal and Sen, 1973), bacteriophage susceptibility (Dahar *et al.*, 1993), absence of the appropriate strains in the field (Nassar *et al.*, 1972), incompatibility between rhizobia and host plant (Saparrow and Ham, 1983), antagonistic effect of other organisms in the rhizosphere (Ibrahim *et al.*, 1971), delay of rhizobia activity until flowering (Cackett, 1965), and heavy application of nitrogen fertilizers (Mahmoud *et al.*, 1971 ; Nassar *et al* ., 1972 ; Crespo *et al.*, 1987).

One of the possible approaches to enhance nodulation and N_2-fixation in beans is to introduce a soil microorganism that might contribute significantly to biofertility of the agricultural lands. Yeast are among the soil microflora that play an important role in association with other micro-residents and growing plants.

In Egypt, few studies are available concerning the possibility of increasing the effectiveness of nodulation and N_2-fixation in bean plants by yeast treatment. Therefore, the aim of the present study was to evaluate the effect of inoculation with *Saccharomyces cerevisiae* on nodulation and growth of two bean varieties with different *Rhizobium* inoculants.

2. MATERIALS AND METHODS

2.1. The soil used

A clay loamy soil (clay 38%; silt 27%; sand 35%; pH 7.5; EC 0.6 dS m^{-1}; organic matter 0.5%; Total-N 0.08%) was collected from the Experimental Field Station, ARC, Giza. Soil was air dried, sieved and mixed thoroughly with sand (1:1). Soil analysis was done as described by Black *et al.* (1965). Plastic pots were filled with the soil samples at a rate of 10 kg pot^{-1}.

2.2. Seeds

Seeds of two bean varieties Giza 6 (local variety) and Nebrasca (foreign variety) were used and selected to be similar in size and weight as possible.

2.3. Microbial strains

Six strains of rhizobia representing *Rhizobium leguminosarum* bv *phaseoli* were used as inoculants in this study. ARC 301 and ARC 302 are local strains obtained from ARC Culture Collection; NIFTAL 182 obtained from Niftal-Project-Faculty of Tropical Agriculture, Univ. of Hawai, USA; 3612 obtained from Rothansted Experimental Station, Harpenden, UK.strain 1291 was obtained from Rhizobium Research, Dept. of Soil Science, Univ. of Minesesota, USA, and 3629 was obtained from Canada Agricultural Station, Canada.

2.4. Preparation of *Rhizobium* inoculum

Rhizobium strains were grown on yeast extract mannitol (YEM) medium (Vincent, 1970) at 30°C for 3 days until early log phase (5×10^9 cfu/ml), then transferred to sterile fine peat neutralized with 5% $CaCO_3$. Seeds were inoculated with various inoculants at a rate of 400 g inoculum / 35 kg seeds/fed for Giza 6 variety and per 45 kg seeds/fed for Nebrasca variety using Arabic gum solution (16%) at a rate of 400 ml fed^{-1}.

2.5. Yeast

A commercial product of *Saccharomyces cerevisiae*,kindly provided by Soil, Water and Environ. Res. Instit. ARC, Giza, was used in this study.

2.6. Experimental layout

A greenhouse experiment was conducted in ARC at Giza, Egypt. The soil was supplemented with superphosphate at a rate of 1 g pot^{-1}. The experiment included eighteen treatments, nine treatments for each cultivar with 6 replicates as follows :- Uninoculated plants (control) ; plants fertilized with the recommended doses of NPK fertilizers ; plants (either cv.Giza 6 or Nebrasca) inoculated with mixed strains of (182 and ARC 301) or mixed strains of (USA 1291 and 3612)or mixed estrains of (ARC302 and 3629)all mixed strains received 20 Kg N fed^{-1} ; plants (either cv. Giza 6 or Nebrasca)were inoculated with the same *Rhizobium* strains together with *Saccharomyces cerevisiae* and received 20 kg N fed^{-1}. Plants (either cv. Giza 6 or Nebrasca) supplemented by *Saccharomyces cerevisiae* only and recommended doses of NPK fertilizers.Seven seeds of bean (*Phaseolus vulgaris*) from each cultivar were sown in each pot. Pots were arranged in a randomized complete block design. Pots were watered as needed. Yeast suspension was added over head soil after 15 and 21 days of planting at a rate of 5 L fed^{-1}. Plants were thinned to 3 plants per pot after one week. Nitrogen fertilizer was added two weeks after sowing at a rate of 1 g pot^{-1} as ammonium sulphate (20.5% N), while potassium sulphate was incorporated at a rate of 0.5 g pot^{-1}. Plant samples were collected after 60 days of planting to determine the number and dry weight of nodules, N_2-as activity on plant root and dry weight of shoots. N_2-ase activity of plant roots was determined using the Acetylene Reduction Assay as described by Hardy *et al.* (1973), using Dani 1000 Gas Chromatography.

At harvest, straw and seed yields were determined. Nitrogen and phosphorus contents of shoots, seeds and straw were determined according to the methods described by Page *et al.* (1982). Data were subjected to analysis of variance using the statistical analysis system computer packge M-STAT-C (Snedecor and Cochran, 1980).

3. RESULTS AND DISCUSSION

Nodulation, growth and yield of two varieties of beans as affected by the application of *Saccharomyces cerevisiae* were evaluated.

3.1. Nodulation status

Data presented in Table (1) show the effect of inoculation with 3 different *Rhizobium* inoculants on nodulation parameters of two varieties of beans in the absence or presence of *Saccharomyces cerevisiae*. Results showed that the soil tested was devoid of native or the appropriate strains nodulating bean. This observation was also reported by Fawaz *et al.* (1970) and Nassar *et al.* (1972). Nodulation and N_2-fixation were inhibited in plants received the recommended doses of NPK as a mineral fertilizers; application of high concentrations of N, generally reduced nodulation and N_2-fixation in beans confirming the results of Trinchant and Rigaud (1984) and Buttery *et al.* (1987). Bean roots showed a variable number of nodules depending on the type of inoculant. Bean seeds (Giza 6 and Nebrasca) inoculated with the strain USA 1291; and the foreign strains scored the minimum number, dry weight of nodules and N_2-ase activity among all the treatments.

In Giza 6 seeds inoculated with *Rhizobium* strains only, the maximum number, dry weight of nodules and N_2-ase activity were obtained with plants inoculated with ARC 302 and the foreign strain 3629. In Nebrasca, the maximum values were obtained in plants inoculated with the strains (182 and ARC 301).

Saccharomyces cerevisiae over-head soil inoculation had a stimulative effect on nodulation status. It is clear from Table (1) that the addition of *Saccharomyces cerevisiae* besides inoculation of bean seeds (cv. Giza 6) with (ARC 302 and 3629) rhizobial strains produced the highest number, dry weight of nodules and N_2-ase activity on plant roots. In Nebrasca variety, *Saccharomyces cerevisiae* combined with seed inoculation with (182 and ARC 301) resulted in the highest number, dry weight of nodules and N_2-ase activity. The stimulative effect of *Saccharomyces cerevisiae* was more pronounced in Nebrasca variety. These findings were also observed by Tuladhar

Table (1): Nodulation status of bean plants as affected by inoculation of *Saccharomyces cerevisiae* and *Rhizobium* inoculation.

Bean varieties / Treatments	Giza 6			Nebrasca		
	No. of nodules/ plant	D.wt. of nodules (mg/plant)	N_2-ase activity µmole C_2H_4/plant/hr.)	No. of nodules /plant	D.wt. of nodules (mg/plant)	N_2-ase activity µmole C_2H_4/plant/hr.)
Control (uninoculated)	0.0	0.0	0.0	0.0	0.0	0.0
Recommended doses of NPK	0.0	0.0	0.0	0.0	0.0	0.0
182 + ARC 301 + 20 kg N fed^{-1}	17.0	51.0	43.6	32.0	102.0	187.7
USA 1291 + 3612 + 20 kg N fed^{-1}	10.0	15.0	16.9	11.0	30.0	64.0
ARC 302 + 3629 + 20 kg N fed^{-1}	25.0	95.0	146.6	16.0	38.0	46.0
S. cerevisiae + Recommended dose of NPK	0.0	0.0	0.0	0.0	0.0	0.0
S. cerevisiae + 182 + ARC 301 + 20 kg N fed^{-1}	28.0	96.0	160.4	51.0	192.0	235.0
S. cerevisiae + USA 1291 + 3612+ 20 kg N fed^{-1}	17.0	45.0	34.2	22.0	85.0	69.0
S. cerevisiae + ARC 302 + 3629 + 20 kg N fed^{-1}	35.0	183.0	222.9	29.0	134.0	145.0
L.S.D. 0.05	**11.51**	**8.66**	**13.46**	**4.00**	**9.20**	**35.61**

and Subba Rao (1985), who reported increases in nodulation and other symbiotic parameters of forage legumes (*Trifolium alexandrium* and *Medicago sativa*) due to the combined inoculation of yeast (*Saccharomyces cerevisiae*) and specific *Rhizobium* spp. Additionally, Ghalab (1996) reported that the applied *Bradyrhizobium japonicum* for soybean was improved by the incorporation of *Saccharomyces cerevisiae* into the introduced inoculum.

3.2. Dry matter production and N-content

The results in Table (2) show the response of the two bean varieties to inoculation with different *Rhizobium* strains in the presence or absence of *Saccharomyces cerevisiae* in respect to shoot biomass and N yields. In treatments without *Saccharomyces cerevisiae*, the highest dry weight and N-content of shoots of cv. Giza 6 were obtained for plants supplied by the recommended doses of NPK followed by those inoculated with mixed *Rhizobium* strains (ARC 302 and 3629). Under 20 kg N fed^{-1} in Nebrasca variety, plants inoculated with different *Rhizobium* strains in absence of *Saccharomyces cerevisiae* were the superior. The highest dry weight and N-content of shoots were obtained in plants received the full doses of NPK followed by the plants inoculated with mixed strains of (182 and ARC 301) under 20 kg N fed^{-1}.

In respect to plants treated with *Saccharomyces cerevisiae*, the maximum dry weight and N-content of shoots were obtained when amended with *Saccharomyces cerevisiae* and full doses of NPK fertilizers. Addition of *Saccharomyces cerevisiae* and seed inoculation with different strains of *Rhizobium* resulted in rather promotive effects on both cultivars compared to the inoculation with *Rhizobium* strains alone.

Generally, from the results in Table (2), it is clear that inoculation with *Saccharomyces cerevisiae* combined with application of the recommended doses of NPK gave the best results in terms of dry matter of shoots and N-content in both varieties followed by *Rhizobium* inoculation with (ARC 302 and 3629) and supplied by *Saccharomyces cerevisiae* under 20 kg N fed^{-1} in Giza 6 as well as in

Table (2): Effect of application of *Saccharomyces cerevisiae* on dry weight of bean plants after 60 days from planting.

Bean varieties / Treatments	Giza 6		Nebrasca	
	D.Wt. of shoots (g/plant)	N-content of shoots (mg/plant)	D.Wt. of shoots (g/plant)	N-content of shoots (mg/plant)
Control (uninoculated)	1.9	33.6	2.1	40.5
Recommended doses of NPK	5.0	146.2	5.3	160.4
182 + ARC 301 + 20 kg N fed^{-1}	3.4	83.0	4.1	114.9
USA 1291 + 3612 + 20 kg N fed^{-1}	2.7	59.6	2.9	64.5
ARC 302 + 3629 + 20 kg N fed^{-1}	4.3	115.1	3.8	93.7
S. cerevisiae + Recommended dose of NPK	5.7	172.4	5.8	175.8
S. cerevisiae + 182 + ARC 301 + 20 kg N fed^{-1}	3.7	99.9	5.5	157.5
S. cerevisiae + USA 1291 + 3612+ 20 kg N fed^{-1}	3.1	73.7	3.5	85.6
S. cerevisiae + ARC 302 + 3629 + 20 kg N fed^{-1}	5.1	152.4	4.1	105.9
L.S.D. 0.05	**0.46**	**13.75**	**0.27**	**7.71**

plants inoculated with (182 and ARC 301) and amended with *Saccharomyces cerevisiae* under 20 kg N fed^{-1} in the case of Nebrasca variety. It is necessary to mention that no significant differences were recorded in the plants which received NPK plus *Saccharomyces cerevisiae* and those inoculated with (ARC 302 and 3629) plus *Saccharomyces cerevisiae* in Giza 6 and those inoculated with (182 and ARC 301) plus *Saccharomyces cerevisiae* in Nebrasca variety. These observations mean that the possibility of using a mixture of (ARC 302 and 3629) plus 20 kg N fed^{-1} together with *Saccharomyces cerevisiae* is better than heavy dressing of NPK plus *Saccharomyces cerevisiae*. This indicates the important role of yeast application in enhancing nodulation and growth of bean plants. This could be attributed to its greater content of minerals particularly NPK and certain natural hormones as reported by Subba Rao (1984).

3.3. Straw and seed yields and their N-contents

Data in Table (3) demonstrate the influence of different *Rhizobium* inoculants on straw and seed yields besides their N-contents of both bean cultivars. Similar trend was observed as reported in dry matter and N yield of shoots. For cv. Giza 6, among all the treatments, plants amended with *Saccharomyces cerevisiae* and full doses of NPK gave the highest straw and seed yields as well as N-contents followed by the plants inoculated with (ARC 302 and 3629) in the presence of *Saccharomyces cerevisiae* under 20 kg N fed^{-1}. In Nebrasca variety, the highest straw and grain yields as well as N-content were recorded in the plants received the full doses of NPK and amended with *Saccharomyces cerevisiae* followed by treatments inoculated with (182 and ARC 301) in the presence of *Saccharomyces cerevisiae* and received 20 kg N fed^{-1}.

It is clear from Table (3) that there was non-significant differences between plants received full doses of NPK and treated with *Saccharomyces cerevisiae* and those either inoculated with (ARC 302 and 3629) in the presence of *Saccharomyces cerevisiae* under 20 kg N fed^{-1} in Giza 6 or the plants inoculated with (182 and ARC 301) plus application of *Saccharomyces cerevisiae* under 20 kg N fed^{-1} in Nebrasca variety. Again, it is preferable to use a mixture of *Rhizobium* strains and *Saccharomyces cerevisiae* to obtain good

Table (3): Straw and seed yields of bean plants as affected by *Saccharomyces cerevisiae* and *Rhizobium* inoculation.

Bean varieties / Treatments	Giza 6				Nebrasca			
	Straw yield (g/plant)	N-content (mg/plant)	Seed yield (g/pot)	N-content (mg/pot)	Straw yield (g/plant)	N-content (mg /plant)	Seed yield (g/pot)	N-Content (mg/pot)
Control (uninoculated)	3.6	41.2	6.6	154.1	3.8	45.3	6.8	188.2
Recommended doses of NPK	7.1	127.2	9.2	368.0	7.4	90.1	9.7	389.6
182 + ARC 301 + 20 kg N fed^{-1}	5.2	82.8	8.6	283.8	6.4	110.5	10.9	420.3
USA 1291 + 3612 + 20 kg N fed^{-1}	4.8	63.7	7.4	233.6	4.8	63.3	8.9	282.9
ARC 302 + 3629 + 20 kg N fed^{-1}	6.3	111.6	10.8	387.5	5.4	84.9	9.1	299.4
S. cerevisiae + Recommended dose of NPK	8.1	156.3	11.8	485.5	8.3	160.7	11.6	479.8
S. cerevisiae + 182 + ARC 301 + 20 kg N fed^{-1}	6.0	107.5	10.1	360.9	8.0	159.5	12.3	488.3
S. cerevisiae + USA 1291 + 3612+ 20 kg N fed^{-1}	5.7	87.6	8.8	325.2	5.8	88.7	9.5	314.4
S. cerevisiae + ARC 302 + 3629 + 20 kg N fed^{-1}	7.9	155.4	11.9	471.9	6.6	111.9	10.5	357.4
L.S.D. 0.05	**0.32**	**7.06**	**0.87**	**51.53**	**0.56**	**39.72**	**0.74**	**32.79**

nodulation, growth, straw and seed yields instead of heavy fertilization with NPK combined with *Saccharomyces cerevisiae*.

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تحسين النمو والتعقيد والمحصول لنباتات الفاصوليا عن طريق تلقيح التربة بالخميرة " *Saccharomyces cerevisiae* "

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ملخص

أجريت تجربة لدراسة إمكانية زيادة فاعلية ثلاثة أنواع مختلفة من لقاحات الريزوبيا وذلك باضافة الخميرة لصنفين من بذور الفاصوليا.

وقد أوضحت النتائج أن عدد العقد الجذرية والوزن الجاف لها ونشاط أنزيم النيتروجينيز للمجموع الجذرى للنبات والوزن الجاف للمجموع الخضرى وكذلك محتوى النتروجين قد زاد عند إضافة الخميرة الى التربة. وقد زاد أيضا محصول القش والحبوب ومحتواهما النتروجينى عند استخدام الخميرة وذلك لكل من الصنفين (جيزة ٦ و نيبراسكا). كانت أعلى النتائج فى كلا الصنفين للمعاملات التى سمدت بالجرعات الموصى بها من السماد النتروجينى والفوسفورى والبوتاسى مع إضافة الخميرة ويأتى بعد ذلك النباتات التى لقحت بالـ *R. leguminosarum* (ARC 302 and 3629) فى وجود الخميرة بجانب التسميد بـ ٢٠ كجم ن للفدان وذلك فى صنف جيزة ٦ والنباتات التى لقحت بالـ (182 and ARC 301) *R. leguminosarum* فى وجود الخميرة بجانب التسميد بـ ٢٠ كجم ن للفدان بالنسبة لصنف نيبراسكا ولم يوجد فرق معنوى واضح فى النتائج السابق ذكرها.

وبناء على ذلك ، فأن نتائج البحث الحالى تشير الى أن تلقيح بذور الفاصوليا للصنفين بلقاح الريزوبيا المختلط مع إضافة الخميرة يكون أفضل من استخدام الجرعات الكاملة الموصى بها من السماد النتروجينى والفوسفاتى والبوتاسى مع إضافة الخميرة.

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