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FABA BEAN YIELD RESPONSE TO RHIZOBIAL INOCULATION PLANT - GROWTH PROMOTING RHIZO - BACTERIA AND YEAST

Emara, H.A.; A. A. Abo El-Soud; Nabila M. Radwan and S. A. El-Sayed*
Depart. of Microbiol.; Soils, Water and Environ. Res. Inst., Agric. Res. Center; Giza, Egypt.

ABSTRACT

A pot experiment under greenhouse conditions was conducted in clayey loam and sandy soils to evaluate the effect of *Pseudomonas fleuroscens* (PS) or *Saccharomyces cerevisiae* (Ye) on the efficiency of *R. leguminosarum* bv. *viceae* (Rh) to nodule and fix nitrogen in faba bean plants. Significant increase of rhizobial nodules number and nodules dry weight was recorded with mixed cultures of Rh, Ps and Ye in clayey loam soil in comparison with rhizobial inoculated plants. While, non-significant increase was recorded in sandy soil. High significant increase of nitrogenase activity was obtained with mixed cultures of Rh, Ps and Ye in either clayey loam or sandy soils (115.6 or 119.5 μmole g^{-1} dry nodule hr^{-1}). Consequently, high nitrogen content of faba bean plants was determined (416.6 and 392.2 mg nitrogen/plant).

INTRODUCTION

On a global scale, N_2-fixing legumes are the major source of nitrogen to the biosphere although estimates of the actual amounts vary considerable (Evana and Barber, 1977). In developing countries, legumes are often an integral part of pasture, forest and agricultural ecosystems. To break the hold of poverty, keep apace with population growth and improve the standard of living, farmers in developing countries must increase food and fiber production, without a concomitantly significant increase in inputs.

Biological processes capable of improving agricultural productivity, while minimizing soil loss and ameliorating adverse edaphic conditions, are essential. An important consideration for optimizing N_2-fixation in the legume-rhizobial symbiosis is the response of the microsymbiant and the nodule to a dynamic soil environment (Harold *et al*., 1992).

Plant- growth promoting yeasts enhancing the growth parameters and yield of many kinds of fruits and vegetables were reported (Subba Rao, 1984; Nijjar, 1985; Ferguson *et al*., 1987; Idso *et al*., 1995; Ahmed *et al*., 1997 and Abdel-Ghany *et al*., 2001). The various effects of yeasts soil amendment due to their content of amino acids, vitamin B- complex as well as their ability to secrete growth substances (Larson *et al*., 1962). Makary and El-Sayed (2000) found an increase of growth parameter and yield of faba bean inoculated with *Saccharomyces cerevisiae* in saline soil. The efficiency of rhizobacteria to improve *Rhizobium* nodulation on leguminous plants was also reported (Li and Alexander, 1990; Sturz *et al*., 1997; Srinivasan *et al*., 1997; Andrade *et al*., 1998 and Moenne *et al*., 1998).

The aim of the current study was to investigate the effect of plant-

growth promoting bacteria or yeast on the efficiency of *Rhizobium leguminosarum* bv. *viceae* to nodule and fix nitrogen on faba bean plants.

MATERIALS AND METHODS

An experiment was conducted in pots (30- cm) using two types of soils (Table, 1) under greenhouse conditions, to study the interaction between rhizobial inoculation and plant-growth promoting rhizobacteria or yeast. Before planting, rhizobial treated seeds of faba bean cv. Blanka were inoculated with peat-based inoculant of *Rhizobium leguminosarum* bv. *viceae* strain ARC 201F (Biofertilization Production Unit; Soils, Water and Environment Research Institute; ARC; Egypt), using seeds coating technique. *Pseudomonas fleuroscens* – 191 or *Saccharomyces cerevisiae* – 109 (Provided from Agric. Res. Center) were used as plant- growth promoting microorganisms. They were added after seed germination as soil amendments at a rate of 15 ml culture (10^9 cells/ml)/pot. Soil amendments of plant-growth promoting microorganisms were repeated three times (15, 30 and 45 days of planting).

The pots were divided into two groups. Each group contained eight treatments. The first group was filled with clayey loam soil and the other one was filled with sandy soil.

The following treatments were conducted:

1- Inoculated with rhizobia (Rh) + 20 Kg N/fed (ammonium sulphate).
2- Inoculated with *Psedo.* (Ps) + 20 Kg N/fed (ammonium sulphate).
3- Inoculated with Yeast (YE) + 20 Kg N/fed (ammonium sulphate).
4- Inoculated with (Rh and Ps) + 20 Kg N/fed.
5- Inoculated with (Rh and Ye) + 20 Kg N/fed.
6- Inoculated with (Rh, Ps and Ye) + 20 Kg N/fed.
7- control (without inoculation) + 20 Kg N/fed.
8- Full dose of nitrogen (40 Kg N/fed.)

All treatments were received the recommended dose of phosphorus (200 kg/fed.) in the form of superphosphate (15.5% P_2O_5) and potassium sulphate (48%, KO_2) at the rate of 100 kg/fed. Other agricultural practices were performed as recommended. The pots were arranged in rhandomized complete block design with six replicates for two samples (Fifty and eighty days of planting).

Plants of faba bean were uprooted and assayed for number and dry weight of nodules as well as dry weight of shoots. Nitrogen content of plant shoot using micro-kjeldhal method described by Walker and Black (Piper, 1950) was determined. Acetylene reduction of nitrogenase activity for plants was estimated by using Dani 1000 Gas Chromatograph according to Hardy et al. (1973). Data were subjected to statistical analysis according to Steel and Torrie (1980) using the statistical analysis system computer.

Table (1): Mechanical and chemical properties of soils used in greenhouse experiment.

Properties	Soil-I	Soil-II
Sand %	25.77	90.72
Silt %	35.90	2.15
Clay %	36.23	7.13
$CaCO_3$ %	1.24	0.64
Textural class	Clayey loam	Sandy
PH	7.80	7.39
E.C. (dS m^{-1})	1.10	0.28
O.C. %	0.56	0.18
T.N.%	0.136	0.028
Soluble cations (meq l^{-1})		
Ca^{++}	4.22	0.44
Mg^{++}	2.88	0.25
Na^{+}	3.00	1.70
K^{+}	1.19	0.45
Soluble anions (meq l^{-1})		
CO_3^{--}	0.00	0.00
HCO_3^{-}	3.28	0.77
Cl^{-}	4.33	0.50
SO_4^{--}	3.68	1.58

RESULTS AND DISCUSSION

From data presented in Table (2), it could be concluded that *Saccharomyces cerevisiae* caused significant increase in root nodules number as well as dry weight of nodules of rhizobial inoculated faba bean plants in comparison with that inoculated with *Rhizobium leguminosarum* bv. *viceae* alone. Thus, root nodules number and nodules dry weight of rhizobial inoculated plants grown in clay loam soil were increased from, 58.7 nodule/plant and 430 mg/plant to 75.7 nodule/plant and 532 mg/plant, respectively. While, plants grown in sandy soil were increased from, 37.0 nodule/plant and 366 mg/plant to 42.0 nodule/plant and 540 mg/plant after 50 days of planting. The corresponding figures after 80 days of planting were from 65.7 nodule/plant and 515 mg/plant to 81.0 nodule/plant and 556 mg/plant, respectively, for inoculated faba bean grown in clay loam soil. With respect to efficiency of rhizobia to nodule faba bean plants in sandy soil after 80- days, data showed that number and dry weight of nodules were increased from 41.0 nodules/plant and 375 mg/plant to 49 nodules /plant and 552 mg/plant, respectively.

Combined mixture of both *Saccharomyces cerevisiae* and *Pseudomonas fleuroscens* with *Rhizobium leguminosarum* bv. *viceae* was also caused significant increase of both nodules number and dry weight.

Table (2): Influence of *Saccharomyces cerevisiae* (Ye) or/and *Pseudomonas fleuroscens* (Ps) combined with *Rhizobium leguminosarum* bv *viceae (Rh)* on the efficiency of rhizobial (Rh) nodulation and nitrogenase activity of faba bean, grown in sandy or clay loam soils under greenhouse conditions.

* Treatments	No. of nodules/plant		D.W. of nodules mg/plant		Nitrogenase activity (μ mole ethylene g^{-1} dry nodule hr^{-1})	
	50- day	80- day	50- day	80- day	50- day	80- day
	Clayey loam soil					
Rh	58.7	65.7	430	515	90.6	61.5
Ps	32.0	34.3	318	370	13.7	12.0
Ye	31.7	36.0	402	414	63.4	30.5
Rh + Ps	63.7	67.3	510	535	93.6	43.0
Rh + Ye	75.7	81.0	532	556	109.6	82.8
Rh + Ps + Ye	80.3	84.7	591	643	115.6	115.0
** Control	20.0	21.7	205	249	20.3	16.3
*** Full dose of nitrogen	19.3	22.7	180	200	13.7	12.3
L. S. D. 0.05	7.7	5.6	72	89	11.7	8.8
	Sandy soil					
Rh	37.0	41.0	366	375	60.6	45.9
Rs	16.0	17.3	159	163	18.4	17.2
Ye	17.7	19.3	184	212	38.7	36.0
Rh + Ps	39.7	46.0	459	502	64.6	46.5
Rh + Ye	42.0	49.0	540	552	92.8	70.0
Rh + Ps + Ye	45.3	52.7	551	562	119.5	103.0
** Control	7.3	0.7	75	78	15.9	14.4
*** Full dose of nitrogen	6.3	9.0	61	67	0.0	0.0
L. S. D. 0.05	7.74	5.25	49	63	12.5	9.7

*** All treatments were received half dose of nitrogen (20 kg /fed.),**
**** Uninoculated and received half dose of nitrogen (20 kg /fed.),**
***** Uninoculated and received full dose of nitrogen (40 kg /fed.).**

The obtained results also revealed that nodules number and dry weight of rhizobial inoculated faba bean in clay loam soil were highly affected. Thus, nodules number were 80.3 and 84.7 nodules/plant after 50 and 80 days of planting, respectively. The corresponding figures for nodules dry weight were 591and 643 mg/plant, respectively. While, nodules number formed on faba bean plants grown in sandy soil were 45.3 and 52.7 nodules/plant after 50 and 80 days of planting. High nodules number in clay loam soil were detected, and this may be attributed to high native rhizobia in clay loam soil (Abo El-Soud, 1992).

The effect of *Sacch. cerevisiae* on root nodulation of rhizobial inoculated plant could be attributed to its capability to produce ethylene in rhizosphere region, which enhances plant root distribution, consequently, increasing the root surface area exhibited to rhizobia invasion (Dasilva *et al*., 1974; Arshed and Frankenberger, 1989).

With respect to nitrogenase activity, *Sacch. cerevisiae* induced highly

significant increase in enzyme activity. Therefore, nitrogenase activity of rhizobial nodulated plants was increased from 90.6 μmole ethylene g^{-1} dry nodule hr^{-1} to 109.6 μmole ethylene g^{-1} dry nodule hr^{-1} after 50 days of planting in clay loamy soils. The same highly nitrogenase activity was recorded with combined mixture of both *Sacch. cerevisiae* and *Ps. fleuroscens* with *Rhizobium leguminosarum* bv. *viceae*. The same behavior was observed with plants grown in sandy soil. The activation of *Saccharomyces cerevisiae* as a soil amendment may be due to their ability to secrete amino acids and cytokinins as well as Vitamin B-complex, which influence on both rhizobia growth and faba bean plant growth (Larson *et al*., 1962; Makary and El-Sayed 2000).

Table (3): Influence of *Saccharomyces cerevisiae* (Ye) or/and *Pseudomonas fleuroscens* (Ps) combined with R*hizobium leguminosarum* bv. *viceae* (Rh) on the growth parameter and N-content of rhizobial inoculated faba bean grown in clay loam or sandy soils under greenhouse conditions.

* Treatments	D.W. of shoots (g/plant)		N-content of shoots (mg/plant)	
	50- day	80- day	50- day	80- day
	Clayey loam soil			
Rh	8.6	9.5	326.8	396.0
Ps	8.4	8.6	313.6	321.1
Ye	8.0	9.2	352.6	392.1
Rh + Ps	8.4	9.2	310.0	344.9
Rh + Ye	8.5	9.2	329.3	366.7
Rh + Ps + Ye	8.8	9.9	378.9	415.6
**Control	6.0	7.5	180.9	252.3
***Full dose of nitrogen	8.9	9.0	339.7	347.8
L. S. D. 0.05	0.9	0.6	30.9	21.7
	Sandy soil			
Rh	7.4	8.9	263.5	317.4
Ps	6.4	7.9	208.6	267.5
Ye	7.0	8.0	276.6	298.4
Rh + Ps	7.3	8.1	276.5	291.0
Rh + Ye	7.6	8.1	280.4	296.4
Rh + Ps + Ye	8.0	8.6	339.4	392.2
**Control	4.9	6.0	135.5	175.0
***Full dose of nitrogen	7.2	8.6	266.0	307.4
L. S. D. 0.05	0.4	0.7	24.2	27.5

*** All treatments were received half dose of nitrogen (20 kg /fed.),**
**** Uninoculated and received half dose of nitrogen (20 kg /fed.),**
***** Uninoculated and received full dose of nitrogen (40 kg /fed.).**

The high recorded nitrogenase activity of either *Sacch. cerevisiae* or the combined mixture of *Ps. fleuroscens* and *Sacch. cerevisiae* with *Rhizobium* was reflected on N-content of shoots where, it increased from 180.9 and 252.3 to 329.3 and 366.7 mg/plant with yeast and rhizobial

inoculated plants grown in clay loam soil after 50 and 80 days of planting, respectively (Table 3). The corresponding figures with sandy soil were from 135.5 and 175.0 to 280.4 and 296.4 mg/plant at the same order. The same behaviors were also recorded with faba bean plants inoculated with combined mixture of yeast, *Pseudomonas* and rhizobia in both clay loam and sandy soils.

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استجابة محصول الفول البلدى للتلقيح بالريزوبيا والخميرة والريزوبكتيريا المنشطة لنمو النبات

حمدى على عمارة و علاء الدين عبد الحميد أبوالسعود ونبيلة محمد رضوان وسمير على السيد
معهد بحوث الأراضى والمياه والبيئة ـ مركز البحوث الزراعية ـ الجيزة ـ مصر.

تم إجراء تجربة أصص تحت ظروف الصوبة البحثية بإستخدام نوعين من التربة هما : التربة الرملية والتربة الطمبية –وذلك لدراسة تأثير السيدوموناس والخميرة علي كفاءة الرايزوبيا من حيث تكوين العقد الجذرية وتثبيت النيتروجين في نبات الفول البلدي مع التسميد بنصف الجرعة المقررة من السماد نيتروجينى (كبريتات الأمونيوم). وتم تقدير عدد العقد البكتيرية ووزنها الجاف والوزن الجاف للمجموع الخضرى والمحتوى النيتروجينى له وكذلك نشاط أنزيم النيتروجينيز. وقد أوضحت النتائج المتحصل عليها أن التلقيح بخليط من بكتيريا *R. leguminosarum* مع خميرة *Sacch. cerevisiae* أدى إلى زيادة معنوية فى العقد الجذرية وذلك فى الأرض الطمبية بالمقارنة بالريزوبيا فقط. بينما الزيادة كانت غير معنوية فى الأرض الرملية ، وبالنسبة لأنزيم النيتروجينيز أوضحت النتائج زيادة معنوية جدا بالنسبة للتلقيح بخليط الريزوبيا والخميرة والسيدوموناس فى كلا من الأرض الطمبية والرملية (١١٥,٦ ، ١١٩,٥ ميكرومول ايثيلين لكل جرام عقدة فى الساعة. وهذا أدى إلى زيادة فى المحتوى النيتروجينى لنبتات الفول البلدى (٤١٦,٦ ، ٣٩٢,٢ ملليجرام نيتروجين لكل نبات).