"السيانوبكتريا- الريزوبيا- البقوليات" نسيج بيئى فريد فى الأراضى الرملية

رسالة مقدمه من

خديجة طلعت عبد العظيم
بكالوريوس فى العلوم الزراعية (تكنولوجيا حيوية) كلية الزراعة – جامعة القاهرة ،٢٠٠٣

للحصول على

درجة الماجستير

فى

العلوم الزراعية
(ميكروبيولوجيا زراعية)

قسم الميكروبيولوجيا الزراعية
كلية الزراعة
جامعة القاهرة
مصر

٢٠١٠

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"CYANOBACTERIA- RHIZOBIA- LEGUMES" A UNIQUE ENVIRONMENTAL WEAVE IN SANDY SOILS

By

KHADEGA TALAAT ABD EL-AZEEM

B.Sc.Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2003

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APPROVAL SHEET

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ABSTRACT

Twelve isolates of *Rhizobium* sp. specific to peanut were obtained from root nodules of peanut and six isolates of *R. Leguminosarum* were obtained from nodules of faba bean. All isolates were maintained on yeast extract mannitol (YEM) agar medium then purified and identified for the use in biofertilization treatments. Similarly, 35 cultures of filamentous heterocystous cyanobacteria blonging to genera *Nostoc, Anabeana* and *Calothrix* from soil samples in which the legume hosts were cultivated. Only 3 cultures representing the previous genera were selected and introduced into the applied treatments.

Two pot experiments were conducted in the green house of ARC, Giza to evaluate the response of two cultivars from peanut, (Georgia and Giza 5) and faba bean, (Giza 40 and Masr 1) to rhizobial inoculation and cyanobacterial application. The results indicated that biomass of both cultivars for both legumes significantly increased due to rhizobial inoculation with strains kh8, kh11 (for peanut), Fb4 and Fb6 for faba bean. In addition, soil treatment with cyanobacterial culture filtrates of *Nostoc, Anabeana* and *Calothrix* did enhance growth and nodulation of both lrgumes. For example, peanut cv. Georgia exhibited higher dry weights of both roots (1.93 mg plant^{-1}) and shoots (6.43mg plant^{-1}), as well as better nodulation parameters (241 nodules plant^{-1} of 0.51mg nodules dry weight). Also, data revealed that N-content of peanut for both tested cultivars increased as a result of mixed rhizobial inoculation with kh8 and kh11 and cyanobacterial treatments. In this respect, 146.1 and 148.2 mg plant^{-1} were obtained for cv. Giza 5, respectively.

Regarding the response of faba bean cultivars to the applied treatments , it was found that shoot nitrogen content, root dry weight and nodules dry weight for faba bean plants cv. Giza 40 reached 171.9 mg plant^{-1}, 31.8 mg plant^{-1} 2.9 mg nodule^{-1}. while, the respective parameters were 178.6 , 29.9 and 2.4 for cv. Masr1, when cyanobacteria was involved with rhizobial cultures for the biofertilization treatment, respectively.

On the other hand, when cyanobacterial filtrates were applied to some treatments, both peanut and faba bean plants exhibited better growth and nodulation criteria. It is, therefore, suggested that in addition to rhizobial inoculation, soil treatment with cyanobacterial filtrates as a source of phytohormones led to significant enhancement of both growth and nodulation of peanut and faba bean.
Keywords: Rhizobia, cyanobacteria, biofertilization, peanut, vicia faba.

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المستخلص العربى

تناولت هذه الدراسة عزل ١٢ سلالة من *Rhizobium* sp المتخصصة للقول السوداني من العقد الجذرية لنباتات الفول السوداني، وكذلك ٦ سلالات من *R. leguminosarum* من العقد الجذرية لنباتات الفول البلدي، وقد تم تنميتها وعزلها وحفظها على بيئة آجار المانيتول ثم تم تنقيتها وتعريفها لإستخدامها في معاملات التسميد الحيوي. وبالمثل فقد تم الحصول على ٣٥ مزرعة من السيانوبكتريا التابعة لأجناس، *Anabaena, Nostoc, Calothrix* من عينات التربة المزروعة بكل من الفول السوداني والفول البلدي. وقد تم اختيار ٣ سلالات ممثلة للأجناس السابقة لاستخدامها في معاملات تجارب الأصص. هذا وقد تم تقييم استجابة كل من الفول السودانى صنفى (جورجيا، جيزه٥) والفول البلدى صنفى (مصر ١، جيزة ٤٠) فى تجربتى أصص للتلقيح بالريزوبيا مع المعاملة براشح السيانوبكتريا كمصدر لبعض الهرمونات المنظمة للنمو. وقد أظهرت النتائج أن الوزن الجاف لكل من جذور وسيقان نباتات الفول السودانى قد زاد معنويا نتيجة التلقيح بسلالتى الريزوبيا Kh8, Kh11 . كذلك أوضحت النتائج أن المعاملة براشح بعض سلالات السيانوبكتريا قد أدى إلى تشجيع نمو وتعقيد نباتات الفول السودانى حيث أظهرت النباتات أعلى القيم فى الوزن الجاف الجذور (١.٩٣ملجم/نبات) والسيقان (٦.٤٣ملجم/نبات) وكونت أكبر عدد من العقد الجذرية (٢٤١عقده / نبات).

من جهة أخرى ، أثبتت النتائج أن المحتوى النتروجينى لنباتات الفول السودانى للصنفين المختبرين زاد نتيجة التلقيح بالسلالين kh11 , kh8 والمعاملة براشح السيانوبكتريا حيث وصلت هذه القيم إلى ٩٩ ، ١٤٦ ملجم/ نبات فى صنفى جورجيا وجيزة ٥ على الترتيب. بالنسبة لمدى استجابة أصناف الفول البلدى المختبره للمعاملات المختلفة فقد ثبت أن المحتوى النتروجينى للسيقان ، الوزن الجاف للجذور، الوزن الجاف للعقد للفول البلدى صنف مصر ١ وصل إلى١٧٨.٦ ملجم/ نبات، و٢٩.٩٣ ملجم/ النبات، ٢.٤ملجم/ للعقد على الترتيب فى حين أن صنف جيزة ٤٠ وصل إلى ١٧١.٩ملجم/ النبات و٣١.٨ملجم/ النبات و ٢.٩ملجم/ للعقد على الترتيب .

على الوجه الأخر ، أثبتت النتائج أنه عند استخدام راشح سلالات السيانوبكتريا المختلفة (*Anabaena, Nostoc, Calothrix*)– كمصدر للهرمونات النباتية– فى بعض المعاملات فقد أدت إلى تحسين مظاهر كل من النمو والتعقيد فى الفول السودانى والفول البلدى فى جميع الأصناف المختبره.

وبناء عليه فقد إنتهت الدراسة إلى أن التلقيح بالريزوبيا بجانب معاملة التربة براشح السيانوبكتريا يمكن أن يؤدى إلى تحسين كل من نمو وتعقيد وجوده نباتات الفول السودانى والفول البلدى.

الكلمات الدالة: الريزوبيا– السيانوبكتريا– التسميد الحيوى– الفول السودانى – الفول البلدى.

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DEDICATION

*I would like to dedicate this thesis to my Mother, Father, Mother in-law "**Salwa**", Father in-law. Special dedication goes to my dear husband "**MOHAMED**" my son "**Omar**". They all gave me so much love and support that made this achievement easy and possible.*

Kh. T.

CONTENTS

INTRODUCTION

Concerning the gradual increases in climatic changes risks, the better use of the natural resource base, less use of chemicals and efficient use of irrigation water have become increasingly important goals of sustainable agriculture in Egypt. It is established that biological nitrogen fixation is a world wide economical and sustainable nitrogen supply to legume crops. It may reduce the expenses with chemical nitrogen fertilizers, as well as eliminating the negative impact of them on the environment.

Faba bean is an annual legume known botanically as *Vicia faba* L. It does not possess any components toxic to animal or human and it is possible to feed the bean to all types of livestock or poultry provided it is cracked or crushed. The faba bean is about 25% protein, and is higher in energy than soya bean (Oplinger *et al*., 2010). The same authors added that the large broad bean seeds are often preferred , the seed coats are often removed by hand before eating. Skinned beans are cooked, salted and used for sandwich filling in North Africa. In Egypt and other Mid-Eastern countries, faba bean is eaten as a staple food by many strata of the society, and the increasing population of Middle-Eastern people in the U.S. may be a potential market for fababeans.

On the other hand, the peanut, grown primarily for human consumption, has several uses as whole seeds or is processed to make peanut butter, oil, and other products. The

seed contains 25 to 32% protein and 42 to 52% oil. A pound of peanuts is high in food energy and provides approximately the same energy value as 2 pounds of beef, 1.5 pounds of cheddar cheese, or 36 medium-size eggs (Putnam *et al*., 2010). Also, peanut has been long used as a dietary component particularly for its high oil content. Its uses in industry have been focused particularly in oil extraction. It thrives well in the newly reclaimed soil specially calcareous and sandy soils, hence its cultivation can be extended as much as needed and as followed by other circumstances without interfering in the crop rotation of old cultivated land. Since as sandy soils are relatively lacking fertility, appropriate biofertilization program should be applied (El- Boraie *et al*., 2009).

Therefore, the present study was designed, in pot experiments, to study the influence of inoculation with *Rhizobium leguminosarum* and *Rhizobium* sp. for both faba bean and peanut , respectively, to evaluate their effect on plant growth and nodulation. Also, soil treatment with some diazotrophic cyanobacterial filtrates belonging to the genera *Nostoc*, *Anabeana* and *Calothrix* , as a source of some required phytohormones , *e.g.* indole acetic acid , gibberellic acid and abscicic acid, were also evaluated as an approach to enhance both growth and nodulation of the tested legumes.

REVIEW OF LITRATURE

1. Ecophysiology of rhizobia in their ecosystems

Peanut *(Arachis hypogaea* L.) and faba bean (*Vicia faba*) are considered of the most important legumes for oil and protein production, respectively, which cultivated and thrive in the newly reclaimed sandy soil and valley soils in Egypt. Rashid and Ryan (2004) stated that Mediterranean type soils generally have free $CaCo_3$, high pH and low organic matter and consequently, nutrients disorders in these soils are the most important limiting factor to crop production, second only to moisture stress. Major problems are deficiencies of nitrogen and phosphorus; however, recent researchs have revealed that micro nutrients problems are also hampering crop production (Ahmed and El-Abagy, 2007; El- Habbasha *et al.,* 2007; El- Gizawy and Mehasan, 2009).

The introduction of new rhizobia inocula into soil containing native *Rhizobium* populations frequently results in only a small proportion of nodules containing the introduced strain. This is due to a competition with ineffective indigenous strains and other rhizospheric bacteria (Mcloughlin *et al.,* 1985). The nodulation process involves signal exchange between the host and the bacterium plant growth and nodulation by rhizobia are promoted by certain rhizobacteria (Bai *et al.,* 2002 and Rao and pal, 2003).

In Taiwan, research work on the selection of efficient rhizobial strains for inoculation started in 1958. Collection, isolation and

subsequent selection of effective rhizobial strains and its uses in agriculture have yielded fruitful results. Since marked variations were observed among rhizobial strains (Young and Chao, 1983), selected a number of pure rhizobial strains from lupin, alfalfa, peanut, and soybean, and conducted a wide range of fied experiments to select the most effective inoculants. Yield was significantly increased when lupin, alfalfa, peanut, and soybean were inoculated with selected rhizobial strains compared to those non-inoculated plants.

On the other hand, few field experiments were conducted to determine the effects of single and mixed inoculations with *Rhizobium* and Arbuscular-Mycorrhiza AM (Young *et al.,* 1988). They reported that inoculation with rhizobial strains alone increased N_2-fixation and soybean yield. While, inoculation with rhizobial strain singly, or in combination with AM, without N fertilizer applications, significantly increased soybean yield. Results from the other experimental sites also showed that a mixed inoculum of *Rhizobium* and AM can be an efficient biological fertilizer that lead to considerable increases in soybean yields. Therefore, it was suggested that the combined effect of the mixed inoculum was a striking finding in the field of soybean biofertilization and that AM might have provided the essential P for the growth of soybean plants.

As agricultural production intensified over the past few decades, producers became more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However, increasing use of chemical inputs causes