RESPONSE AND COMPATIBILITY OF SOME FABA BEAN (*Vicia faba L.*) CULTIVARS TO INOCULATION WITH SALINITY-TOLERANT RHIZOBIAL STRAIN UNDER HIGH SALINITY CONDITIONS

BY

El-Gremi*, Sh. M. A., E. B. A. Belal* and M. F. El-Nady**

Agric. Microbiology* and Agric. Botany ** Branches, Dept. of Agric. Botany, Fac. Agric., Kafr El-Sheikh, Tanta Univ.

ABSTRACT

Pots experiments were carried out at Dept. of Agric Botany, Fac. of Agric. Kafr El-Sheihk, Tanta Univ., Egypt during the winter season of 2004/2005 to study the response and compatibility of four faba bean cultivars to nodulation with the the *Rhizobium leguminosarum* biovar. *viciae* rhizobial strain (E1) previously isolated, identified and adapted against the high concentration of NaCl. In this study, seed treatment with Peat-based preparation of (E_1) at the time of planting in sandy soil irrigated with 200 mM NaCl concentration exhibited significant differences in the plant growth and nodulation parameters as well as crude protein % of dry seeds between the cultivars under study. The response of cultivars Giza Blanca and Giza 957 to rhizobial nodulation (464.67 and 394.33 nodules/plant, respectively) was more compatible with strain (E1) than Sakha 1 and Reina Mora (50.33 and 39.33/plant, respectively). Success in nodulation resulted in significant improvements in both the plant growth and the dry seed crude protein % parameters as well as the total microbial counts in the rhizosphere of the growing plants.

The absence of nodules on the non-inoculated plants revealed the necessity of using the effective and salinity-tolerant rhizobial strain (E1) to inoculate faba been under saline conditions.

Keywords: *Vicia faba*, Cultivars, *Rhizobium leguminosarum* biovar. *viciae*, Nitrogen fixation, Salinity.

INTRODUCTION

Faba bean (*Vicia faba* L. Family: Leguminosae, Subfamily: Lotoidae) is one of the most important legume crops in Egypt. It's an important crop in the Mediterranean area, offering high-quality protein and increasing the input of combined N_2 into the soil as used in crop rotation with cereal crops (FAO, 1999 and Farag *et al.*, 2005). The Rhizobium-legume symbiosis is suggested to be economic and environmental-safe solution of nitrogen defect in reclaimed arid soil (Zahran, 1999). This symbiotic association between legumes and

rhizobia is the most biocatalytic link for the flow of nitrogen between the largest potential available N-reservoir, the atmosphere type of soil and the living world (Paau, 1989).

In order to hardness potential benefit of the rhizobial inoculation in commercial agricultural, the consistency of their performance must be improved. This requires research in many diverse areas as these biological systems involve complex interactions among the inocula, the host and the soil conditions.

Symbiotic N_2-fixation is adversely affected as a result of presence of soil or irrigation water salinity (Ibrahim *et al.*, 1970; Salem *et al.*, 1981; Abdallah *et al.*, 1986 and El-Nady & Belal, 2005) or using different genotypes from the same host (Velagaleti & Marsh 1989, Craig *et al.*, 1991, Cordovilla,*et al.* 1995 and Ghobrial *et al.*, 1995).

In previous work (El-Nady & Belal, 2005) the inoculation with the rhizobium strain E1 of faba bean (*Rhizobium leguminosarum* biovar *viciae*) adapted against salinity increased the N_2-fixiation and the growth parameters as well as the crude protein of faba bean var. Kassasin 1 under salted sandy soil compared with the original strain. Therefore, the present investigation was designed to evaluate the response of different faba bean cultivars to inoculation with strain (E1) under salinity conditions.

MATERIALS AND METHODS

This work was carried out at Dept. of Agric. Botany, Fac. of Agric. Kafr El-Sheihk, Tanta Univ, Egypt. Rhizobium strain E1 of faba bean (*Rhizobium leguminosarum* biovar *viciae)* was previously isolated from active nodules initiated on healthy faba bean plants and adapted against the high concentration 200 mM of NaCl (El-Nady & Belal, 2005). It was applied at the time of planting as seed treatment. Peat-based Rhizobium inoculum was prepared as described by Vincent, (1970). The faba bean seeds of Giza Blanca, Giza 957, Sakha 1 and Reina Mora cultivars were inoculated according to Belal, *et al.*, (1996) before sowing in 30 cm inner diameter pots containing sandy soil on the 23rd October 2004. Pots sowed with non-inoculated seeds served as control treatments. Pots were irrigated with water supplemented with 200 mM NaCl, each treatment was represented by 10 replicates. NaCl concentration was tested in the soil two weeks intervals in order to be adjusted. The cultural practices, fertilization and pest control were carried out as commonly used. The Climatic features occurred during the growth season 2004/2005, are shown in Table (1).

Ninety days after planting, plant height, leaf area, number of flowers and pods and fresh and dry weight of roots and shoots were estimated The blade of the 4th leaf was used to determine the photosynthetic pigments (chlorophyll a, b and total) according to Inskeep & Blom, (1985). Number of root nodules as well as fresh and dry weight of nodules were also estimated at 60 days from planting. At the end of the growing season, the crude protein % was assayed in the dry seeds by the Kjeldahl method (AOAC, 1990) as total –N, and the factor 6.25 was used to convert the total-N to its crude-protein equivalent. At two times from planting (30 and 60 days), the total microbial counts were determined in rhizosphere samples using dilution series of poured standard-plate count agar medium consisted of tryptone 5g, yeast extract 2.5g, glucose 1g, agar 15g/L dist. water (pH 7±1) according to DSMZ-catalogue media (2004) with replacing dextrose by glucose.

Table (1): Averages of monthly records of the climatic features in the local Meteorological station, Sakha Station covering the growth season 2004/2005.

Month	Mean of air temperature °C		Mean of relative humidity %		Solar Rad. Mega Joule/m^2
	Max	Min	7:30	13:30	
October	29.3	15.8	76.4	47.4	12
November	25.7	10.2	84	52	12.8
December	20.2	7.8	85.4	54.8	7
January	19.5	6.2	85	55	9.8
February	21.5	7	92.9	56	13.8
March	22.2	7.4	83.7	46.5	15
April	26.3	10.7	82.4	46.2	22.2

Statistical analysis:

The experiment was designed as complete randomized design and the obtained data were subjected to the proper statistical procedures for analysis of variance according to Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

In previous work (El-Nady & Belal, 2005), the rhizobium strain (E1) of faba bean was isolated from active nodules initiated on healthy faba bean plants and identified as *Rhizobium leguminosarum* biovar

viciae. The strain (E1) was adapted against the high concentration of salinity under sterilized and non sterilized conditions. Inoculation with strain (E1) enhanced the N_2-fixiation and the plant growth parameters as well as the crude protein of the faba bean Kassasin 1 cultivar planted in salted sandy soil compared with the original strain. This strain was selected for further applications to verify the response of other recommend faba bean cultivars for their higher yield components (Farag *et al.*, 2005) towards inoculation by this strain under salinity stress.

Data in Table (2) show highest values of growth parameters in the inoculated plants with *R. leguminosarum* bv. *viciae* compared with the non-inoculated plants. Inoculation with E1 significantly increased length of plant in Giza Blanca, Giza 957 and Sakha 1 cultivars, while an insignificant increase of plant length was recorded in Reina Mora cultivar. Giza 957 and Giza Blanca recorded significant increases in plant length, leaf area and number of flowers/plant by inoculated plants with E1. In the respect of number of pods/plant, Giza Blanca, inoculated with E1 gave a significant increase (3.99 pods/plant), while an insignificant increases were recorded in the other tested faba bean cultivars (2.99, 2.11 and 2.07 for Giza 957, Sakha 1 and Reina Mora, respectively). Fresh and dry weights of root and shoot were increased in all tested faba bean cultivars inoculated with E1. The highest values of fresh and dry weight of root were found in Giza Blanca cultivar (22.06 and 9.63g, respectively). The highest values of shoot fresh weight were recorded in Giza 957 (25.15g) and Giza Blanca (23.23g) and the highest value of dry weight of shoot was obtained in Giza Blanca (7.67g). These results are in agreement with our previous study, it was also observed that inoculation of faba bean cutivar (Kassasin 1) with this strain increased significantly the growth parameters under the same concentration from NaCl (El-Nady and Belal, 2005).

Concerning to photosynthetic pigments, concentrations of chlorophyll a, b and the total chlorophyll were significantly increased in the inoculated plants. Data presented in Table (3) revealed that the highest value of total chlorophyll was found in the inoculated plants of Giza Blanca cultivar (15.2). Logically, it was due to the increase in N_2-fixation since nitrogen is an essential element for synthesis of photosynthetic pigments. Chlorophyll pigments are derived from tetrapyrroles. Tetrapyrroles are large rings each contains four pyrole rings which consists of four carbon and one nitrogen atoms, each (Moore, *et. al.*, 1998).

able (2): Growth parameters of the four tested faba bean cultivars inoculated with *Rhizobium leguminosarum* biovar *viciae* (strain E1) 90 days after sowing in salinity conditions.

Treatments	Plant length (cm)	Leaf area (cm^2)	No. of flowers/plant	No. of pods/ plant	Root weight/plant (g)		Shoot weight /plant (g)	
					Fresh	Dry	Fresh	Dry
Giza Blanca								
Non-inoculate	76.29 e	25.85 c	3.23 e	3.27 b	17.78 b	7.03 c	20.84 c	6.16 b
Inoculate	85.47 b	27.23 b	6.31 b	3.99 a	22.06 a	9.63 a	23.23 b	7.67 a
Giza 957								
Non-inoculate	82.13 c	21.97 d	5.35 c	2.96 c	11.31 d	4.05 f	18.77 d	4.22 d
Inoculate	96.36 a	28.72 a	8.13 a	2.99 c	21.00 a	6.15 d	25.15 a	5.74 c
Sakha 1								
Non-inoculate	76.28 e	13.10 f	3.36 e	2.13 d	6.92 e	4.93 e	14.69 f	5.80 c
Inoculate	80.37 d	17.39 e	4.17 d	2.11 d	11.14 d	6.95 c	15.59 e	5.80 c
Reina Mora								
Non-inoculate	76.29 e	13.53 f	3.31 e	2.09 d	14.82 c	8.54 b	16.04 e	5.87 c
Inoculate	76.34 e	17.18 e	4.12 d	2.07 d	16.16 b	9.42 a	19.40 d	5.94bc

Values having the same alphabetical letter within column significantly are not different at $P < 0.05$.

Table (3): Chlorophyll concentration of the non-inoculated and inoculated plants of four tested faba bean cultivars.

Treatments	Chlorophyll pigments of the leaf (mg/l)		
	a	b	Total
Giza Blanca			
Non-inoculate	5.81 f	1.83 ab	7.79 f
Inoculate	12.46 a	2.15 ab	15.20 a
Giza 957			
Non-inoculate	5.67 f	1.41 b	7.40 f
Inoculate	7.65 c	2.91 ab	10.28 c
Sakha 1			
Non-inoculate	6.35 e	2.91 a	9.01 e
Inoculate	8.47 b	2.42 ab	11.10 b
Reina Mora			
Non-inoculate	6.83 d	1.93 ab	9.69 d
Inoculate	7.20 d	1.91 ab	10.01 cd

Values having the same alphabetical letter within column significantly are not different at $P < 0.05$.

Under salinity conditions, the compatibility between the used strain (E1) and the inoculated faba bean cultivars could be verified through nodulation. Data presented in Table (4) show variation in nodulation between the different tested cultivars reflecting the response of cultivars to inoculation by the strain (E1) under salt stress. Inoculated plants of Giza Blanca and Giza 957 gave the highest results in term of the number of active nodules/plant reached 464 and 394, respectively. On the other hand, less nodulations were recorded on the other two cultivars (Sakha 1 and Reina Mora) where nodules appeared small in size (Fig. 1), and majority of them were inactive. When they were examined for the red color of leghaemoglobin, the absence of leghaemoglobin indicated negative results for the presence of the nitrogenase enzyme. Under this saline concentration, the latter two cultivars (Sakha 1 and Reina Mora) showed a degree of salt-sensitivity as the root-hairs of both cultivars were impaired. Many reports

confirmed that Rhizobium-host compatibility, presence of good developed root-hairs and suitable conditions for infection are essential for active nodulation. Many reports confirmed that, tolerance of the legume host to salt is the most important factor in determining the success of compatible *Rhizobium* strains to form successful symbiosis under conditions of high soil salinity (Zahran & Sprent 1986, Velagaleti & Marsh 1989, Craig *et al.*, 1991 and Cordovilla, *et al.*, 1995).

Table (4): Symbiotic N2-fixation parameters of the non-inoculated and inoculated plants of four tested faba bean cultivars.

Treatments	Nitrogen fixation parameters		
	No. of nodule/plant	Root nodule weight/plant (g)	
		Fresh	Dry
Giza Blanca			
Non-inoculate	0.0c	0.0 e	0.0 e
Inoculate	464.67 a	6.34 b	1.54 b
Giza 957			
Non-inoculate	0.0 c	0.0 e	0.00e
Inoculate	394.33 a	8.12 a	2.38 a
Sakha 1			
Non-inoculate	0.0 c	0.0 e	0.0 e
Inoculate	50.33 b	1.42 d	0.29d
Reina Mora			
Non-inoculate	0.0 c	0.0 e	0.0 e
Inoculate	39.33 b	0.98 c	0.42 c

Values having the same alphabetical latter within column are not significantly different ($P < 0.05$).

Although seed protein contents are yield components specific for each *Vicia faba* genotype (Farag et al, 2005), significant increase in Giza Blanca seed crude protein % (up to 27.41%) was achieved by inoculation with E1 strain (Fig.2).

Fig. (1): Root systems of non-inoculated (left) and inoculated (right) faba bean cultivars with salinity tolerant isolate of *R. leguminosarum* bv. *viciae* 60 days after seeds sowing. A- Giza Blanca. B- Giza 957. C- Sakha 1. D- Reina Mora

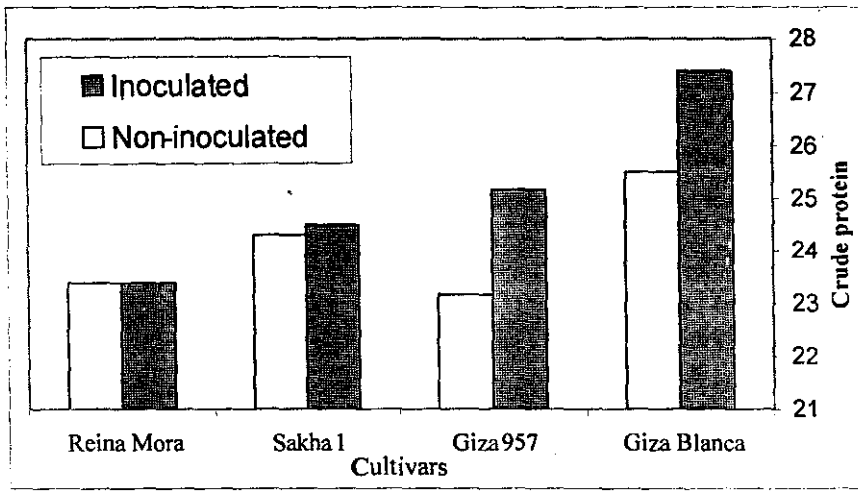

Fig. (2): Crude protein % in seeds of four faba bean as affected by inoculation with the salinity tolerant *R. leguminosarum* bv. *viciae* strain (E1).

The biotic inhabitants of soil displays its healthy conditions for cultivating. So, the total microbial counts was determined in the soil during growth season. In the rhizosphere soil of plants treated with the *Rhizobium leguminosarum* bv. *viciae* strain E1, the total microbial flora increased as the age of plant increased (Fig. 3). Significant increase was recorded in case of the most nodulation-compatible cultivars Giza Blanca and Giza 957 ($15x10^5$ and $14x10^5$ cfu/g, respectively). Similar results were obtained by Badr El-Din & Sahab (1986) and El-Nady&Belal (2005). *R. leguminosarum* bv. *viciae* can fix the atmospheric free nitrogen and make it easier for the broad bean plants and other microorganisms in the rhizosphere, in addition, the root-nodule bacteria produce growth promoting substances (Sekine *et al.*, 1989) which may encourage microbial population in the rhizosphere.

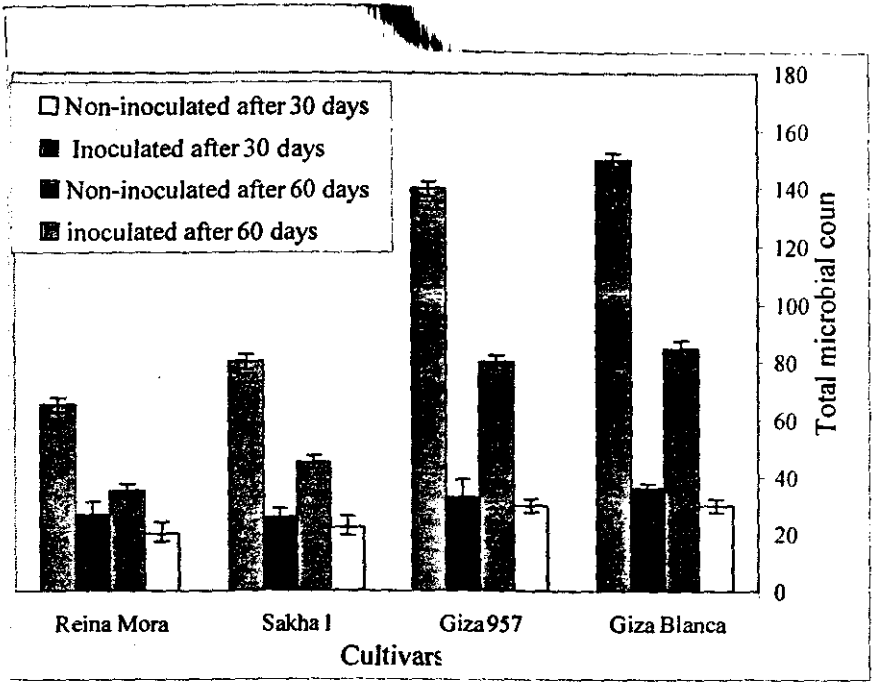

Fig. (3): Total microbial flora in the rhizosphere of faba bean cultivars as affected by inoculation with the salinity tolerant *R. leguminosarum* bv. *viciae* strain (E1) at different growth times (counts x 10^4 cfu/g dry soil).

The obtained results indicate the benifits of using such promising strains like E1 having salt-tolerance character for inoculation of the compatible faba bean host cultivare Giza Blanca and Giza 957 under salt stress conditions dominant at the new reclaimed land in Egypt to improve growth and yield components.

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الملخص العربي

استجابة وتوافق بعض أصناف الفول البلدي للتلقيح بعزلة متحملة للملوحة من بكتيريا العقد الجذرية تحت ظروف الملوحة العالية

شكرى محمد على الجريمى* السيد بلال عبدالمنطلب بلال* محمد فتحي النادي **

فرعي الميكروبيولوجيا زراعية* و النبات زراعي**
قسم النبات الزراعي – كلية الزراعة بكفر الشيخ – جامعة طنطا

أجريت تجربة أصص بقسم النبات الزراعي – كلية الزراعة بكفر الشيخ – جامعة طنطا في الموسم الشتوي ٢٠٠٤/٢٠٠٥ لدراسة مدي استجابة وتوافق أربع أصناف من الفول البلدي لتكوين العقد الجذرية بواسطة سلالة من *Rhizobium leguminosarum* bv. *Viciae* (E1) والتي سبق عزلها وتعريفها ثم تكييفها لتتحمل التركيزات العالية من كلوريد الصوديوم:

أظهرت معاملة البذور بمستحضر البكتيريا E1 المحملة على البيتموس وزراعتها في تربة رملية تم ريها بتركيز ٢٠٠ ملليمول من كلوريد الصديوم إلى ظهور اختلافات معنوية في المقاييس الخاصة بكل من النمو النباتي والتعقيد وكذلك في النسبة المئوية لمحتوى البروتين الخام في البذور الجافة بين الأصناف تحت الدراسة.

كانت استجابة صنفي جيزة بلانكا وجيزة ٩٥٧ للتعقيد (٤٦٤,٦٧ و ٣٩٤,٣٣ عقدة/نبات ، على الترتيب) أكبر من صنفي سخا ١ والرايناموراا (٥٠,٣٣ و ٣٩,٣٣ عقدة /نبات، على الترتيب) مما يدل على ان الصنفين الأولين اكثر توافقا مع السلالة E1. وقد أدى نجاح التعقيد إلى تحسن معنوي في مقاييس كل من نمو النبات والنسبة المئوية لمحتوى البذور الجافة من البروتين الخام وكذلك في العدد الكلي للميكروبات في رايزوسفير النباتات النامية.

أظهر غياب العقد في النباتات الغير ملقحة ضرورة التوصية باستعمال سلالة الريزوبيوم E1 الفعّالة والمتحملة للملوحة في تلقيح الفول تحت الظروف الملحية.