17 *Egypt. J. Microbiol*. 37, No. 3, pp. 247- 262 (2002)

Assessment of Symbiotic Performance of Several Bean Cultivars Inoculated With Their Specific Rhizobia

H. Moawad and Wafaa M. Abd El-Rahim
Agricultural Microbiology Department, National Research Center, Cairo, Egypt .

A GREEN house experiment was carried out to study the symbiotic performance of six rhizobial strains with five bean cultivars. These studies were done in order to evaluate the response of these cultivars to rhizobial inoculation and to assess competition between the indigenous and inoculated strains. In these studies soils of two different textures were used: a silty loam collected from Ismailia and aclay soil from Giza region. The dual inoculation generally produced higher values of plant dry weight and nodule formation, and the duel cultivar gave the highest values of plant dry weight in silty loam soil. This cultivar gave the best nodulation and dry weight in clay soil in the co-inoculation treatment using isolate Ph7 and strain CE3. Royallmel cultivar was the best in nodulation by dual inoculation with Ph7 and strain CE3. The availability of nitrogen was critical for nodulation success. The capability of strains to nodulate bean plants differed with cultivar tested. The strain CE3 occupied more than 50% of nodules in silty loam soil while it was less competitive in clay soil. All tested cultivars were nodulated partially by native rhizobia.

Keywords : Bean, Rhizobia, Symbiosis, Egyptian soils.

French bean is one of the most important legume crops of the family *leguminaceae* in Egypt, as well in Latin America and major parts Africa. This crop, as are other legume plants are nodulated by nitrogen fixing organisms belong to Family *Rhizobiaceae* (Jordon and Vincent, 1984). Today the rhizobia include the genera *Azorizobium, Bradyrhizobium, Mesorhizobium, Allorhizobium, Rhizobium,* and *Sinorhizobium.* Members of these genera belong to alpha sub-class of the proteobacteria (Pablo *et al.,* 1998). Improvement of common bean production in the Mediterranean area requires selection of effective rhizobial strains and bean cultivars well adapted to the conditions of the semi-arid climate prevailing in this region (Ilham *et al.,* 2001). The common bean (*Phaseolus vulgaris* L.) is often subjected to various environmental constraints in the soil) Kaouthar *et al.,* 2001). The characterization of *Phaseolus* symbionts isolated from Mediterranean soils and analysis of genetic factors related to pH tolerance have been studied (Priefer *et al.,* 2001). Sonia *et el.* (2002) evaluated the biochemical events in root nodules which increase yield, when bean is inoculated with a *Rhizobium etli* mutant (CFN037). CFN037-inoculated plants had 22% more nitrogen than did wild-type (CE3)-inoculated plants. Manhart and Wong (1979) studied the nitrate reductase activity of some species of rhizobia, and found that *Rhizobium trifolii, R. phaseoli,* and *R. leguminosarum* did not express nitrate reductase activities (NAR) in the root nodules, but NAR was express when grown in the presence of nitrate. In bacteroids of *R. trifolii, R. leguminosarum,* and *R. phaseoli,* high N_2- fixation activities were not accompanied by high NAR activities .

Maximizing symbiotic N_2 fixation requires a greater understanding of the ecology of plant-rhizobial systems. One of the major limitations in the study of this symbiosis is the difficulty in recognizing strains of rhizobia in their natural habitats. Several different techniques have been suggested for rhizobial strains identification. Among them, intrinsic antibiotic resistance (IAR), (Moawad and Bahlool, 1992). Khmel'nitskii and Zlotnikov (1982) studied the effect of several antibiotics on rhizobia of four-fast-growing (*R. phaseoli, R. leguminosarum, R. trifolii* and *R. meliloti*) and three slow growing rhizobia, (*R. vigna, R. japonicum* and *R. lupini*). It was found that *R. meliloti, R. japonicum, R. vigna and R. lupini* had multiple drug resistance.

The aim of this work was to study the performance of three rhizobial isolates and three standard strains with specific bean cultivars as well as the strain-cultivar interactions using six-bean rhizobial strains and five commercially used bean cultivars.

Material and Methods

Pot experiments were carried out to study the symbiotic performance of six rhizobial strains with five bean cultivars. Six rhizobial strains were tested in this experiment. Three local isolates (Ph7, Ph9, Ph14) from Ismailia Governorate-

Egypt were isolated from the nodules of field grown beans as described by Somasegaran and Hoben . (1985) Foreign strains of bean rhizobia were obtained from International Institute of Genetics and Biophysics (IIGB), Naples, Italy. Wild-type strain CE3 and two of its antibiotic resistant mutants are showed in Table 1.

TABLE 1 . Genotype and antibiotic profile of reference strains.

Strain	Genotype	Antibiotic Profile
CE3	Wild type	Nal
2012	Tn5 mutant	Nal/Km
TR(101)	Wild type containing vector	Nal/Tet

Local obtained strains were evaluated for their intrinsic antibiotic resistance (IAR) pattern against 6 different antibiotics (Table 2). Stock solutions of most of the antibiotics were prepared as 10mg/ml in sterile distilled water. Naladixic acid was dissolved in 1M NaOH, while rifampicin and erythromycin were dissolved in 95% ethanol. Appropriate volumes of the stock solutions were added to sterilized yeast mannitol agar (YMA) medium (Somasegaran and Hoben, 1985) at 50-60oC. Single strains were inoculated using a sterile 21 pin metal replicator on to the surface of petri dishes containing different antibiotics. Petri dishes were incubated for 48 hrs at 28°C, and the growth was scored by visual inspection as compared with positive controls on media without antibiotics (Moawad and Bohlool, 1992). The antibiotic concentration showing the maximal differences among isolates was chosen for further analysis.

TABLE 2. Concentrations of antibiotics used for testing IAR pattern.

Antibiotics	Abbreviation used	Concentrations (µg /ml)
Ampicillin	Amp.	40
Nalidixic	Nal.	50
Neomycin	Neo.	10
Rifampicin	Rif.	30
Spectinomycin	Spe.	20
Tetracycline	Tet.	5

Bean Cultivars

Five *Phaseolus vulgaris* cultivars were used in this study. *P. vulgaris* Bronco, Royallmel, Paultista, Samamtha and Duel, all obtained from certified germoplasm at the Institute of Vegetable Research Center, ARC, Ministry of Agriculture, Egypt.

The experiment was done in plastic pots 25cm in diameter. These pots were filled with 7 Kg of silty loam soil collected from the Ismailia region and a clay soil collected from the Giza region. These two regions are the main sites for bean cultivation in Egypt. The characteristics of used soils are showed in Table 3.

TABLE 3. Physico - chemical analysis of used soils .

Analyses/Location	Ismailia	Giza
pH	7.9	8.13
Ec (dSm^{-1})	0.15	0.27
Organic Carbon (%)	0.18	0.19
Organic matter (%)	0.31	0.32
Total nitrogen (%)	0.01	0.13
Available Phosphorus (ppm)	6.2	10.8
Calcium (meq 100 g^{-1})	2.0	4.3
Magnesium (meq 100 g^{-1})	2.3	13.3
Texture	silty loam	clay

Seed germination

Seeds of bean cultivars were surface sterilized by soaking in 75% ethanol for 10 seconds, followed by soaking in 3% hydrogen peroxide for 3-4 minute. Seeds were washed several times with sterile water and germinated in sterile petri dishes containing wet sterile filter paper. After seed germination, non-contaminated small seedlings were transplanted to the soils in pots. Bean seedlings were thinned to two plants in each pot two weeks later.

Foreign strains and local isolates of bean rhizobia were grown in yeast extract mannitol (YEM) broth on rotary shaker for 3-4 days at 28°C to a population density of about 10^8 cells/ ml. Two ml of broth cultures of each tested rhizobial strains were mixed with 250 ml of water and added to each pot before cultivation. Two treatments without any inoculation, one without fertilization; and the other fertilized with nitrogen by the rate of 30 kg/acre were included for comparison. Plants were gently uprooted and roots were washed with tap water. 60 d after planting the location of nodules on roots were determined and nodules were weighed after drying at 70°C to constant weight.

Enumeration of rhizobia in used soils

Total rhizobial numbers in soils were determined using the serial dilution (MPN) technique.

Assessment of competition

Nodules were collected from bean plants to estimate the most competitive strains for nodule occupation. Nodules were washed several times with soap to remove adhering particles of soils, soaked in 70% ethanol for 2-3 min, and washed several times with sterile water. The washed nodules were picked into one drop of sterile water and crushed to release constituents. Crushate of nodules were streaked on YEM agar plates amended with Congo red dye. Isolates were tested on YEM agar amended with antibiotics using a replica platting technique to select strains resistant to antibiotics to differentiate between native isolates and foreign strains.

In these studies several treatments were evaluated encompassing several combinations of rhizobial mixtures. The treatments were as follows :

1 .Uninoculated plants.
2 .N-fertilized plants (30 Kg N/acre).
3 .Inoculation with strains Ph7 plus CE3.
4 .Inoculation with strains Ph9 plus 2012.
5 .Inoculation with strains Ph14 plus TR101.

Sixty days after planting, the following measurements were done: nodule dry weight (mg/plant) as indication to inoculation response, plant dry weight (g/plant) as indication of plant growth response to inoculation and competition for nodulation between strains CE3 and Ph7, based on nodule occupancy by CE3 strain assessed by antibiotic marker.

The results of the experiment were statistically analyzed using analysis of variance and Duncans Multiple range test Mstatc (Michigan State university).

Results

Intrinsic antibiotic resistance (IAR) profiles were used to differentiate bean rhizobia. The local isolates number Ph7, Ph9 and Ph14 had the same antibiotic resistance pattern with ampicillin, neomycin, rifampicin and tetracycline at 40, 10, 30 and 5 μg/ml, respectively (Table 4). However, nalidixic at 10 μg/ml inhibited growth of all three local isolates. Opposite to the pervious results, with concentration 20 μg/ml of spectinomycin; isolates number Ph7 and Ph9 gave good growth, but isolate Ph 14 did not grow (Table 4).

Table 5 shows the nodulation and growth response of Bronco bean cultivar to inoculation with six rhizobial strains in the two soils collected from the major bean growing areas in Egypt.

TABLE 4 . Rhizobial strains and their differentiating markers .

Strains No.	Source	Intrinsic Antibiotic Resistance (IAR mg/l)					
		Amp (40)	Nal. (50)	Neo. (10)	Rif (30)	Spe. (20)	Tet. (5)
Ph 7	Ismailia	-	-	-	-	+	-
Ph 9	Ismailia	-	-	-	-	+	-
Ph 14	Giza	+	-	+	+	-	+

TABLE 5. Response of *P. vulgaris* cv Bronco to inoculation in two soils with six *Rhizobium* strains.

Inoculation Treatments	Silty loam soil		Clay soil	
	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)
Uninoculated	2.28 c	88.2 c	2.19 c	28.9 b*
N-fertilized	2.46 b	37.0 d	2.54 a	0.0 d
Ph9+ 2012	2.47 b	172.4 a	2.39 b	16.6 c
Ph7+CE3	2.12 d	151.5 b	1.95 d	39.2 a
Ph14+TR101	2.60 a	153.0 b	1.40 d	0.0 d

* Values have the same letter are not significantly different.

The results show that Bronco cultivar performed better in the silty loam soil than in the clay soil. The nodulation by native rhizobia was significantly suppressed by N-fertilization in both soils. Nodulation significantly increased in clay soil when inoculated with a mixture of Ph7 and CE3. However, the nodulation was much lower than in the silty loam soil where the response to inoculation was significant in all three inocula mixture treatments. The treatment of Ph9+2012 gave the highest nodule formation. The growth performance was also better in the silty loam soil than in clay soil. The N-fertilization treatment was better than the uninoculated control in both soils. The comparatively high plant biomass in uninoculated soils is due to the presence of indigenous rhizobia in the soil; as the counts of rhizobia in both soils showed that in the silty loam and clay soils contained $6X10^4$ and $8X10^3$ cells of rhizobia/g soil, respectively.

Table 6 shows positive plant dry matter accumulation with the Paultista cultivar in all inoculation mixtures in silty loam soil, as compared with uninoculated plants. The nodulation response, however, was not significantly positive with the mixture of Ph7+CE3. This cultivar did not show positive response to inoculation in the clay soil, and plant dry weight either was similar or lower as compared with the control.

Table 7, shows that bean cultivar Royallmel responded significantly to inoculation with the strain mixtures Ph9+2012 and Ph7+CE3 in both soils. The dual inoculation with Ph14 plus TR101 induced significant increase in plant dry weigh in the silty loam soil, while there not was an increase in plant biomass in the clay soil as compared with the uninoculated control. None of the inoculant mixtures enhanced plant growth over the N-fertilized treatment. This may be due to the rapid effect of N-fertilization on plant biomass accumulation during the growth period of the plants (60d). The nodulation response was significantly positive in clay soil, where as, the nodule dry weight was greater in silty loam soil than in clay soil.

Table 8, shows that highest plant biomass yield was recorded in the silty loam soil inoculated with Ph9+2012. The nodule dry weight was also much higher in the silty loam soil than in clay soil in all inocula mixture treatments.

TABLE 6 . Response of *P. vulgaris* cv Paultista to inoculation in two soils with six *Rhizobium* strains.

Inoculation Treatments	Silty loam soil		Clay soil	
	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)
Uninoculated	1.98 e	209.2 c	2.46 b	73.5 b
N-fertilized	2.84 a	135.4 e	2.63 a	30.0 d
Ph9+ 2012	2.44 b	241.0 b	2.46 b	74.9 b
Ph7+CE3	2.19 d	191.8 d	2.29 d	43.7 c
Ph14+TR101	2.24 c	247.3 a	2.41 c	83.9 a

* Values have the same letter are not significantly different.

TABLE 7. Response of *P. vulgaris* cv Royallmel to inoculation in two soils with six *Rhizobium* strains.

Inoculation Treatments	Silty loam soil		Clay soil	
	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)
Uninoculated	2.13 c	219.4 b	2.59 d	51.9 d
N-fertilized	2.79 a	130.2 e	3.42 e	16.5 e
Ph9+ 2012	2.33 b	218.8 c	2.88 c	68.4 c
Ph7+CE3	2.30 b	248.9 a	2.63 a	89.3 b
Ph14+TR101	2.30 b	170.4 d	2.30 d	151.5 a

* Values have the same letter are not significantly different.

TABLE 8. Response of *P. vulgaris* cv Duel to inoculation in two soils with six *Rhizobium* strains.

Inoculation Treatments	Silty loam soil		Clay soil	
	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)
Uninoculated	2.59 c	254.7 a	2.73 ab	112.6 b
N-fertilized	2.53 d	112.7 e	2.76 a	0.0 e
Ph9+ 2012	3.06 a	235.6 c	2.46 c	24.4 d
Ph7+CE3	2.66 b	143.5 d	2.63 b	193.9 a
Ph14+TR101	2.58 c	248.5 b	2.00 e	77.2 c

* Values have the same letter are not significantly different.

Contrary to the previous four cultivars, Samamtha bean performed better in clay soil than in silty loam (Table 9). The growth response to inoculation was significant in silty loam soil with all inocula mixtures. The best nodulation was recorded in Ph14 +TR101 mixture in both soils. The nodulation in clay soil was significantly increased with three inocula mixtures.

The competition between inoculant strain and native rhizobia was done only on treatment having strain CE3 in combination with the local isolate Ph7. We decided to restrict this competition study to that treatment as other treatments have the same CE3 strain slightly modified for certain antibiotic resistant marker. This particular treatment was used as a model to see whether the inoculant strain CE3 (wild type bean rhizobia from Italy) can compete for nodulation either with other inoculant strain (Ph7) or the native bean rhizobia strains.

The competition between R. etli strain CE3 and Ph7 isolate, as well as, the other native rhizobia for nodulation of five commercial bean cultivars is presented in Table 10 . The data show that in silty loam soil, strain CE3 occupied between 50-80% of nodules formed on the five cultivars. The Paultista cultivar showed the highest compatible macro symbiotic for CE3 strain. In the clay soil, strain CE3 was less competitive for nodulation as compared with the other inoculant strains in mixture or and native rhizobia stains in soil.

TABLE 9 . Response of *P. vulgaris* cv Samamtha to inoculation in two soils with six *Rhizobium* strains.

Inoculation Treatments	Silty loam soil		Clay soil	
	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)	Plant dry weight (g/plant)	Nodule dry weight (mg/plant)
Uninoculated	1.59 d	187.2 c	2.61 b	28.7 d
N-fertilized	1.99 b	0.0 e	2.74 a	NA
Ph9+ 2012	2.38 a	198.8 b	2.58 b	69.2 b
Ph7+CE3	2.38 a	134.3 d	2.38 c	63.5 c
Ph14+TR101	1.84 c	205.4 a	2.79 a	79.4 a

* Values have the same letter are not significantly different.

TABLE 10. Competition for nodulation of five bean cultivars in two Egyptian soils (percentage of nodule occupancy by strain CE3).

Soil type	Bean cultivars				
	Bronco	Paultista	Samamtha	Royallmel	Duel
Silty loam	60	80	70	66	50
Clay soil	36	51	46	43	53

Discussion

The IAR patterns of common bean rhizobial with six antibiotics were different. The isolates Ph7, Ph9 and Ph14 had the same pattern with four antibiotics. The pattern of antibiotic resistance of each strain was found to be a stable property by which rhizobia could be recognized (Josey *et al*., 1979) Khmel'nitskii and Zlotnikov (1982), who studied the effect of several antibiotics on four -fast -growing rhizobia and three slow growing rhizobia. It was shown that four of these species had multiple drug resistance. Several different techniques had been suggested for rhizobial strains identification. Among them, (IAR), was used in this paper to identify *Leucaena* rhizobia (Moawad and Bohlool, 1992).

Many studies have examined the inoculation efficiency of beans in different soil conditions (Caixian *et al.*, 2001; Moez *et al.*, 2001; Mariam *et al.*, 2001; Vincent and Jean-Jacques, 2001; Sonia *et al.*, 2002). Also Aguilar *et al.*, (2001) found that the inoculation of some efficient bean genotypes with rhizobia increased the yield in the field in two successive bean-cropping seasons.

In our work the performance of six rhizobial strains with five bean cultivars in two types of soils was studied .In our study soil experiment with the clay soil showed a reduction in nodule formation with Bronco cultivar with most treatments. The best treatment was Ph9+2012 which produced the highest values of plant dry weight. The Duel cultivar in treatment Ph7+CE3 recorded the highest values for nodule formation (193.9 mg/plant) in the clay soil. This was also reported by Sonia *et al.* (2002) who evaluated the biochemical parameters in root nodules which lead to increase in yield. They found that the inoculation with *Rhizobium etli,* mutant (CFN-037) increased nitrogen by 22% as compared to inoculation with the wild-type strain CE3.

In silty loam soil the best cultivar was Duel, which gave the highest value of plant dry weight with the rhizobial mixture Ph9+2012. In this soil the cultivar Royallmel recorded the highest value of nodule dry weight but was not efficient in N_2-fixation. These results are in accordance with the finding of Andrade *et al.,* (2002) who studied some cultural and biochemical characteristics of *Phaseolus* nodulating isolates. These isolates recorded significant increase in yield particularly with liming of the soil.

Our results indicated that owing to the uninoculated plants contained a number of nodules on their roots, presence of indigenous rhizobia. These results agreed with Aguilar *et al.* (1998) who characterized a collection of rhizobial isolates from nodules of wild beans (*Phaseolus vulgaris* var. *aborigineus*) growing in virgin soils of different geographically separate sites in the northwest Argentina on the basis of host range and their growth .All tested cultivars were nodulated by native isolates. These results are also in agreement with the findings of Trabelsi and Sifi (2000) who showed that symbiotic nitrogen fixation (SNF) of the common bean is limited by the low numbers and/or the absence of the indigenous rhizobia, and also in agreement with M'hamedi *et al.* (1997) who isolated of genetically characterized 152 native common bean rhizobia strains which variable of rhizobial strains for selecting highly effective strains.

The availability of nitrogen in soil is one main factor that influences the nodule formation with majority of cultivars in this study. The application of mineral nitrogen inhibited nodulation which has reported by many different, Rosas and Bliss (1986), Srivastava and Ormorod (1987), Buttery and Patak (1989), Buttery *et al.* (1990) and Umali (1991). The capability of strains to nodulate bean plants differed according to the cultivar tested.

In this study nodulation competitiveness was estimated with the use of antibiotic marker strains. The most competitive strain was CE3 with the majority of studied cultivars. This is an agreement with Tas *et al.,* (1996) and Onishchuk *et al.* (2001). Aouani *et al.* (1997) reported that from 14 native rhizobia strains isolated from Tunisia, at least one rhizobia was found to be more efficient than CIAT 899. Also those studied the dynamics of *Rhizobium* competition for nodulation of many legume crops (Broughton *et al.,* 1980; Triplett and Sadowsky, 1992; Moawad *et al.*, 1994; Tas *et al.*, 1996; Monibe *et al.*, 1998). In our study the percentage of nodule occupying ranged between 50 and 80 % with five cultivar in the silty loam soil, but in clay soil was only between 36 to 53 % with Bronco and Duel cultivars, respectively. Lopez-Garcia *et al.* (2002) found that nodule occupancy with a similarly established rhizobial population recovered from the vermiculite was significantly less competitive.

It could be concluded that for improving the common bean production in these two soils under this study is based on the selection of effective rhizobial strains and bean cultivars to be adapted with the conditions of cultivated areas.

Acknowledgment : This study is part of PhIMED project was supported by INCO/EU funds (grant/IC18 CT98 0313). The authors are grateful to Dr. U. Priefer project team leader and all the staff member of the project.

References

Aguilar, O.M., Lopez, M.V. and Riccillo, P.M. (2001) The diversity of rhizobia nodulating beans in Northwest Argentina as a source of more efficient inoculant strains. *Biotechnology*. 4; 91(2-3), 181.

Aguilar, O.M., Lopez, M.V., Riccillo, P. M., Gonzalez, R .A., Pagano, M., Grasso, D.H., Puhler, A. and Favelukes, G. (1998) Prevalence of the *Rhizobium etli* like allele in genes coding for 16S rRNA among the indigenous rhizobial populations found associated with wild beans from the Southern Andes in Argentina. Appl. *Environ .Microbiol*., **64** (9), 3520 .

Andrade, D .S., Murphy, P. J. and Giller, K .E. (2002) The Diversity of *Phaseolus* nodulating rhizobial populations is altered by liming of acid soils planted with *Phaseolus vulgaris* (L) in Brazil. *Appl .Environ .Microbiol*., **68** (8), 4025

Aouan, M.L., Jebara, M. and Ghrir, R. (1997) Competitiveness between local isolates and reference strains of common bean rhizobia and interaction with cultivars and NaCl stress. In *FYSAME Annual Report* No. I, INRA-France.

Broughton, W.J., Van-Egeraat, A. W. and Lie, T.A. (1980) Dynamics of *Rhizobium* competition for nodulation of *Pisum sativum* cv. Afghanistan. *Can. J. Microbiol*., **26** (4), 562.

Buttery, B.R. and Parak, S.J. (1989) Identification and characterizations of common bean *Phaseolus vulgaris* (L) lines well nodulated in the presence of high nitrate. *Plant and Soil*, **119**, 237.

Buttery, B.R., Parak, S. J. and Ahanvantari , B.N. (1990) Effect of combined nitrogen *Rhizobium* strain and substrate on a supernodulating mutant of *Phaseolus vulgaris* (L). *Can. J. Plant Sci*., **70**, (4), 955

Caixian, T., Philippe, H., Benoit, J., Zed, R. and Jean-Jacques, D. (2001) Effect of phosphorus deficiency on the growth, symbiotic N_2 fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes *Agronomie.* **21**, 683.

Ilham, B., Fatiha, B., Abdelkarim, F. M. and Jamal, A. (2001) Selection of osmotolerant and effective strains of *Rhizobiaceae* for inoculation of common bean (*Phaseolus vulgaris*) in Moroccan saline soils. *Agronomie* **21**, 591.

Jordan, D.C. and Vincent, J.M. (1984) Family III. *Rhizobiaceae.* In: R. Krieg and J. G. Holt (Eds.). *Bergey's Manual of systematic Bacteriology,* volume 1. pp. 234-244. The Williams and Wilkins Co. Baltimore, USA.

Josey, D.P., Beynon, J.L., Johnson, A.W.B and Beringer, J.E. (1979) Strain identification in *Rhizobium* using intrinsic antibiotic resistance. *Journal of Applied Bacteriology,* **46**, 343.

Kaouthar, S., Jean-Jacques, D., Mokhtar, H. and Chedly, A. (2001) Genotypic variability for tolerance to salinity of N_2-fixing common bean (*Phaseolus vulgaris*). *Agronomie* **21**, 675.

Khmel'nitskii, M.I .and Zlotnikov, K.M. (1982) Antibiotic resistance of nodular bacteria. *Antibiotiki* . **27** (6) , 418 .

Lopez-Garcia, S .L., Vazquez , T .E., Favelukes, G .and Lodeiro, A .R. (2002) Rhizobial position as a main determinant in the problem of competition for nodulation in soybean. *Environ .Microbiol.*; **4** (4), 216 .

Manhart, J .R. and Wong , P.P. (1979) Nitrate reductase activities of rhizobia and the correlation between nitrate reduction and nitrogen fixation. *Can J Microbiol* . **25** (10), 1169 .

Mariam, K., Lina, P., Mariam, S., Carmen, L. and Antonio, O. (2001) Ammonium assimilation and urea metabolism in common bean (*Phaseolus vulgaris*) nodules under salt stress. Agronomie. **2**, 635.

M'hamdi, R., Mars ,M., Amarger, N. and Laguerre, G. (1997) Diversity of rhizobia nodulation bean un Tunisia. In *FYSAME Annual Report* . No. I INRA-France.

Moawad, H. and Bohlool, B. B. (1992) Characterization of rhizobia from *Leucaena. World Journal of Microbiology and Biotechnology,* **8**, 387.

Moawad, H., Khalafallah, M.A. and Badr El-Din. (1994) Field screening for nitrogen fixing and competitive abilities of *Bradyrhizobium japonicum* strain nodulating soybean In: *Recent Development in Biological Nitrogen Fixation in Africa,* M. Sadiki and A. Hilali (Ed), IAV Hassan II, Rabat, Morocco, pp. 151 -163, 1994.

Moez, Jebara, Jean-Jacques Drevon and Mohamed Elarbi Aouani. (2001) Effects of hydroponic culture system and NaCl on interactions between common bean lines and native rhizobia from Tunisian soils. Agronomie. 21, 601.

Monibe, M., Moawad, H., Fayez, M., Khalafallah, M. and Shames El-Deen ,A. (1998) Competition between the most promising local and standard type strains of bean rhizobia nodulating cv. Giza 6 using Gus gene and FA techniques. In: *Third Arab Conference on Modern Biotechnology and Areas of Application in the Arab World,* 14-17: December, Cairo, Egypt 1998.

Onishchuk, O.P, Kurchak, O.N., Sharypova, L.A., Provorov, N.A. and Simarov, B.V. (2001). Analysis of various types of competition in Tn5-mutants of alfalfa *rhizobium* bacteria (*Sinorhizobium meliloti*) *Genetika.* 37(11), 1507.

Pablo, V., Jan, L.W.R., Frans J. de Bruijn and , Dietrich W. (1998) Genotypic characterization of bradyrhizobium strains nodulating endemic woody legumes of the canary islands by PCR-restriction fragment length polymorphism analysis of genes encoding 16S rRNA (16S rDNA) and 16S-23S rDNA intergenic spacers, repetitive extragenic palindromic PCR genomic fingerprinting, and partial 16S rDNA sequencing *Appl. Environ. Microbiol.,* 64. 2096.

Priefer, U.B., Aurag, J., Boesten, B., Bouhmouch, I., Defez, R., Filali-Maltouf, A., Miklis, M., Moawad, H., Mouhsine, B. Prell, J., Schluter, A. and Senatore, B. (2001) The characterization of *Phaseolus* symbionts isolated from Mediterranean soils and analysis of genetic factors related to pH tolerance. *Journal of Biotechnology.*,91, 223.

Rosas, J.C. and Bliss, F.A. (1986) Host plant associated with estimates of nodulation and nitrogen fixation in common bean. *Hort. Sci*. 21, 287.

Sonia, S., Lourdes, B., Alberto, C., José-Luis, O., Mario, R. and Miguel Lara-Flores. (2002) *Rhizobium etli* Mutant Modulates Carbon and Nitrogen Metabolism in *Phaseolus vulgaris* Nodules. *MPMI*, 15,728.

Somasegaran, P. and Hoben, H. J. (1985) *Methods in legume Rhizobium Technology.* NifTAL, Paia Maui, HI, USA.

Srivastava, H. S. and Ormorod, D. P. (1987) Effects of nitrogen dioxide and nitrate nutrition on nodulation, nitrogenase activity, growth and nitrogen content of bean plants. *Plant Physiol.* **81**,737.

Tas, E., Leinonen, P., Saano, A., Rasanen, L.A., Kaijalainen, S., Piippola, S. and Hakola, S., Lindstrom, K. (1996) Assessment of competitiveness of rhizobia infecting *Galega orientalis* on the basis of plant yield, nodulation and strain identification by antibiotic resistance and PCR. *Appl. Environ. Microbiol.*, **62** (2), 529.

Trabelsi, M. and Sifi , B. (2000) Screening for common bean *Rhizobium* symbioses adapted to adverse ecological conditions in Tunisia. In Proceeding of the Regional Symposium on Agro-Technologies Based on Biological Nitrogen Fixation For Desert Agriculture, Egypt. Edited by M. Fayez and N. A. Hegazi. 337-346.

Triplett, E. W. and Sadowsky M. J. (1992) Genetics of competition for nodulation of legumes. Annu. Rev. *Microbiol.*, **46**, 399.

Umali, M. G. (1991) Early response of some legume trees Rhizobium inoculation and combined nitrogen Coll. Forest. Univ. Philippines.

Vincent, V. and Jean-Jacques Drevon (2001) Genotypic variability in phosphorus use efficiency for symbiotic N_2fixation in common bean (*Phaseolus vulgaris*). *Agronomie.* **21**, 691.

(Received 7/9 2002;
accepted /10/2002)

تقييم الأداء التكافلي لعدد من أصناف الفاصوليا لتلقيح بالريزوبيا المتخصصة لها

حسن معوض، وفاء محمد عبد الرحيم
قسم الميكروبيولوجيا الزراعية، المركز القومي للبحوث ، القاهرة،
مصر.

تم إجراء تجربة أصص لدراسة الأداء التكافلي لستة سلالات من ريزوبيا الفاصوليا مع خمسة من اصناف الفاصوليا بتقييم استجابة هذه الأصناف للتلقيح و كفاءة السلالات المستخدمة في المنافسة الرزوبيا المتوطنة فى. وقد استخدم فى هذه التجربة نوعان مختلفان من الاراضي: تربة سلتيه طمييه تم تجميعها من محافظة الإسماعيلية وتربة طينية من محافظة الجيزة. وعموما نتج من التلقيح المزدوج بالسلالات زيادة فى الوزن جاف للمجموع الخضري و تكوين العقد الجذرية. و من خلال هذه التجربة سجل الصنف Duel أعلى قيم للوزن الجاف للمجموع الخضري فى التربة السلتيه الطمييه. و أعطى هذا الصنف أيضا أفضل النتائج للتعقيد و الوزن الجاف للنمو الخضري تربة الطينية و ذلك بمعاملة التلقيح المزدوجة من مخلوط سلالتي الريزوبيا Ph7 و CE3 .

كما أوضحت النتائج أن الصنف Royallmel كان الأفضل فى تكوين العقد الجذرية وذلك باستخدام التلقيح المزدوج بالسلالتينV CE3 + Ph . كما وجد آن كلا من نوعي التربة المستخدمة قد احتويت على الريزوبيا المتوطنة ولكن كانت الحاجة إلى تلقيح واضحة في التربة الطينية، وذلك راجعاً لزيادة نسبة النتروجين فى هذه التربة حيث شكل عائقا لنجاح عملية التعقيد.

أوضحت الدراسة اختلاف معدلات تكوين العقد الجذرية فيما بين الأصناف المختلفة. ومن خلال دراسة القدرة التنافسية لسلالة CE3 في اللقاح أتضح أن هذه السلالة كانت قادرة على تكوين أكثر من٥٠ ٪ من العقد المكونة على جذور النباتات في التربة السلتيه الطمييه وكانت أقل منافسة في التربة الطينية.