4 *Egypt. J. Microbiol.* **41**, pp. 49 - 59 (2006)

Ecological Studies on Soil Indigenous Rhizobia in Egypt. IV. Nitrogen Fixation Ability of Native Rhizobia and Competition Among Antibiotic-Marked Rhizobial Isolates Compatible to Clover, Chickpea and Pea Crops

H.K. Abd El- Maksoud, I. Hosney* and Fatma H. Abd El- Zaher

*Agricultural Microbiology Department, National Research Centre and *Microbiology Department, Faculty of Agriculture, Cairo University, Cairo, Egypt.*

THE ABILITY of 131 rhizobial isolates to fix N_2 was tested under greenhouse condition in sterilized fertile soil. Majority of the isolates failed to nodulate their host plants (clover, chickpea and pea). Remnants isolates (56.0, 4.8 and 40.0% in respective) successfully formed nodules resulted in significant differences (p=0.05) in symbiosis expressed as N-uptake plant^{-1}. A number of 9, 12 and 23 isolates of clover rhizobia were high, moderate and poor N fixers, respectively. On the other hand, only one isolate of chickpea rhizobia could nodulate the roots and improved plant growth. Among pea rhizobia 11 isolates could form root nodules, 4,2 and 5 isolates proved to be high, moderate and weak N fixers.

The potentiality of 5 streptomycin (str.)-marked rhizobial strains to compete with indigenous soil rhizobia in developing nodules on roots of clover, chickpea and pea was investigated. Spontaneous str. – resistant indigenous rhizobia of the three plants could be recovered in the tested soil. Tolerable sti. concentration of these native rhizobia was taken in consideration when competition ratios of the tested strains were calculated.

Keywords: Legumes, Rhizobia, N_2 Fixation, Competition.

Since nitrogen is often the limiting nutrient in soil, the use of appropriate strains of *Rhizobium* to supply nitrogen to the plant is economically and ecologically justified. That is why the natural nodulating population of those rhizobial isolates collected to assess their potency in N_2 fixation . The relation between rhizobia and legumes is the most significant contribution, that a soil bacterium can improve agricultural production. This symbiosis has the potentiality to free the host legumes from entirely dependence on nitrogenous fertilizer, as well as increasing soil fertility. The full realization of this potential depends on maximizing the contribution of each partner, attending to specificity in the association and providing conditions for plant growth and nodule function. The nitrogen gains from the atmosphere will depended on the intrinsic capacity of the host and the effectiveness of the root nodules (Somasegaran and Hoben, 1994). Therefore, the development of this symbiosis between the host and the bacterial symbionts has a major importance to agriculture in nitrogen-deficient environments.

Competition for nodule occupancy between strains of rhizobia is a complex and controversial area in the study of the legume-*Rhizobium* symbiosis. Many ecological variables, intrinsic characteristics of the rhizobia themselves and genetic determinants of the host contribute to the success or failure of rhizobial strains to occupy a significant proportion of nodules formed under a given set of conditions.

The competition between the introduced *R. leguminasarum* bv. *trifolii* and the indigenous rhizobia early studied by Martenson *et al.* (1987) ; Ames-Gottfired and Christie (1989) who concluded that the ratio of nodules occupancy of the highly competitor inoculated in case of dominant soil indigenous rhizobia were ineffective. Somasegaran *et al.* (1988) indicated that competition among rhizobia was influenced by the plant growth medium, plant genotype and strain efficiency, but not inoculation rate. In contrary, Fabiano and Arias (1991) carried out an experiment on the ability of native rhizobia to inoculate clover roots in the presence of two commercial rhizobial inocula , they found that the ratio between the number of nodules occupied with the native rhizobia and those occupied with the introduced strain was proportional to their respective inoculum densities. On the other hand, others (Thies *et al.*, 1992) demonstrated that the most significant environmental variable controlling the competition success was the size of the indigenous rhizobial population. In recent study conducted by Josic *et al.* (2002) on clover to assess polymorphism among 42 native isolates derived from different locations, they found wide diversity among the different isolates in their potentiality of N_2 fixation. Di-Bonito *et al.* (1990) assessed the ability of various strains of *R.* spp. to nodulate different chickpea genotypes using the indirect immuno-fluorescent technique, they concluded that all strains increased the nodule numbers on the inoculated plants and the different strain/cultivar combinations exhibited a significant variation in the nodulation and plant productivity.

In a study on competition phenomenon between rhizobia and pea plant, Fesenko *et al.* (1995) surveyed 53 effective *R. leguminosarum* bv. *viceae* within 481 isolates. Three categories were defined for these 53 isolates: high-competitive (32), modurate- competitive (10) and low- competitive strains (11) . They found no correlation between the competitiveness power and symbiotic efficiency.

Abd El-Maksoud *et al.* (2003) and Hosney *et al.* (2002) performed some experiments on the native rhizobia compatible to clover, chickpea and pea plants concerned with salt and pesticides tolerance as well as intrinsic antibiotic resistance (IAR). They concluded that no correlation seems to exist between salt tolerance among different isolates and locations, the isolates also exhibited variable resistance towards the different concentrations of pesticides. They also stated that the all isolates possessed IAR character, but the number of antibiotics resisted varied and the resistance power of most isolates decreased with the increase in antibiotic concentration.

This study is concerned with the N_2 fixation performance of some indigenous rhizobial isolates well matched to clover, chickpea and pea crop collected from different ecological areas as well as evaluation of their competition potency using the antibiotic marked technique.

Material and Methods

Rhizobial isolates of winter legume crops clover, chickpea and pea were isolated from the root nodules of growing plants at flowering stage. Root samples were collected from 31 sites in 10 governorates allocated at Nil Delta and Upper Egypt. The isolates were purified and then enumerated up to their host. A total number of 131 rhizobial isolates represented to the three legume crops were characterized. Their efficacy to fix atmospheric N_2 and competition potency were evaluated.

Ability to fix atmospheric nitrogen

The rhizobial isolates belonging to clover (*R. leguminasarum* bv. *trifolii*, 81 isolates), chickpea (*R.* spp. , 21 isolates) and pea (*R. leguminasarum* bv. *viceae*, 29 isolates) were assayed for their ability to nodulate and fix N_2 in symbiosis with their respective host legumes.

Three pot experiments were performed using a sterilized Nile fertile soil packed into sterilized plastic pots (600g soil pot^{-1}). Each pot was provided with 0.5g superphosphate. Sterile water was used to raise moisture up to 60 % WHC. Seeds were selected for uniformity in size and weight, then surface sterilized with ethanol and calcium hypochlorite 6% (Somasegaran and Hoben, 1994). Seeds were aseptically located on the surface of water agar 7 % in petri dishes for 1-2 days. Pregerminated seeds were sown in the pots at the rate of 10 seeds for clover and 5 seeds of either chickpea or pea. Inoculation was carried out by pipetting 1ml of 3 day-old *Rhizobium* culture on seed surface before insertion in soil. A batch of uninoculated pots was similarly prepared for each test plant as control. Another batch was also inoculated with known rhizobial culture (RT 112; cp 105 and TAL 202) as reference strains for clover, chickpea and pea, respectively. Three pots were prepared for each bacterial isolates. Pots were kept under greenhouse condition with intermittent irrigation with N-free Hogland's solution. After 60 days, plants were removed and roots were carefully washed to remove soil particles. Nodules were separated and scored per plant. Fresh and dry weight of nodules and whole plant were recorded. Plant total-N was determined by the micro-Kjeldahl method according to Black (1982) (In case of clover, 5 representative isolates were chosen from the total number of each efficiency rank).

Competitive potency of Rhizobium isolates

A pot experiment was conducted under greenhouse conditions to investigate the ability of 5 rhizobial strains, as antibiotic-marked, to compete with the native rhizobia and to achieve a successful symbiosis with their selective host legumes.

Development of antibiotic-marked strains

Rhizobial cultures of clover (114 &116), chickpea (cp105) and pea (164 & TAL 202) were chosen according to their ability to develop large number of effective nodules on their respective host legumes and characterized by a high performance in N_2 fixation. Adaptation of these bacteria to tolerate a high concentration of str. was carried out according to the method given by Schwinghamer and Dudman (1973) and Somasegaran & Hoben (1994). A stock solution of str. was prepared by dissolving 200 mg str. sulfate in 50 ml sterile distilled water (4 mg ml^{-1}), then the solution stored in the refrigerator. Ten-ml aliquots of yeast manitol broth (YMB) distributed in test tubes were aseptically provided with a known volume of the antibiotic stock solution to obtain a concentration of 40 mg L^{-1}. Tubes were inoculated with 0.2 ml of 3 days-old *Rhizobium* culture and incubated at 28 C°. When visual bacterial growth was recognized, usually within 3-5 days, a lopful of the culture was taken to inoculate another tube contains YMB medium with a concentration of 80 mg str. per liter. After a relative prolonged incubation time, the developing bacteria were transferred on YMA slant tubes contain the same concentration of the antibiotic.

Estimation of competitive potency of streptomycin –marked rhizobia

Pregermination seeds were sown in plastic pots filled with a nonsterile fertile clay soil (600 g pot^{-1}) at the rate of 10 seeds of clover, 5 seeds of either chickpea or pea per pot. Pots were inoculated with 1ml of 3 days-old culture of the selected antibiotic-marked *Rhizobium* strain. The inoculum was added as drops over the seeds and then covered with a thin layer of sand. Pots were randomly distributed in the greenhouse and irrigated with N-free Hougland's solution at intervals. After 60 days, plants were uprooted and nodules detached, scored and surface sterilized as mentioned before. Representative 10 nodules of each plant were crushed in a well of a sterile plastic tray. A lopful of the extract was used to inoculate previous-poured plates contain YMA medium supplemented with 20, 40, 60 and 80 mg str. L^{-1}. Some plates were performed with antibiotic-free medium. Plates were examined after 5-8 days. A competitive ratio for each tested strain was calculated according to the equation given by Noelle Amarger and Lobreau (1982) as follows:

$$\text{Competition ratio} = \frac{\text{Count of antibiotic-marked } Rhizobium}{\text{Count of total grown } Rhizobium} \times 100$$

Results and Discussion

Ability to fix atmospheric nitrogen

Indigenous rhizobia isolated from nodules of clover, chickpea and pea plants were tested for their ability to nodulate their hosts besides their efficiency to fix atmospheric N_2 symbiotically. A relatively large number of isolates (35, 20 and 18 of clover, chickpea and pea *Rhizobium*, respectively) failed to respond with the host may be deduced to the relatively long period of preservation in the refrigerator . These isolates which represent about 43.2, 95.2 and 62.1 % of the respective original culture collection were excluded. Efficacy of the infective isolates is shown in Table 1. Three *Rhizobium* strains namely: RT 112, cp 105 and TAL 202 were used as reference organisms .These strains were known to be active symbionts with clover, chickpea and pea plants, respectively. Statistical analysis showed significant differences between tested clover isolates in both dry weight of nodules and plants as well as the net effect of symbiosis expressed as N-uptake by plant. The variations in number and weight of nodules were reflected on plant growth, where plant dry weight ranged from 22 up to 170 mg plant^{-1} against 20 mg in the uninoculated plants. It is noteworthy that plant vigorousness seems to be consistently correlated with the number and weight of nodules in most cases. Plant N ranged between 2.0 to 5.4 % which could be considered within the normal N percentage of legumes. N uptake, which is the interaction between plant dry weight and its N %, could be practically considered as the most reliable parameter to judge rhizobia's efficiency in symbiotically dinitrogen fixation. A little number of isolates (20%) was highly efficient, where N uptake was above 3 mg N plant $^{-1}$. Whereas, moderate quantities of N uptake (2-3 mg plant^{-1}) were accumulated with 26% of isolates. However, the majority of clover isolates (54%) proved to be weakly effective as N fixers where N uptake was less than 2 mg N plant^{-1}. These data are in accordance with those obtained by Jesen (1987) who mentioned that, the indigenous populations contain ineffective or poorly effective as well as highly effective strains. The ineffective strains may be act as competitor to the effective ones in root infection of legume hosts, therefore N_2 fixation in some legume fields is apparently scant.

Results secured clearly point out that, Egyptian soils naturally harbour reasonably effective indigenous clover *Rhizobium*. At the same time, many of the strains prevailing may be of low efficiency or may be ineffective ones which fail in forming root nodules. It is also interesting to maintain that the distribution of indigenous clover *Rhizobium* in Egyptain soils follows the same pattern previously stated by Moawad *et al.* (1998) who mentioned that as 25% of *Rhizobium* to have a low degree of effectiveness, 50% to have moderate ability and only 25% to be fully effective.

TABLE 1. Effect of seed inoculation with the indigenous rhizobial isolates on nodule formation and plant N-uptake .

Isolate No.	**Nodule plant^{-1}	Nodule weight mg plant^{-1}		Plant weight mg plant^{-1}		***N-uptake mg plant^{-1}	Efficiency* %
		fresh	dry	fresh	dry		
Clover*							
Control				150.0	20.0	024	
low effic.							
5	1.1	0.40	0.29	230.0	32.3	0.65	25 isolates (54%)
21	1.0	0.40	2.02	462.0	22.0	0.77	
47	1.2	2.20	1.95	377.3	30.5	1.55	
117	1.9	9.20	2.40	781.0	32.0	1.04	
183	3.0	0.60	0.40	647.0	35.0	1.16	
med. effic.							
13	2.4	1.00	0.80	490.0	66.0	2.19	12 isolates (26%)
34	5.0	4.95	2.80	696.0	68.5	2.50	
48	3.0	2.06	1.00	330.0	81.0	2.43	
100	2.2	1.29	0.75	380.0	76.0	2.15	
121	1.0	2.90	2.40	665.0	104.0	2.73	
high effic.							
6	1.5	1.50	1.40	460.6	92.0	3.03	9 isolates (20%)
31	1.5	0.30	0.15	536.0	105.0	3.13	
41	8.0	2.58	2.40	518.0	160.0	6.55	
116	3.5	5.40	1.80	504.3	120.0	3.92	
120	9.7	20.30	6.40	3495.0	170.0	6.03	
RT 112	2.0	3.50	1.30	838.0	75.0	2.05	
LSD			0.39		7.22	0.36	
Chickpea*							
Control	-	-	-	522.0	71.4	0.80	
139	3.4	10.2	3.2	1770.0	242.0	7.29	
cp. 105	5.0	13.0	4.0	3182.0	518.0	16.41	
Pea*							
Control	-	-	-	940.0	190.0	2.25	
low effic.							
147	6.4	16.2	4.0	3205.3	475.3	14.62	5 isolates (46 %)
148	1.5	6.1	2.4	988.0	275.0	7.03	
152	4.4	7.0	1.5	4834.6	615.0	18.58	
155	7.0	29.0	4.6	3728.0	363.3	14.70	
162	24.0	27.0	9.8	3280.0	445.3	19.28	
med. effic.							
151	23.4	55.6	10.3	5310.0	505.0	21.35	2 isolates (18 %)
160	2.17	4.8	1.1	5910.6	1020.0	26.22	
high effic.							
154	14.4	46.0	11.0	6874.0	775.0	31.60	4 isolates (36%)
157	15.0	20.0	6.0	8270.0	1200.0	50.53	
161	21.0	27.4	7.5	647.4	1105.0	44.72	
164	22.0	56.4	12.0	5140.0	677.0	32.47	
TAL202	8.0	31.0	5.4	4111.3	482.7	19.95	
LSD 0.05			0.15		19.0	2.28	

* Mean of three replicates   * efficiency% = proportion of effective isolates

** Nodule average number

*** N-uptake

	Clover	Pea
low	< 2	<20
medium	2-3	20-30
high	>3	>30

Regards to chickpea *Rhizobium*, only one isolate could form nodules while the majority failed to associate with plant roots. This unexpected result due to the conditions prevailing in the present experiment as preservation on the artificial medium resulted in loosing the rhizobia their efficacy to nodulate the host. However, the only effective isolate could nodulated 100 % of the cultivated plants markedly improved plant growth as indicated by the marked increase in plant dry weight. N% and accordingly N uptake by plant, although this isolate was less efficient than the reference strain cp 105.

With respect to pea *Rhizobium* isolates, it was found that out of the 29 investigated cultures, 11 isolates successfully formed nodules on pea roots. Plant growth was significantly affected according to variation between the bacterial isolates. Three isolates resulted in larger dry weight (1020: 1200 mg. plant $^{-1}$) while two isolates, on the other hand, showed poor symbiosis resulted in plant dry weight of less than 400 mg. plant^{-1} . However, six isolates (54.5%) were moderately efficient ones since plant dry weight ranged between 445-775 mg.plant^{-1}. The N uptake by pea plants was accordingly correlated to the accumulated plant biomass.

The general trend of these obtained results points out to abundance of indigenous rhizobia in Egyptian soils but many of these bacteria proved to be of moderate or even poor N fixers (Moawad *et al.,* 1998). However, the traditional farming in Egypt generally depends upon these native rhizobia to perform symbiotic N_2 fixation in most leguminous crops, particularly clover. Therefore, it may be more convenient to disseminate the application of inoculation technology in order to achieve maximum benefit of this important phenomenon. Of course, this needs further evaluation studies including the net outcome of biofertilization with recommended highly efficient rhizobial strains particularly in fertile old soils.

Competition potency of some rhizobia isolates

Because of the large indigenous population of rhizobia that are not fully effective in N_2 fixation, it is not surprising that supplemental inoculation with the selective rhizobial strains commonly results in highly significant agronomic response. As well known, the success in seed inoculation with rhizobia depends on the competitiveness among strains and the ability to survive in soil in addition to the compatibility with the host plant. Moreover, the competition for nodule occupancy between the introduced strains in plant rhizosphere is governed by certain environmental factors.

To assess the competitive potency of certain *Rhizobium* strain, the antibiotic-marked technique has been applied by many workers (Rynne *et al.,* 1991; Issa & Wood, 1995; Simon & Kalalova, 1996; Swelim *et al.,* 1996 and Josic *et al.,* 2002). It is evident that, there is a number of spontaneous str.- resistant isolates in the soil giving rise to the formation of 16.7 and 23.3 % of the nodules on the uninoculated clover and chickpea plants, respectively when using 20 mg str. L^{-1} medium (Table 2). At higher antibiotic concentrations, no spontaneous str.-

resistant isolates could be recovered. At 40 mg str.L^{-1}, the competition ratio of clover rhizobial isolates nos. 114 and 116 were 63.3% and 80.0%, respectively. The corresponding percentages obtained for the 60 mg str. were somewhat lower, being 56.7% and 73.3%. On the other hand, the occupancy percentage of the chickpea strain cp 105 reached to 83.3% and 76.7% at 40 and 60 mg. str. L^{-1}, respectively.

TABLE 2. Competitive potency of streptomycin-marked rhizobial isolates.

Isolate No.	Nodule characteristics			Rhizobial growth on YMA medium + str. (mg.L^{-1})							
	Nodule No. plant^{-1}	weigh mg plant^{-1}		20		40		60		80	
		Fresh	dry	No.	%	No.	%	No.	%	No.	%
Clover (10 plants pot^{-1})											
Control	10.3	25.9	6.2	1.6	16.7	0.0	0.0	0.0	0.0	0.0	0.0
114	19.0	33.7	7.5	8.7	86.7	6.3	63.3	5.7	56.7	0.0	0.0
116	27.3	34.6	8.6	8.3	83.3	8.0	80.0	7.3	73.3	0.0	0.0
Chickpea (5 plants pot^{-1})											
Control	13.3	45.6	12.2	2.3	23.3	0.0	0.0	0.0	0.0	0.0	0.0
Cp 105	15.7	46.0	14.9	8.7	86.7	8.3	83.3	7.7	76.7	0.0	0.0
Pea (5 plants pot^{-1})											
Control	19.7	50.3	20.7	1.3	13.3	1.3	13.3	1.0	10.0	0.0	0.0
164	27.0	57.9	29.7	9.0	90.0	8.7	86.7	8.0	80.0	0.0	0.0
TAL202	23.7	53.3	27.1	9.7	96.7	7.7	76.7	6.7	66.7	0.0	0.0

Respects to nodule occupancy by spontaneous str.- resistant native pea *Rhizobium* amounted to 13.3% of nodules developed on uninoculated control plants. Meanwhile, competition ratios for isolate no. 164 were 90.0, 86.7 and 80.0 % correspondent to 20,40 and 60 str. L^{-1}, respectively.

Finally, a high occupancy rate of introduced rhizobia takes place in all the three examined leguminous plants indicating a quite possible success of seed inoculation regardless of the population of the original native rhizobia in the old fertile soil of Nile Delta. The relation between rhizobia and legumes is the most significant contribution, that a soil bacterium can improve agricultural production. This symbiosis has the potentiality to free the host legumes from entirely dependence on nitrogenous fertilizer, as well as increasing soil fertility .

References

Abd El-Maksoud, H.K., Hosney, I. and Abd El-Zaher, Fatma H. (2003) Ecological studies on rhizobia indigenous to soils in Egypt. 2. Pesticides tolerance. *Egypt. J. Microbiol.* **38**, 2, 145.

Ames-Gottfred, N.P. and Christie, B.R. (1989) Competition among strains of *Rhizobium leguminasarum* bv *trifolii* and use of a diallel analysis in assessing competiton. *Appl. Environ. Microbiol.* **55** (6), 1599.

Black, C.A. (1982) *"Methods of Soil Analysis"*. Part 2, Chemical and microbiological properties, Madison, Wisconsin, USA.

Di-Bonito, R., Porreca, G. and Mannelli, S. (1990) Competition for nodulation of *Rhizobium* spp.(*Cicer arietinum* L.) inoculated on different cultivars of chickpea. *Proc. of the Internat. Conf. on the mechanisms between soil-plant- microorganisms in the rhizosphere,* pp.405-409.

Fabiano, E. and Arias, A. (1991) Competition between a native isolate of *Rhizobium leguminasarum* bv *trifolii* and two commercial inoculant strains for nodulation of clover. *Plant and Soil,* **137** (2), 293.

Fesenko, A. N., Provorov, N.A., Orlova, I. F., Orlov, V.P. and Simarov, B.V. (1995) Selection of *Rhizobium leguminasarum* bv *vieeae* strains for inculation of *Pisum sativum* L. cultivars: Analysis of symbiotic efficiency and nodulation competitvenes. *Plant and Soil,* **172** (2), 189.

Hosney, I., Abd El-Maksoud, H.K. and Abd El-Zaher, Fatma H. (2002) Ecological studies on rhizobia indigenous to soils in Egypt. 1. Salt stress. *Egypt J. Microbiol.* **37** (2), 173.

Issa, S. and Wood, M. (1995) Multiplication and survival of chickpea and bean rhizobia in dry soils : The influence of strains, matric protential and soil texture. *Soil Biology and Biochemistry,* **27** (6), 785.

Jesen, E.S. (1987) Inoculation of pea by application of *Rhizobium* in the planting furrow. *Plant and Soil,* **97** (1), 63.

Josic, D., Popovic, V., Mladenovic, D.S. and Konstantinov, K. (2002) Biodiversity among indigenous *Rhizobium leguminasarum* bv *trifolii* revealed by genotypic and phenotypic methods. *Symp. (9),* 17th WCSS, 14-21 August, 2002, Thailand.

Martenson, A.M., Gustafsson, J.G. and Ljunggren, H.D. (1987) Competition in soil between effective strains of *Rhizobium leguminasarum* bv *trifolii* in the nodulation of clover, *Trifolium pretense* L., studied with ELISA. *J. Gen. Microbiol.* **133** (12), 3467.

Moawad, H., El-Din, SMSB, Abdel-Aziz, R. A., Hardarson, G. and Broughton, W. (1998) Improvement of biological nitrogen fixation in Egyptian winter legumes through better management of *Rhizobium*. *Plant and Soil,* **204** (4), 95.

Noelle Amarger and Lobreau, J.P. (1982) Quantitative study of nodulation competitiveness in *Rhizobium* strains. *Appl. Environ. Microbiol.* **44,** 583.

Rynne, F.G., Dilworth, M. J. and Glenn, A. R. (1991) The effect of chloramphenicol resistance mutation on the nodulation of *Rhizobium leguminasarum* bv *trifolii*. *Soil Biol. Biochem.* **23** (9), 903.

Schwinghamer, E. A. and Dudman, W.F. (1973) Evaluation of spectinomycin resistance as a marker for ecological studies with *Rhizobium* spp. *J. Appl. Bact.* **36,** 263.

Simon, T. and Kalalova, S. (1996) Preparation of antibiotic resistance mutants of rhizobia and their use. *Rostlinna-Vyroba*, **42** (9), 385.

Somasegaran, P. and Hoben, H.J. (1994) *"Methods of Legume- Rhizobium Technology"*. United States Agency For International Development Contract No. DNA 0613.

Swelim, D. M., Kuykendall, L. D., Hashem, F. M., Abdel-Wahab, S. M. and Hegazi, N. I. (1996) Competitiveness of *Rhizobium* sp. [*Leucaena leucocephala* (lam.) Dewit] strains and their genetically marked derivatives. *Lett. Appl. Microbiol.* **22,** 443.

Thies, J.E., Bohlool, B. B. and Singleton, P.W. (1992) Environmental effects on competition for nodule occupancy between introduced and indigenous rhizobia and among introduced strains. *Can. J. Microbiol.* **38,** 493.

(*Received* 3/8/2005;
accepted 4/6/2006)

دراسات بيئية على الريزوبيا المتوطنة بالأراضى المصرية . ٤- القدرة على تثبيت النيتروجين الجوى والمنافسة بين العزلات فى تكوين العقد الجذرية على نباتات البرسيم والحمص و البسلة

حسين كامل عبد المقصود ، اسماعيل حسنى* و فاطمة حلمي عبد الظاهر
قسم الميكروبيولوجيا الزراعية – المركز القومي للبحوث و *قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة القاهرة – مصر .

تم عزل ١٣١ مزرعة ريزوبيا من على جذور ثلاثة نباتات بقولية (البرسيم، الحمص والبسلة) من مناطق مختلفة شملت محافظات (البحيرة ، الغربية ، المنوفية ، القليوبية ، الجيزة ، المنيا ، أسيوط ، سوهاج ، قنا واسوان) لاختبار قدرتها على تثبيت النيتروجين الجوى و القدرة التنافسية بين السلالات فى تكوين عقد على جذور النبات العائل و ذلك من خلال معاملة بعض السلالات التى اثبتت كفاءة عالية فى عملية التثبيت للنيتروجين بتركيزات مختلفة ومتدرجة من المضاد الحيوى الاستربتوميسين وذلك لسهولة التعرف عليها فيما بعد الاختبار.

اختبرت جميع العزلات (١٣١ عزلة ، ٨١ برسيم ، ٢١ حمص و ٢٩ بسلة) بالنسبة لقدرتها على تثبيت النيتروجين الجوى تحت ظروف الصوبة فى تربة

معقمة. وقد أظهرت النتائج فشل نسبة عالية من السلالات فى تكوين عقد جذرية على النبات العائل (٣٥ برسيم، ٢٠ حمص و١٨ بسلة) أظهر التحليل الاحصائى وجود اختلاف مؤكد (٥%) بين العزلات التى نجحت فى تكوين العقد الجذرية، من حيث أوزان العقد والنباتات وكذلك المحتوى الكلى للنيتروجين/ نبات. وهذا الاختلاف يرجع بالدرجة الأولى لتباين كفاءة السلالات.

بالنسبة لريزوبيا البرسيم وجدت سلالتين ذات كفاءة عالية فى تثبيت الينتروجين وقد أمكن تقسيم السلالات الى عالية الكفاءة (٩)، متوسطة الكفاءة (٤١) ومنخفضة (٢٣). أما فى الحمص فكانت هناك سلالة واحدة أمكنها تعقيد الجذوروتحسين النمو. و قد أمكن ايضا تقسيم ريزوبيا البسلة الى ذات كفاءة عالية (٤)، متوسطة (٢) ومنخفضة (٥).

وتوضح الدراسة أن كفاءة كثير من الريزوبيا المتوطنة فى الأراضى المصرية منخفضة ولا تفى بحاجة النبات البقولى من عنصر النيتروجين ولذلك فإن عملية التلقيح بالسلالات عالية الكفاءة تصبح ضرورة لنجاح عملية التعقيد.

اختبرت عدد ٥ سلالات من الريزوبيوم التى أثبتت قدرة خاصة على تحمل تركيزات من المضاد الحيوى "الاستربتوميسين" من حيث قدرتها على المنافسة مع العزلات الأخرى من التربة تحت ظروف الصوبة لقحت فيها بذور النبات العائل بالسلالات المختبرة و تم زراعتها فى تربة غير معقمة وقد أدى التلقيح إلى تحسين عملية التعقيد وصحب ذلك زيادة فى الوزن الجاف للعقد الجذرية مابين ٢١,٠% إلى٤٣,٥% فى النباتات الثلاثة المختبرة مقارنة بالكنترول. وقد أمكن التعرف على وجود ريزوبيا متوطنة ذات قدرة ذاتية على تحمل تركيزات عالية من الاستربتوميسين، وقد أخذ ذلك فى الإعتبار عند تقييم قدرة السلالات المختبرة على التنافس.

وقد تم الاستفادة من هذه الخاصية (التحمل لتركيزات العالية من المضاد الحيوى) فى التعرف على سلالات الريزوبيا ذات القدرة التافسية العالية لحسم إختيار السلالات ذات الكفاءة العالية فى تثبيت النيتروجين الجوى بهدف الوفاء باحتياجات النبات البقولىالعالية من النيتروجين ترشيدا لاستخدام الأسمدة الكيماوية والحد من التلوث.