

FIELD ASSESSMENT OF THE COMPATIBILITY BETWEEN FIVE *Bradyrhizobium japonicum* STRAINS WITH FOUR SOYBEAN CULTIVARS.

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ABSTRACT

A field experiment was conducted in a clayey soil to study the effect of inoculation with five *Bradyrhizobium japonicum* strains namely, (USDA110, USDA HH303, UK 3407, USDA 118 and ARC500) to four soybean cultivars differing markedly in morphological traits (Clark, Crawford, Giza35 and Mc-call) on nodulation, growth, yield and yield components. Results revealed that the most effective symbiosis (leading to nitrogen fixation and good seed and straw yields and protein content) was achieved by careful selection of the appropriate strain for each cultivar. Strain USD A110 was the most efficient strain in N₂-fixation and in increasing seed and straw yields on each cultivar followed by USDA HH303, USDA 118, ARC 500 and UK 3407 strains, respectively. The tested soybean cultivars were differed in their response to inoculation. The highest responses were reported by Clark followed by Giza 35, Crawford and Mc-call cultivars, respectively. Generally, soybean inoculation with any strain of *B. japonicum* gave high responses in all tested parameters compared to those due to uninoculated plants and amended with the recommended dose of nitrogen fertilizer.

Keywords: *Bradyrhizobium japonicum*, symbiotic N₂ fixation, soybean cultivars, nodulation, N-content, crude protein and seed & straw yields.

INTRODUCTION

Soybean is one of the most important leguminous crops. It has long been considered as one of the five scored grains on account of its exceptional food value. The grain of present day cultivars contains on average 40% protein that contains a dozen of amino-acids, which are essential for man and animals, notably lysine, tryptophane, methionine and cystine and 21% oil of excellent quality on dry weight basis.

The symbiotic association between legumes and rhizobia is the most important bio-catalytic link for the flow of atmosphere-N and the living world (Paau, 1989).

In order to harness the potential benefit of rhizobial inoculation in commercial agriculture, the consistency of their performance must be improved. This requires researches in many diverse as those of biological systems, which involved complex interaction among the host, soils and their microbial inhabitants and environment. The formation of effective nodules is complex and highly regulated process that requires coordinated expression of number of genes in the bacterial symbiont and the host plant.

Soybean was introduced to Egypt in the 1960's but was not able to be produced in commercial scale until the 1970's. Farmers grow it as a non-legume rely on heavy N-fertilization to obtain high yield (Nassib *et al.*, 1983).

Because soybean is a newly introduced crop, Egyptian soils are void of native *Bradyrhizobium japonicum* in contrast to the traditional food legume crops such as faba beans, lentils and berseem clover as their native rhizobia are ubiquitous in Egyptian soils (Ghobrial *et al.*, 1995).

In Egypt it is found that soybean yield is more than doubled when effectively inoculated by effective rhizobia strains. Douka *et al.* (1986) reported that soybean inoculation with specific rhizobia strain can save more than 200 kg N/ha. Many of the published reports showed that the relationship between soybean cultivars and *Bradyrhizobium* strain is one of the most important factors influencing biological N₂-fixation (Schmidt *et al.*, 1986 and Ghobrial *et al.*, 1995).

Successful inoculation of soybean with *Bradyrhizobia* is one of the most important factors that determine the seed yield. The response of different soybean cultivars to inoculation with different effective strains of *Bradyrhizobium japonicum* may be variable. Some strains give good nodulation on a soybean cultivar, while other strains fail to form nodules.

The present work aims to study the compatibility of five *Bradyrhizobium japonicum* strains with four commercial soybean cultivars grown in Egyptian clayey soils. As well as to study the effect of inoculation with rhizobia on soybean morphological characters, straw and seed yields, seeds N-content, protein content and nodulation status.

MATERIALS AND METHODS

Bacterial strains:

Five *Bradyrhizobium japonica* strains namely, (USDA110, USDA HH303, UK 3407, USDA 118 and ARC500) were supplied by Microbiology Dept, Soils, Water and Environ. Res. Inst., (ARC), Giza, Egypt. *Bradyrhizobium* strains were grown each separately in Yeast Extract Mannitol (YEM) culture medium (Vinscent, 1970). *Bradyrhizobium* strains cultures were incubated at 28°C for three days on rotary shaker until early log phase of 10⁹ viable cell mL⁻¹.

Experimental Techniques:

Preparation of inoculants:

Vermiculite supplemented with 10% Irish peat was packed in poly ethylene bags (300g carrier per bag), then sealed and sterilized by Gamma irradiation (5.0 x 10⁶ rads.). Bacterial culture was injected into sterilized vermiculite to satisfy 60% water holding capacity.

Soybean genotypes:

Soybean seeds of four commercial genotypes namely, (Clark, Crawford, Giza35 and Mc-call) were kindly provided by Legumes Res. Dept., Field Crops Res. Inst., (ARC), Giza, Egypt.

Field experimental layout:

A field experiment was carried out at Zarzoura Agric. Res. Station, Ittay El-Baroud, Behaira Governorate, during summer season 2004 to study the effect of inoculation with different *Bradyrhizobium japonicum* strains on

nodulation, growth, yield and yield components of the four soybean genotypes. Physical and Chemical properties of experimental soil were determined according to Jackson (1973) and are presented in (Table 1).

The following treatments were conducted:

- 1- Uninoculation + recommended dose of nitrogen (60 kgN / fed.)(100%N).
- 2- Inoculation with *Brady. Japonicum* (USDA 110) + 15kg N /fed. (25%N).
- 3- Inoculation with *Brady. Japonicum* (USDA HH303) + 15kg N/fed. (25%N).
- 4- Inoculation with *Brady. Japonicum* (UK 3407) + 15kg N/fed. (25%N).
- 5- Inoculation with *Brady. Japonicum* (USDA 118) + 15kg N/fed. (25%N).
- 6- Inoculation with *Brady. Japonicum* (ARC 500) + 15kg N/fed. (25%N).

Table (1): Chemical and mechanical analyses of soil used for the field experiment

Properties	Value
Chemical analysis :-	
E.C. (dS/m at 25°C)(soil:water extract 1:5)	1.2
PH (soil:water suspension 1:5)	
CaCo3	7.8
Total nitrogen %	2.67
Organic matter %	0.20
Organic carbon %	1.84
C/N ratio	1.07
	5.43
Soluble cations (meq/L):-	
Ca ⁺⁺	
Mg ⁺⁺	2.10
Na ⁺	4.90
K ⁺	4.70
	0.06
Soluble anions(meq/L):-	
CO3 ⁻⁻	ND [*]
HCO3 ⁻	0.00
Cl ⁻	3.30
SO4 ⁻⁻	1.40
	7.06
Mechanical analysis :-	
Sand	
Silt	15.50
Clay	14.80
Texture	69.70
	Clay

*Not Detected

Soybean genotypes seeds were individually inoculated with the tested *B. japonicum* strains at a rate of 300g / 40 kg seed each using Arabic gum solution (16%) as sticking agent.

A split plot design with three replications was used. Plot area was 7.2 m². While the main plots included soybean genotypes and bio-fertilization treatments were assigned to sub plots. All treatments received calcium super phosphate (15.5% P₂O₅) at rate 100 kg/fed. Nitrogen fertilizer in the form of urea (46.5 % N) was used as a source of nitrogen.

Plant samples were uprooted after 60 days from cultivation to determine the number and dry weight of nodules, dry weight of shoots following oven

drying at 70°C up to a constant dry weight and nitrogen content of shoots was determined according to the methods described by Page *et al.* (1982).

At harvest (120 days from sowing) ten soybean plants were randomly sampled from the second inner tow rows of each plot to determine the following parameters:

- 1- Plant height (cm).
- 2- Number of tillers per plant.
- 3- Number of pods per plant.
- 4- 100-seeds weight (g).
- 5- Crude protein content of seed according to (A.O.A.C., 1990).

The middle three rows of each plot with 3 m² in area were harvested to determine the straw and seed yields (ton/feddan). The obtained data were subjected to the analysis of variance as described by Sendecor and Cochran (1980).

RESULTS AND DISCUSSION

Data in Table (2) showed that successful nodulation was achieved in all inoculation treatments due to all tested soybean cultivars.

Table (2): Number and dry weight of nodules of four soybean cultivars inoculated with different *Bradyrhizobium japonicum* strains after 60days of sowing

Character	Number of nodules/plant				Dry weight of nodules(mg/plant)			
	Clark	Crawford	Giza 35	Mc-call	Clark	Crawford	Giza 35	Mc-call
Treatments Soybean cultivars								
Uninoculated+(control)100% nitrogen fertilizer	4.000	6.000	7.000	5.000	44.000	66.000	77.000	55.000
Inoculated with <i>Bradyrhizobium japonicum</i> usda110+25% nitrogen fertilizer	31.000	28.000	60.000	21.000	341.000	308.000	660.000	231.000
Inoculated with <i>Bradyrhizobium japonicum</i> USDAHH303+25% nitrogen fertilizer	22.000	19.000	38.000	25.000	242.000	209.000	413.000	275.000
Inoculated with <i>Bradyrhizobium japonicum</i> UK3407+25%nitrogen fertilizer	16.000	13.000	19.000	11.000	167.000	143.000	209.000	121.000
Inoculated with <i>Bradyrhizobium japonicum</i> USDA118+25% nitrogen fertilizer	33.000	17.000	31.000	13.000	363.000	133.333	304.333	198.000
Inoculated with <i>Bradyrhizobium japonicum</i> ARC500+25% nitrogen fertilizer	17.000	16.000	25.00	16.00	183.333	176.00	304.333	176.00
L.S.D. 0.05	12.1491	7.0179	11.5156	5.2351	133.4498	77.1692	132.1324	57.586

Highly significant increases were obtained in number and dry weight of nodules for all soybean inoculated treatments. On the other hand only a few nodules were found in the uninoculated plants due to weak establishment of native *Bradyrhizobium japonicum* in Egyptian soils.

The highest increases of nodule number ranged from 366.7 (USDA110 x Crawford) to 757.1% (USDA 110 x Giza 35) against the lowest increases of 300.0 (UK 3407 x Clark) to 116.7% (UK 3407 x Crawford).

Concerning *B. japonicum* strains, results showed that USDA 110 strain had the highest nodule numbers for all tested soybean cultivars compared to the other *B. japonicum* strains except for (USDA 118 x Clark).

The responses of all inoculation treatment in both soybean cultivars in dry weight of nodules gave similar results as those obtained by nodules numbers. These findings were in accordance with those obtained by Ghobrial *et al.* (2002) and Mehesen (2004).

The abovementioned results indicated a positive response to inoculation with strain USDA 110 being the superior strain with all tested cultivars.

Data in Table (3) presented the dry weight of shoot (g/plant) and shoot N-content (mg/plant). The dry weight of shoots increased significantly due to four soybean cultivars inoculated with different *B.japonicum* strains. The increases ranged between 31.00 (USDA 100 x Clark) and 1.71% (UK 3407 x Crawford) compared to those obtained by uninoculated ones and those supplemented with full nitrogen fertilizer dose. These results are in agreement with those obtained by Ghobrial *et al.* (2002) and Mehesen (2004).

Inoculation with *B. japonicum* USDA 110 had recorded the favorite shoot dry weight, since it gave the highest increases, while the lowest ones were due to inoculation with *B.japonicum* UK 4307 strain. On the other hand, the effectiveness of *B.japonicum* strains can be ranked in descending order as USDA 110, USDA HH 303, USDA 118, ARC 500 and finally UK 3407.

The inoculation of all tested soybean cultivars with *B. japonicum* USDA 110 strain gave the highest increases in shoot N-content being in the range of 81.69 of Giza 35 to 46.61% of Crawford, but the lowest increment percentages were obtained by all tested soybean cultivars inoculated with *B. japonicum* UK 3407 strain, the increases ranged between 20.88 of Clark and 9.23% of Crawford compared to those obtained by the uninoculated plants and/or those received the recommended dose of nitrogen fertilizer.

Data in Table (4) presented the number of pods/plant and 100-seed weight (g). Results revealed that irrespective of cultivars, inoculated plants with different *B.japonicum* strains gave higher responses compared to those uninoculated plants and/ or those fertilized with 100% nitrogen. All inoculated soybean cultivars gave the highest increases being in the range of 75.8 to 33.8% due to the inoculation with *B. japonicum* USDA 110 strain, while soybean cultivars inoculated with *B. japonicum* UK 3407 strain recorded the lowest increases being in the range of 17.20 to 5.9%.

Table (3): dry weight and N-content of shoot of four soybean cultivars inoculated with different *Bradyrhizobium japonicum* strains after 60 days of sowing

Character Soybean cultivars	Dry weight of shoot (g/plant)				Shoot N-content (mg/plant)			
	Clark	Crawford	Giza35	Mc-call	Clark	Crawford	Giza35	Mc-call
Uninoculated+(control)100% Nitrogen fertilizer	18.700	24.900	19.200	14.000	384.667	498.00	412.800	274.400
Inoculated with <i>Bradyrhizobium japonicum</i> usda110+25% nitrogen fertilizer	24.500	29.300	25.000	17.500	580.667	730.133	750.000	413.000
Inoculated with <i>Bradyrhizobium japonicum</i> USDAHH303+25% Nitrogen fertilizer	23.600	27.800	23.600	16.400	554.600	653.30	637.200	403.467
Inoculated with <i>Bradyrhizobium japonicum</i> AJK3407+25%nitrogen fertilizer	20.500	25.333	20.700	14.500	440.800	543.967	486.467	326.267
Inoculated with <i>Bradyrhizobium japonicum</i> USDA118+25% nitrogen fertilizer	22.500	27.000	22.500	16.000	596.267	621.000	562.500	371.067
Inoculated with <i>Bradyrhizobium japonicum</i> ARC500+25% nitrogen fertilizer	21.000	26.500	21.500	15.000	472.500	596.267	522.800	353.400
L.S.D.0.05	N.S	N.S	N.S	N.S	132.5026	N.S	119.6508	74.808

Table (4): Number of pods and 100-seed weight of four soybean cultivars inoculated with different *B. japonicum* strains at harvest stage

Character Soybean cultivars	Number of pods/plant				100-seed weight (g)			
	Clark	Crawford	Giza35	Mc-call	Clark	Crawford	Giza35	Mc-call
Uninoculated+(control)100% Nitrogen fertilizer	62.000	84.000	85.000	71.000	9.700	11.550	9.800	10.150
Inoculated with <i>Bradyrhizobium japonicum</i> usda110+25% nitrogen fertilizer	109.000	125.000	135.000	95.000	11.833	13.850	14.000	11.400
Inoculated with <i>Bradyrhizobium japonicum</i> USDAHH303+25% Nitrogen fertilizer	98.000	109.000	131.000	91.000	11.400	12.300	13.850	10.850
Inoculated with <i>Bradyrhizobium japonicum</i> AJK3407+25%nitrogen fertilizer	72.667	69.000	94.000	79.000	9.883	11.600	11.650	10.500
Inoculated with <i>Bradyrhizobium japonicum</i> USDA118+25% nitrogen fertilizer	96.000	104.667	103.000	83.000	10.900	11.900	13.650	10.800
Inoculated with <i>Bradyrhizobium japonicum</i> ARC500+25% nitrogen fertilizer	92.000	97.000	100.000	82.000	10.567	11.600	12.600	10.650
L.S.D.0.05	22.1749	18.0422	20.5691	N.S	1.4174	N.S	2.1022	N.S

Same responses were obtained in 100-seed weight (g) due to soybean cultivars inoculation with different *B. japonicum* strains. However, the superior results were due to soybean cultivars inoculated with USDA 110 strain, but the worst were due to soybean cultivars inoculated with UK 3407.

Strain USDA 110 has been previously reported as the most effective strain in both N₂- fixation and increasing the yield of soybean (Moawad *et al.*, 1988 a & b).

On the other words, the efficient strain was USDA 110 followed by USDA HH 303, USDA 118, ARC 500 and UK 3407 strains.

Irrespective of soybean cultivars, data in Table (5) demonstrated that uninoculated plants and fertilized with 60kg N/fed recorded the lowest values of straw and seed yields due to all tested soybean cultivars. With respect to inoculation, the maximum increases in straw and seed yields were obtained by inoculation with USDA 110 followed by inoculation with USDA HH303, USDA 118, ARC 500 and UK 3407 in descending order.

Regarding the response of the tested soybean cultivars to inoculation, it was found that the highest straw and seed yields were due to inoculation with (USDA HH303 x Mc-call), which recorded increases of 15.0 and 19.7% in both yields, respectively.

Data in Table (5) showed that USDA 110 was the most effective strain in infecting most of the tested soybean cultivars followed by USDA HH303, USDA 118, ARC 500 and UK 3407.

The present results were as similar as those obtained by Dubey (1995), Luna and Planchon (1995) and Araujo *et al.* (1996).

The effect of inoculation with *B.japonicum* strains on soybean crude protein are given in Table (6). Results indicated that uninoculated plants and received 60 kg N/fed recorded the lowest percentage of crude protein (22.820 to 23.127).

Generally, inoculated soybean cultivars amended with 15 kg N/fed recorded significant increases in crude protein percentages as compared with uninoculated plants control. The highest increases of crude protein were in the range of 24.77(USDA 110x Giza 35)to 9.2%(USDA HH 303x Mc-call), while the lowest ones of crude protein being 8.2 (UK 340 x Clark) to 2.6% (UK 340 x Giza 35 and Mc-call).The soybean cultivar Mc-call gave the highest increase with USDA HH303, while soybean cultivar Crawford recorded the lowest increase with ARC 500.The soybean Clark gave the highest percentage increase of crude protein with USDA 118 against the lowest one with UK 3407.

The abovementioned data indicated that strain USDA 110 was the most efficient strain in N₂ fixation and in increasing straw and seed yields of the tested soybean cultivars followed by strains USDA HH303, USDA 118 and ARC 500, while UK 3407 was the least effective strain. Taking into account the low fertilizer recovery by plants, it is suggested that adapted cultivars, which were properly inoculated with effective strains of *Bradyrhizobium japonicum* can fix most of the nitrogen they need for high yields. This is of a great economic importance for crops with high nitrogen-fixation efficiency, like inoculated soybeans, which can fix more than 200kg N/ha (Douka *et al.*, 1986).

Table (5): Straw and seed yield of four soybean cultivars inoculated with different *B. japonicum* strains at harvest stage (120 days from sowing)

Character Treatments	Straw yield (ton/fed)				Seed yield (ton/fed)			
	Clark	Crawford	Giza35	Mc-call	Clark	Crawford	Giza35	Mc-call
Uninoculated+(control)100% Nitrogen fertilizer	3.100	3.460	2.980	1.530	1.215	1.280	1.480	0.660
Inoculated with <i>Bradyrhizobium japonicum</i> usda110+25% nitrogen fertilizer	3.380	3.560	3.160	1.730	1.280	1.360	1.600	0.770
Inoculated with <i>Bradyrhizobium japonicum</i> USDAHH303+25% Nitrogen fertilizer	3.370	3.540	3.140	1.760	1.275	1.340	1.520	0.790
Inoculated with <i>Bradyrhizobium japonicum</i> iUK3407+25%nitrogen fertillier	3.320	3.490	3.000	1.580	1.220	1.300	1.490	0.710
Inoculated with <i>Bradyrhizobium japonicum</i> USDA118+25% nitrogen fertilizer	3.360	3.525	3.120	1.710	1.270	1.325	1.500	0.735
Inoculated with <i>Bradyrhizobium japonicum</i> ARC500+25% nitrogen fertilizer	3.340	3.515	3.100	1.700	1.260	1.315	1.495	0.755
L.S.D. 0.05	0.1628	N.S	0.0814	0.000	N.S	0.000	0.000	0.000

Table(6):Crud prote and nitrogen percentage in seeds of four soybean cultivars inoculated with different *Bradyrhizobium japonicum* strains at harvest stage(120 days from sowing)

Character Soybean cultivars Treatments	Seed crude protein (%)			
	Clark	Crawford	Giza35	Mc-call
Uninoculated+(control)100% Nitrogen fertilizer	22.820	23.127	24.065	23.750
Inoculated with <i>Bradyrhizobium japonicum</i> usda110+25% nitrogen fertilizer	26.565	26.250	30.000	25.313
Inoculated with <i>Bradyrhizobium japonicum</i> USDAHH303+25% Nitrogen fertilizer	26.250	25.630	28.440	25.938
Inoculated with <i>Bradyrhizobium japonicum</i> iUK3407+25%nitrogen fertillier	24.690	24.690	24.690	24.357
Inoculated with <i>Bradyrhizobium japonicum</i> USDA118+25% nitrogen fertilizer	27.190	25.003	26.877	24.880
Inoculated with <i>Bradyrhizobium japonicum</i> ARC500+25% nitrogen fertilizer	25.940	24.130	25.940	24.690
L.S.D.0.05	1.8207	1.6316	1.4371	1.1828

However, the difference between cultivars in symbiotic performance was not only due to the ability to nodulate, but also rather than to a much higher nodule-specific efficiency (Du Teau *et al.*, 1986). In most cases, the reaction of cultivated plants to environmental factors is bounded up with genetic factors.

Many published reports classify soybean cultivars according to their response to the inoculation with different *B. japonicum* strains. Kandil (2003) and El-sawy *et al.* (2006) mentioned that the relationship between variety and *Rhizobium* strains is one of the most important factors influencing biological nitrogen fixation.

Lala and Tilak (2000) and El-Sawy *et al.* (2006) mentioned that symbiotic rhizobial association can meet the entire nitrogen requirement to legumes as the system can siphon out appreciable amount of nitrogen for the atmospheric reservoir and enrich the soil plant with this important but scarce nutrient.

Generally, this study came to a conclusion that it is advisable to inoculate the right *B. japonicum* to the exact and proper soybean cultivar. This is to ensure the highest seed yield with superior quality.

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التقييم الحقلى للتوافق بين خمس سلالات برادى ريزيم جابونيك مع أربعة أصناف فول صويا

بلال عبد السميع أحمد قنديل

معهد بحوث الأراضى والمياه والبيئة ، مركز البحوث الزراعية ، الجيزة

فى تجربة حقلية أقيمت فى تربة طينية استخدم فيها ٤ أصناف من فول الصويا (كلارك ، كراوفورد،جيزة ٣٥ ومالك كول) ، تم تلقيحها بواسطة ٥ سلالات من البرادى ريزيم جابونيك (USDA 110 , USDA HH303 , USDA118 , ARC 500 and UK3407 مقارنة بمعاملة غير ملقحة ومزودة بالجرعة الموصى بها من السماد الأزوتى (٦٠ كيلوجرام أزوت / فدان).

- أدى نشاط معظم المعاملات الملقحة إلى أحسن تثبيت أزوت جوى ، تخزين للمادة الجافة ، ارتفاع فى محتوى الأزوت بالمادة الخضراء ومحصول جيد لكل من البذور والقش وكذلك البروتين الخام فى البذور .

- وقد تم الحصول على هذه النتائج مع السلالات المتوافقة مع كل صنف فول صويا منزرع .

- وقد كانت أكثر السلالات البكتيرية توافقاً مع جميع أصناف فول الصويا هى السلالة (USDA 110)

تليها السلالات (USDA HH 303, USDA 118, ARC 500 and UK 3407) على الترتيب

- اختلفت أصناف فول الصويا المختبرة فى استجاباتها للتلقيح حيث كانت أكثر الأصناف استجابة فى جميع القياسات المأخوذة مع التلقيح بكل سلالات البرادى ريزيم جابونيك هو صنف كلارك تليه الأصناف جيزة ٣٥، كراوفورد ثم مالك كول على التوالى.

بصفة عامة ، يمكن أن يقال أن تلقيح أصناف فول الصويا بأى سلالة من البرادى ريزيم جابونيك أعطى نتائج أعلى فى القياسات المأخوذة مقارنة بأولئك التى تم الحصول عليها من النباتات غير الملقحة والمزودة بالجرعة الموصى بها من السماد الأزوتى (٦٠ كيلو جرام / فدان).