

## SYMBIOTIC NITROGEN FIXATION PERFORMANCE SCREENING OF *R. MELILOTI* ISOLATES FROM SALINE SOIL AGAINST DIFFERENT VARIETIES OF ALFALFA PLANTS IN TWO TYPES OF SOIL

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### ABSTRACT

Two laboratory and greenhouse experiments were conducted to study the efficiency of six *Rhizobium meliloti* isolates (2, 4, 11, 12 and 15) and strain ARC-1 isolated from wild alfalfa plants of salt affected soil to symbiotic performance with 5 varieties alfalfa (*Medicago sativa*) plants (Ismailia 1, Ismailia 2, Nitrogen fixers, Diablo and El-Wady El-gedid). in two soil types.

The results showed that, all isolates withstand high concentration of NaCl more than strain ARC-1. Isolate No.15 was the most resistant one to 7.5% NaCl and different antibiotics concentration.

The data of symbiotic parameters showed that, the highest values of nodules number, nodules dry weight and N<sub>2</sub>-ase activity on plant roots were found with plants inoculated with strain ARC-1 in two soil types. Also isolate No. 12 recorded the highest shoots dry weight and N-content in two soils. The superior interactions were found between ARC-1 and Ismailia 1 for nodulation status and isolate No. 12 and Nitrogen fixers variety for shoot dry weight and N-content.

**Keywords:** Salinity, Alfalfa (*Medicago sativa*), *Rhizobium meliloti*. Symbiotic nitrogen fixation.

### INTRODUCTION

About one-third of land area of the earth is subjected to arid and semi-arid climates and about 15% of the arid and semi-arid lands are salt-affected (Zahran, 1999).

Alfalfa (*Medicago sativa*) is among the commonly cultivated fodder legumes, which grown in large areas of arid and semi-arid environments, and its production magnificence is one of the major targets concerned by agronomists (Passarakli and Huber, 1991).

*Rhizobia* isolated from arid lands are capable to nodulate the legume host under saline condition (Douka *et al.*, 1978).

Although high salt concentrations inhibit nodulation and N<sub>2</sub>-fixation in many legumes, some varieties of legumes, *e.g.*, *Medicago sativa* can successfully fix N<sub>2</sub> under these conditions (Zahran, 1999). Level of salinity that affects the symbiosis between *R. meliloti* and Lucerne (*Medicago sativa*) are lower than those that affect the growth and survival of individual Lucerne genotypes or *Rhizobium* spp. (Mohammed *et al.*, 1989).

Tolerance of the legume host to salt is the most important factor in determining the success of compatible *Rhizobia* strains to form successful symbiosis under high soil salinity conditions, therefore, we need to select

Five selected bacteria isolates and a strain (ARC-1) were used in the experiment and uninoculated plants served as a control. All inoculated bacterial cultures were grown to maximum growth (ca.  $10^9$  cfu ml<sup>-1</sup>). Inoculation was singly surface applied to soil as 10 ml culture pot<sup>-1</sup> once at planting and another 10 ml inoculated after seedling stage. After complete germination, plants were thinned to ten plants pot<sup>-1</sup>. All pots were arranged in a complete randomized design with three replicates.

After 60 days of planting, plants were gently uprooted, nodules were counted and weighed. Shoot dry weight 10 plants<sup>-1</sup> was determined and analyzed for total nitrogen according to Page *et al.*, (1982). Nitrogenase activity (N<sub>2</sub>-ase) of plant roots was determined using the Acetylene Reduction Assay as described by Hardy *et al.*, (1973), using Dani 1000 Gas Chromatography. Data were subjected to analysis of variance (ANOVA) according to the procedure of Snedecor and Cochran (1980).

## RESULTS AND DISCUSSION

### NaCl tolerance:

Nine isolates and one reference strain were tested for their tolerance to different NaCl concentrations. Table (3) showed that all isolates did tolerate high levels of salinity compared to reference strain (ARC-1) which tolerated 3.0% NaCl. This result agree with those obtained by Hua *et al.*, (1982) and Jian *et al.*, (1993) who reported that *Rhizobia* isolated from saline soils survive in inhibitory levels of salinity better than *Rhizobia* isolated from non saline soils. Isolate No. 15 was the most tolerant one, which tolerated 7.5% NaCl, whereas the salt tolerance for the other isolates was in the range of 4.5 to 6.0% NaCl. The majority of tested *Rhizobia* isolates (fast-growers) did withstand a concentration as high as 4.5% NaCl confirming the results of El-Sheikh and Wood (1990) that fast growing *Rhizobia* had higher salt tolerance rate than slow-growing (*Bradyrhizobium*).

### Antibiotic resistance :

Results of resistance and sensitivity to different antibiotics among the examined *Rhizobial* isolates and (ARC-1) strain are illustrated in Table (4). Isolate 15 was highly resistant to all antibiotics examined, while the strain ARC-1 was sensitive to the most antibiotics, *i.e.* ampicillin, kanamycin and streptomycin and intermediate to bactriocin and erythromycin. Strain ARC-1 and isolate 15 exhibited similar behaviors against NaCl concentration test. These results are in accordance with those obtained by Zahran (1991) who reported that the response to salt and antibiotics stress in the *Rhizobium meliloti* might be genetically controlled.

Isolates 11, 12 and 14 were resistant to ampicillin, bactriocin and erythromycin, while were intermediate to kanamycin and streptomycin. In this concern, Somasegaran and Hoben (1994) and Anne *et al.*, (2004) investigated the sensitivity to different antibiotics at different ranges of concentration varied between *Rhizobium* spp. and it was suggested that such variation may be a useful taxonomic character. So, antibiotic resistance

was used as one of the traditional techniques for identification at the species level and the genus level for bacteria (Nakayama, 1999).

**Table (3): Minimum inhibitory concentration (MIC) of NaCl on *R. meliloti* isolates**

NaCl conc. (%)	isolates									Strain ARC-1
	2	3	4	7	8	11	12	14	15	
Control	+	+	+	+	+	+	+	+	+	+
0.5	+	+	+	+	+	+	+	+	+	+
1.0	+	+	+	+	+	+	+	+	+	+
1.5	+	+	+	+	+	+	+	+	+	+
2.0	+	+	+	+	+	+	+	+	+	+
2.5	+	+	+	+	+	+	+	+	+	+
3.0	+	+	+	+	+	+	+	+	+	+
3.5	+	+	+	+	+	+	+	+	+	-
4.0	+	+	+	+	+	+	+	+	+	-
4.5	+	+	+	+	+	+	+	+	+	-
5.0	+	-	+	+	+	+	+	+	+	-
5.5	+	-	-	-	+	+	+	+	+	-
6.0	-	-	-	-	-	+	+	+	+	-
6.5	-	-	-	-	-	-	-	-	+	-
7.0	-	-	-	-	-	-	-	-	+	-
7.5	-	-	-	-	-	-	-	-	+	-
8.0	-	-	-	-	-	-	-	-	-	-

**Table (4): Resistance and sensitivity of *R. meliloti* isolates to different antibiotics**

Isolates	Ampicillin (10 µg)	Bactriocin (10 µg)	Erythromycin (15 µg)	Kanamycin (30 µg)	Streptomycin (50 µg)
Mean diameter of the clear inhibition zone (mm)					
ARC-1	35	10	16	21	41
Isolate 2	30	0.8	0.6	24	42
Isolate 3	15	0.6	0.6	22	25
Isolate 11	10	0.6	0.8	21	14
Isolate 12	10	0.6	0.5	16	13
Isolate 14	0.8	0.4	0.6	12	14
Isolate 15	0.4	0.0	0.0	1.0	1.1

**Nodulation status and N<sub>2</sub>-ase activity:**

Data presented in Table (5) show the effect of inoculation with five different *Rhizobium meliloti* isolates and one reference strain (ARC-1) on nodulation status of five varieties of alfalfa plants. Results showed that, in clayey soil, the greatest number of nodules (171.6) and dry weight of nodules (162.9 mg pot<sup>-1</sup>) were obtained with plants inoculated with strain ARC-1 and isolate 12 which recorded (169.5 and 160.3 mg pot<sup>-1</sup>) for number and dry weight of nodules, respectively. The fewer number and dry weight of nodules were found with plants inoculated with isolate 14 (127.4 nodules and 112.9 mg pot<sup>-1</sup>). On the other hand, the superior interactions were found between strain ARC-1 and variety Ismailia 1 (189.7 nodules and 151.7 mg pot<sup>-1</sup>) and isolate 12 and variety Ismailia 2 (187.7 nodules and 186.0 mg pot<sup>-1</sup>) for number and dry weight, respectively.

The same trend was shown in sandy soil as in clayey soil especially, the behavior of strain ARC-1 (142.6 nodules and 110.6 mg pot<sup>-1</sup>) and isolate 12 (112.2 nodules and 107.5 mg pot<sup>-1</sup>) for number and dry weight of nodules, respectively.

These findings were also observed by Zahran (1999) and Clen and David (2004) who reported that some legumes such as *Medicago sativa* produce indeterminate (meristematic) nodules that were more salt-tolerant than determinate (non-meristematic) ones formed on roots of other legumes. Furthermore, Rhizobia isolated from saline soil survive in inhibitory levels of salinity better than the other collected from soils with no salinity stress. Thus, salt tolerant Rhizobia efficiently colonize roots of leguminous plants grown in saline environments (Wall and Favelukes, 1991).

Regarding N<sub>2</sub>-ase activity, data in Table (6) declared that isolate 12 recorded the highest values of N<sub>2</sub>-ase activity to be 68.85 and 14.90 µmole C<sub>2</sub>H<sub>4</sub> /mg of nodules hr<sup>-1</sup> in clayey and sandy soil, respectively. However, the lowest values of N<sub>2</sub>-ase activity was found in plants inoculated with isolate 2 which recorded 21.10 and 7.24 µmole C<sub>2</sub>H<sub>4</sub> /mg of nodules hr<sup>-1</sup> in clayey and sandy soil, respectively.

#### Shoot dry weights and N-content:

Shoot dry weights and N-contents were significantly different depending upon both the *R. meliloti* isolates and alfalfa cultivars (Table, 7). In general, alfalfa plants grown in clayey soil produced significantly greater total dry weight and N-content in all treatments than those in sandy soil.

Among alfalfa varieties, Nitrogen fixers was found the most responsive one recording 5.55 g pot<sup>-1</sup> for shoot dry weight and 17.62 mg pot<sup>-1</sup> for shoot N-content in clayey soil and 3.18 g pot<sup>-1</sup> and 9.12 mg pot<sup>-1</sup> for shoot dry weight and shoot N-content in sandy soil, respectively. The lowest dry weight and N-content were obtained with uninoculated plants (control) were 3.74 g pot<sup>-1</sup> and 8.99 mg pot<sup>-1</sup> in clayey soil and 1.90 g pot<sup>-1</sup> and 4.82 mg pot<sup>-1</sup> in sandy soil, respectively.

On the other hand, isolate 12 supported the highest dry weight and N-content in alfalfa shoot cultivars to be 5.91 g pot<sup>-1</sup> for dry weight and 20.41 mg pot<sup>-1</sup> for N-content in clayey soil, respectively, and 3.13 g pot<sup>-1</sup> and 10.73 mg pot<sup>-1</sup> in sandy soil followed by strain ARC-1 which recorded 4.78 g pot<sup>-1</sup> and 21.14 mg pot<sup>-1</sup> in clayey soil and 3.08 g pot<sup>-1</sup> and 9.35 mg pot<sup>-1</sup> in sandy soil. These results agree with those obtained by Zahran (1999) and Pieter *et al.*, (2002) who reported that Rhizobia isolated from wild legumes of arid or saline lands might be superior to homologous strains of Rhizobia in effectively nodulating their legume host.

The superior interaction was found between isolate 12 and variety Nitrogen fixers which recorded 8.84 g pot<sup>-1</sup> for dry weight and 25.41 mg pot<sup>-1</sup> for N-content in clay soil and 3.77 g pot<sup>-1</sup> and 16.45 mg pot<sup>-1</sup> in sandy soil.

Levels of salinity that inhibit legumes-Rhizobia symbiosis differ from these harmful for growth of the individual symbionts. Legumes are generally more susceptible to osmotic stress than their specific microsymbionts (Kristin and Walker, 2001).

Table (5): Nodules number and dry weights of alfalfa cultivars inoculated with *R. meliloti* isolates in clayey and sandy soils

Isolates	Number of nodules (No. pot <sup>-1</sup> )					Dry weight of nodules (mg pot <sup>-1</sup> )						
	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>	X <sup>c</sup>	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>	X <sup>c</sup>
Clayey soil												
Isolate 2	169.0	147.7	127.7	157.7	172.7	154.9	170.0	140.0	117.0	108.7	174.7	142.1
Isolate 11	164.0	165.0	136.0	147.0	130.0	148.4	168.0	144.7	124.0	74.0	75.0	117.1
Isolate 12	173.0	187.7	166.7	155.0	165.0	169.5	166.0	186.0	138.0	166.0	145.7	160.3
Isolate 14	100.7	132.7	98.7	142.7	162.0	127.4	100.0	122.7	106.3	104.7	131.0	112.9
Isolate 15	184.0	136.7	165.0	187.7	137.7	162.2	139.7	104.7	149.0	146.0	154.0	138.7
ARC-1	189.7	184.0	155.0	162.7	166.7	171.6	151.7	173.3	159.0	161.7	168.7	162.9
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X <sup>c</sup>	140.0	136.3	121.3	136.1	133.4		127.9	124.5	113.3	108.7	121.3	
Sandy soil												
Isolate 2	114.7	117.7	88.0	108.0	116.7	109.0	83.7	149.7	69.7	94.0	95.7	98.6
Isolate 11	112.0	84.0	104.0	133.7	94.0	105.5	110.0	108.7	95.7	92.0	100.7	101.4
Isolate 12	107.0	141.0	72.0	97.0	144.0	112.2	103.0	132.0	65.0	113.7	124.0	107.5
Isolate 14	93.7	96.0	131.0	105.0	124.0	109.9	93.7	93.0	102.7	98.0	109.7	99.4
Isolate 15	103.0	69.0	134.0	64.7	76.0	89.3	92.0	76.7	106.7	59.0	73.7	81.6
ARC-1	153.6	160.0	147.0	147.7	104.7	142.6	129.7	85.7	120.7	124.0	93.0	110.6
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X <sup>c</sup>	97.9	95.5	96.8	93.7	94.3		87.4	92.3	80.04	82.95	85.24	
L.S.D 0.05												
V			N.S						8.36			
Iso			12.27						9.89			
V x Iso			27.44						22.12			

V<sub>5</sub>: El-Wadi El-gedid

V<sub>4</sub>: Diabolo

V<sub>3</sub>: Nitrogen fixers

V<sub>2</sub>: Ismailia 2

V<sub>1</sub>: Ismailia 1

Table (6): N<sub>2</sub>-ase activity of alfalfa varieties inoculated with different *R. meliloti* isolates

Isolates	N <sub>2</sub> -ase activity $\mu\text{mole C}_2\text{H}_4/\text{mg of nodules hr}^{-1}$ .													
	Clayey soil							Sandy soil						
	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>	X'	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>	X'		
Isolate 2	13.33	12.13	23.89	16.15	39.87	21.10	7.80	7.00	12.40	2.10	6.90	7.24		
Isolate 11	46.83	10.94	70.61	54.58	31.58	42.91	6.20	3.75	2.38	11.89	13.90	7.60		
Isolate 12	67.77	67.70	88.10	56.71	63.99	68.85	8.72	11.47	11.66	24.70	17.84	14.90		
Isolate 14	22.97	49.13	38.10	24.10	70.00	40.86	17.00	9.66	6.27	6.31	4.32	8.71		
Isolate 15	64.72	46.10	54.18	23.27	79.75	53.60	11.11	6.58	5.07	7.61	11.40	8.35		
ARC-1	25.53	33.46	25.38	107.2	52.65	48.84	11.10	10.76	15.93	14.25	26.70	15.75		
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		

Table (7): Shoot biomass and N-content of alfalfa plants inoculated with various *R. meliloti* isolates in different two types of soil

Isolates	Dry weight of shoot (g pot <sup>-1</sup> )						N-content (mg pot <sup>-1</sup> )					
	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>6</sub>	X'	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>	X'
<b>Clayey soil</b>												
Isolate 2	2.67	3.90	5.55	4.40	2.51	3.81	9.70	15.79	18.00	13.10	13.10	13.94
Isolate 11	3.39	4.29	6.40	2.83	3.39	4.10	17.43	15.58	12.63	13.56	9.98	13.84
Isolate 12	4.36	6.39	8.84	6.51	3.44	5.91	18.28	21.02	25.41	25.10	12.23	20.41
Isolate 14	3.69	2.81	5.36	6.35	3.45	4.33	4.36	18.19	19.64	20.85	10.74	14.76
Isolate 15	4.10	4.60	3.63	5.95	3.37	4.23	11.58	14.51	12.10	18.13	11.22	13.50
ARC-1	5.31	4.52	5.39	4.56	4.10	4.78	17.43	23.37	25.53	18.70	20.66	21.14
Control	3.99	3.33	3.71	4.40	3.27	3.74	5.89	6.98	10.32	13.26	8.52	8.99
X'	3.93	4.26	5.55	4.93	3.36		12.10	16.49	17.62	17.52	12.35	
V.			0.147						0.586			
ISO.			0.174						0.693			
V x ISO.			0.389						1.550			
L.S.D <sub>0.05</sub>												
<b>Sandy soil</b>												
Isolate 2	2.54	3.40	2.25	3.53	2.10	2.76	6.94	10.40	8.16	7.89	6.12	7.90
Isolate 11	2.36	3.47	4.30	2.93	1.73	2.96	9.13	10.86	3.31	7.21	6.85	7.47
Isolate 12	3.12	3.36	3.77	3.59	1.79	3.13	9.02	12.87	16.45	9.72	5.57	10.73
Isolate 14	2.71	2.72	3.25	3.14	2.56	2.88	11.30	7.91	8.03	8.49	7.85	8.71
Isolate 15	2.41	2.46	2.62	1.39	2.49	2.29	5.60	6.51	8.34	9.36	4.72	6.91
ARC-1	3.20	3.21	3.43	3.54	2.01	3.08	8.71	7.99	12.20	10.83	7.04	9.35
Control	1.71	2.30	2.62	1.74	1.13	1.90	4.14	5.71	7.35	3.74	3.15	4.82
X'	2.58	2.98	3.18	2.84	1.97		7.83	8.89	9.12	8.18	5.90	
V.			0.126						0.562			
ISO.			0.149						0.665			
V x ISO.			0.334						1.488			
L.S.D <sub>0.05</sub>												
V <sub>1</sub> : Ismailia 1    V <sub>2</sub> : Ismailia 2    V <sub>3</sub> : Nitrogen fixers    V <sub>4</sub> : Diablo    V <sub>5</sub> : El-Wadi El-gedid												

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اختبار كفاءة تثبيت الأزوت تكافليا بين بعض عزلات ريزوبيا البرسيم الحجازي المعزولة من أراضي ملحية في وجود بعض أصناف البرسيم المنزرعة في نوعين من التربة

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١ - قسم بحوث الميكروبيولوجيا الزراعية - معهد بحوث الأراضي والمياد والبيئة - مركز البحوث الزراعية - الجيزة - مصر

٢ - قسم بحوث العلف - معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية - الجيزة - مصر

أجريت تجربتان معمليتان وكذلك تجربة أصص لاختبار كفاءة تثبيت النيتروجين لـ ٦ عزلات من ريزوبيا البرسيم الحجازي (٢، ٤، ١١، ١٢، ١٥) بالإضافة إلى ARC-1 والتي تم عزلها من نباتات البرسيم الحجازي النامية في أراضي ملحية وكذلك على أداء العلاقة التكافلية مع أصناف مختلفة من البرسيم الحجازي (إسماعيلية ١، إسماعيلية ٢، مثبت للأزوت - نيايلو، الوادي الحديد) وذلك باستخدام نوعين من التربة. وقد أوضحت النتائج ما يلي:

تمكنت جميع العزلات من تحمل الملوحة بدرجة أكبر من السلالة ARC-1 وكانت العزلة رقم ١٥ أكثر العزلات تحملا للملوحة (٧,٥% كلوريد صوديوم) وكذلك تحملها للتركيزات المختلفة من المضادات الحيوية المستخدمة.

كما أوضحت النتائج أن أعلى زيادة في عدد ووزن العقد الجذرية الجافة لنباتات البرسيم الحجازي وجدت عند تلقيح النباتات بالسلالة ARC-1. كما أن العزلة رقم ١٢ أعطت أعلى قيم للوزن الجاف للمجموع الخضري والمحتوى النيتروجيني للنباتات وكذلك نشاط أنزيم النيتروجينيز وذلك بالنسبة لكلا النوعين من التربة المستخدمة في الدراسة.

كما أشارت النتائج إلى أن أفضل التداخلات بين العزلات والأصناف كانت بين السلالة ARC-1 والصنف إسماعيلية ١ وذلك بالنسبة لعدد العقد الجذرية ووزنها الجاف والعزلة رقم ١٢ والصنف مثبت للأزوت بالنسبة للوزن الجاف للمجموع الخضري والمحتوى النيتروجيني.