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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_2-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_2-fixation in many legumes, some varieties of legumes, *e.g.*, *Medicago sativa* can successfully fix N_2 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

plant genotypes that are tolerant to salt stress and then match them with the salt-tolerant and effective strain of Rhizobia (Cordovilla *et al.*, 1995).
The aim of the present work was to study the effectiveness of the natively salt tolerant Rhizobia isolates for future application in salt affected soil.

MATERIALS AND METHODS

1. Rhizobia isolates:

Nine isolates of *Rhizobium meliloti* were isolated from alfalfa (*Medicago sativa*) root plants from different saline locations in Sewa Oasis. Isolation and purification of cultures were examined according to the methods described by Vincent (1970). One strain of *R. meliloti* (ARC-1) was provided by Biofertilizers Production Unit, Soils, Water and Environ. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt.

2. NaCl tolerance:

Sodium chloride was used in different concentrations from 0.5% up to 8.0% to identify the salt tolerance of each *Rhizobium* isolates and strain. A set of three plates as replicates for every isolate, each containing a certain concentration of NaCl. Heavy inocula of each culture sizes (ca. 10^6 cfu ml^{-1}) were spotted using micropipettes (each drop equals 20 µl). Plates were incubated at 20-30 °C for 4 days, growth was recorded as positive (visual growth) or negative (no growth). The concentration of NaCl in the basal medium was used as a control.

3. Antibiotics :

BDBBL ™sensi-disc ™antimicrobial susceptibility test discs were used to characterize the antibiotic resistance among isolates and strain of *R. meliloti*. Three replicates of each bacterial culture of 10^6 cfu ml^{-1} were used to be tested with different antibiotics (Ampicillin, Bactriocin, Erythromycin, Kanamycin and Streptomycin) according to the method described by Atlas *et al.*, (1984).

After incubation period (4 days) of plates, the inhibition zones for each antibiotic of each culture were measured and the results were compared with the standard zones to determine the sensitivity, resistance and intermediate for each isolate and strain.

Table(1):Showing zone diameter interpretive standards (Quinn *et al.*, 1994)

Antibiotic	Disc content	Zone diameters, mm		
		Resistant	Intermediate	Susceptible
Ampicillin	10 µg	≤ 13	14-16	>17
Bactriocin	10 units	≤ 8	9-12	> 13
Erythromycin	15 µg	≤ 15	16-20	> 21
Kanamycin	30 µg	≤ 13	14-17	> 18
Streptomycin	10 µg	≤ 11	12-14	> 15

***The discs are American Produced by Becton, Dickinson and were supplied by Difico Lab.**

Pot experiment:

A pot experiment was conducted at the greenhouse of Forage Crops Research Department, Field Crops Res. Inst., Agric. Res. Center (ARC), Giza, Egypt during spring season of the year 2003. The experiment was executed to investigate the effectiveness of these natively salt tolerant Rhizobia isolates for nodulation under two types of soil. Some physical and chemical properties of the used soils according to Piper (1950) are presented in Table (2).

Table (2): Some physical and chemical properties of the studied soils

Property	Values	
	Ismailia	ARC (Giza)
Particle size distribution:		
Sand (%)	90.88	25.77
Silt (%)	2.07	35.90
Clay (%)	7.05	38.33
Texture grade	Sandy	Clayey
Saturation percent (S.P %)	19.30	41.16
pH (1 : 2.5 susp.)	7.49	7.76
E.C (dS m^{-1} at 25°C)	0.38	1.05
Soluble cations (meq L^{-1}) :		
Ca^{++}	0.90	4.22
Mg^{++}	0.55	2.88
Na^{+}	0.95	3.00
K^{+}	0.60	1.19
Soluble anions (meq L^{-1}) :		
$CO^{=}_3$	0.00	0.00
HCO^{-}_3	1.35	3.28
Cl^{-}	0.65	4.33
$SO^{=}_4$	1.00	3.68
Organic matter (%)	0.24	0.59
Organic-C (%)	0.14	0.34
Total-N (%)	0.018	0.048
C/N ratio	7.78	7.08
Total soluble-N (ppm)	16.00	42.18
Available P (ppm)	7.14	13.60
DTPA-extractable (ppm):		
Fe	1.00	5.88
Mn	0.30	3.20
Zn	0.42	1.18
Cu	0.20	0.74

Five varieties of alfalfa (*Medicago sativa* L.) were selected, Ismailia 1, Ismailia 2, Nitrogen fixers, Diablo and El-Wady El-gedid. Seeds were grown in plastic pots of 20 cm diameter filled with 7 kg of two types of soil. The first type was sandy soil collected from Ismailia Experim. Res. Station, ARC, Egypt, while the second one was clayey soil obtained from the field of Agric. Res. Center, Giza, Egypt. Both types of soil were sterilized by H_2O_2 (3%). The soils in pots were supplemented with the recommended dose of superphosphate (15.5% P_2O_5) at a rate of 1.4 g pot^{-1} (200 kg fed^{-1}), while potassium sulphate (48% K_2O) was applied at a rate of 0.7 g pot^{-1} (100 kg fed^{-1}). Ammonium sulphate (20.5% N) was added at a rate of 0.14 g pot^{-1} (20 kg N fed^{-1}) as an activation dose.

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^{-1}). Inoculation was singly surface applied to soil as 10 ml culture pot^{-1} once at planting and another 10 ml inoculated after seedling stage. After complete germination, plants were thinned to ten plants pot^{-1}. 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 $plants^{-1}$ was determined and analyzed for total nitrogen according to Page *et al.*, (1982). Nitrogenase activity (N_2-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_2-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^{-1}) were obtained with plants inoculated with strain ARC-1 and isolate 12 which recorded (169.5 and 160.3 mg pot^{-1}) 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^{-1}). 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^{-1}) and isolate 12 and variety Ismailia 2 (187.7 nodules and 186.0 mg pot^{-1}) 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^{-1}) and isolate 12(112.2 nodules and 107.5 mg pot^{-1}) 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_2-ase activity, data in Table (6) declared that isolate 12 recorded the highest values of N_2-ase activity to be 68.85 and 14.90 µmole C_2H_4 /mg of nodules hr^{-1}. in clayey and sandy soil, respectively. However, the lowest values of N_2-ase activity was found in plants inoculated with isolate 2 which recorded 21.10 and 7.24 µmole C_2H_4 /mg of nodules hr^{-1}. 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^{-1} for shoot dry weight and 17.62 mg pot^{-1} for shoot N-content in clayey soil and 3.18 g pot^{-1} and 9.12 mg pot^{-1} 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^{-1} and 8.99 mg pot^{-1} in clayey soil and 1.90 g pot^{-1} and 4.82 mg pot^{-1} 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^{-1} for dry weight and 20.41 mg pot^{-1} for N-content in clayey soil, respectively, and 3.13 g pot^{-1} and 10.73 mg pot^{-1} in sandy soil followed by strain ARC-1 which recorded 4.78 g pot^{-1} and 21.14 mg pot^{-1} in clayey soil and 3.08 g pot^{-1} and 9.35 mg pot^{-1} 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^{-1} for dry weight and 25.41 mg pot^{-1} for N-content in clay soil and 3.77 g pot^{-1} and 16.45 mg pot^{-1} 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^{-1})						Dry weight of nodules (mg pot^{-1})					
		V_1	V_2	V_3	V_4	V_5	$\bar{X}$	V_1	V_2	V_3	V_4	V_5	$\bar{X}$
		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
$\bar{X}$		140.0	136.3	121.3	136.1	133.4		127.9	124.5	113.3	108.7	121.3	
L.S.D $_{0.05}$	V.			11.10						13.57			
	Iso.			13.03						16.06			
	V x Iso.			29.13						35.91			
		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
$\bar{X}$		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_1: Ismailia 1 V_2: Ismailia 2 V_3: Nitrogen fixers V_4: Diablo V_5: El-Wadi El-gedid

Table (6): N_2-ase activity of alfalfa varieties inoculated with different *R. meliloti* isolates

Isolates	N_2-ase activity µmole C_2H_2 /mg of nodules hr^{-1}.											
	Clayey soil						Sandy soil					
	V_1	V_2	V_3	V_4	V_5	X^-	V_1	V_2	V_3	V_4	V_5	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	48.64	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^{-1})						N-content (mg pot^{-1})					
		V_1	V_2	V_3	V_4	V_5	$\bar{X}$	V_1	V_2	V_3	V_4	V_5	$\bar{X}$
		Clayey soil											
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
$\bar{X}$		3.93	4.26	5.55	4.93	3.36		12.10	16.49	17.62	17.52	12.35	
L.S.D $_{0.05}$	V.			0.147						0.586			
	Iso.			0.174						0.693			
	V x Iso.			0.389						1.550			
		Sandy soil											
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
$\bar{X}$		2.58	2.98	3.18	2.84	1.97		7.83	8.89	9.12	8.18	5.90	
L.S.D $_{0.05}$	V.			0.126						0.562			
	Iso.			0.149						0.665			
	V x Iso.			0.334						1.488			

V_1: Ismailia 1 V_2: Ismailia 2 V_3: Nitrogen fixers V_4: Diablo V_5: El-Wadi El-gedid

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

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أجريت تجربتان معمليتان وكذلك تجربة أصص لاختبار كفاءة تثبيت النيتروجين لـ ٦ عزلات من ريزوبيا البرسيم الحجازى (٢ ، ٤ ، ١١ ، ١٢ ، ١٥) بالإضافة إلى ARC-1 والتي تم عزلها من نباتات البرسيم الحجازى النامية فى أراضى ملحية و كذلك على أداء العلاقة التكافلية مع أصناف مختلفة من البرسيم الحجازى (أسماعيلية ١ ، أسماعيلية ٢ ، مثبت للأزوت ، دياولو ، الوادى الجديد) وذلك باستخدام نوعين من التربة. وقد أوضحت النتائج ما يلى:

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

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

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