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Executive Summary

The overall objective of this project is to increase crop production and adaptation of shrubs and trees to ecosystems in areas affected by salinity, drought and low soil pH, through the isolation, selection, culture and propagation of stress tolerant soil microorganisms in association with plant roots. Towards this goal, a large collection of nitrogen fixing microorganisms was isolated from nodules of the Kyzil-Kum desert (Uzbekistan) leguminous plants *Ammodendron conollyi, Astragalus unifoliolatus* and *Astragalus vilosissimus*. After preliminary screening for their salt-tolerance, a number of bacterial isolates were selected for further studies. Isolates in this sub-collection were characterized according to the following properties: presence of nitrogen fixation (*nif*) genes, presence of nodulation (*nod*) genes, plasmid profile, growth rate, tolerance to salt, growth pH, growth temperature, antibiotic resistance, and production of melanin. All isolates grew within a wide range of temperature and pH values.

Germination, growth characteristics and the tolerance to salt of *Ammodendron conollyi, Astragalus unifoliolatus* and *Astragalus vilosissimus* were studied, The data obtained in these preliminary experiments served as baseline for the subsequent inoculation studies. Next, seedlings of these plants were inoculated with the selected rhizobium isolates. These studies tested (a) the ability of the isolates to cause nodulation in plant species other than that from which they had been obtained, and (b) the effectiveness of nodulation in terms of increase in biomass production. Finally the leguminous crop plant alfafa, and the desert leguminous tree *Acacia saligna* were tested as possible hosts capable of using the salt tolerant rhizobium isolates.

Although it is too early to assess the capacity to produce leguminous crops in desert areas affected by drought and salinity, some of our preliminary results are highly encouraging. Further work is required, including field experiments now in preparation.

Section I

A) Research Objectives

Overall aim and specific objectives

Overall aim: To increase crop production and adaptation of shrubs and trees to ecosystems in areas affected by salinity, drought and low soil pH, through the isolation, selection, culture and propagation of stress tolerant soil microorganisms in association with plant roots.

Specific objectives:

(1) To select and propagate stress resistant strains of nodule bacteria and mycorrhiza to enhance crop production in areas affected by salinity and drought.

(2) To study the mechanisms by which symbiotic microorganisms enhance mineral nutrition and growth of plants in nutrient poor soils and in soils affected by salinity and drought.

(3) To study the effect of symbiotic microorganisms on the adaptation processes of plants to salinity and drought.

(4) To test under pot and field conditions the possibility of creating new microorganism/plant associations to increase stress tolerance of the symbiotic associations.

B) Research Accomplishments

1. Characterization of N_2-fixing organisms isolated from leguminous plants of the Kyzil-Kum desert of Uzbekistan

During the first year of the project, a large number of isolates were obtained from nodules of *Amodendrum Connolly, Astragalus vilosissimus* and *Astragalus unifoliolatus.* In spite of sterilization of nodule surfaces, it is common to obtain rhizosphere bacteria that do not fix nitrogen. The identification of the true nitrogen fixers was done on the basis of a molecular approach, based on the detection of N_2-fixation genes required for nitrogen fixation and nodulation genes needed for symbiotic association. Detection of these types of genes was performed by hybridization of total DNA of the bacterial strains to two probes, *nifHDK* and *nodABC*. The screening was performed by a modification of the colony hybridization procedure, known as "patch hybridization". A total of 180 isolates were grown in patches in large Petri dishes. The grown biomass was transferred to nylon membranes and lysed *in situ*. Hybridization of total DNA from each isolate to the probes was performed by standard procedures, under low stringency conditions. Typical hybridization results to each probe are shown in Fig. 1.

Isolates which hybridized with both probes were then screened for salt tolerance as detected from their capacity to grow on TY solid medium containing up to 2 M NaCl. After this preliminary characterization, 15 bacterial isolates were selected for further studies: four isolates (C8, C11, C*15, and C21) were obtained from nodules of *Amodendrum connollyi*; four isolates (U7, U17, *U30-1 and U*30-2) from nodules of *Astragalus vilosissimus*, and seven (V1, V3, V6, V8, V9, *V9-1 and V30) from *Astragalus unifoliolatus*.

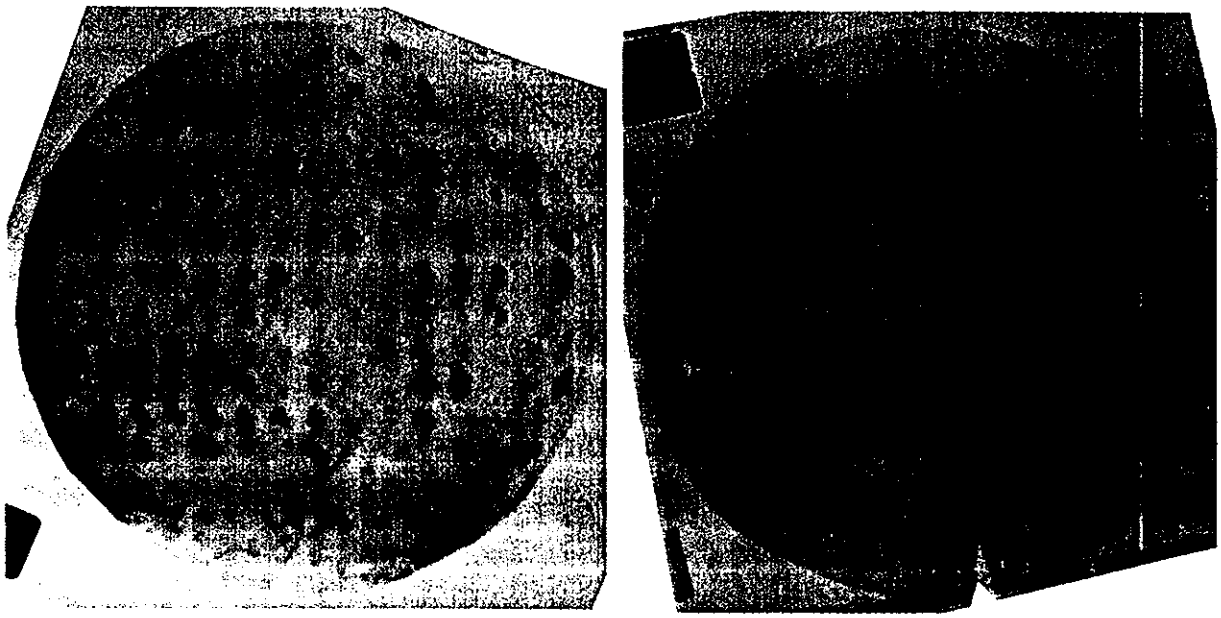

Figure 1: Patch hybridization of bacterial isolates with a *nifHDK* (left) and a *nodABC* (right) probe.

Symbiotic nitrogen-fixing bacteria bear megaplasmids required for symbiotic association and the plasmid content of the selected stains was examined, using a modification of the Eckardt procedure. Plasmid electrophoresis showed that the isolates fell into two different profiles (Fig. 2). Strains C8-1, V1, V3, V8-1, V9 and V9-1 carried three plasmids of approximately 515, 370 and 118 kbp in size, respectively, while the smaller-size plasmid was absent in the remaining strains.

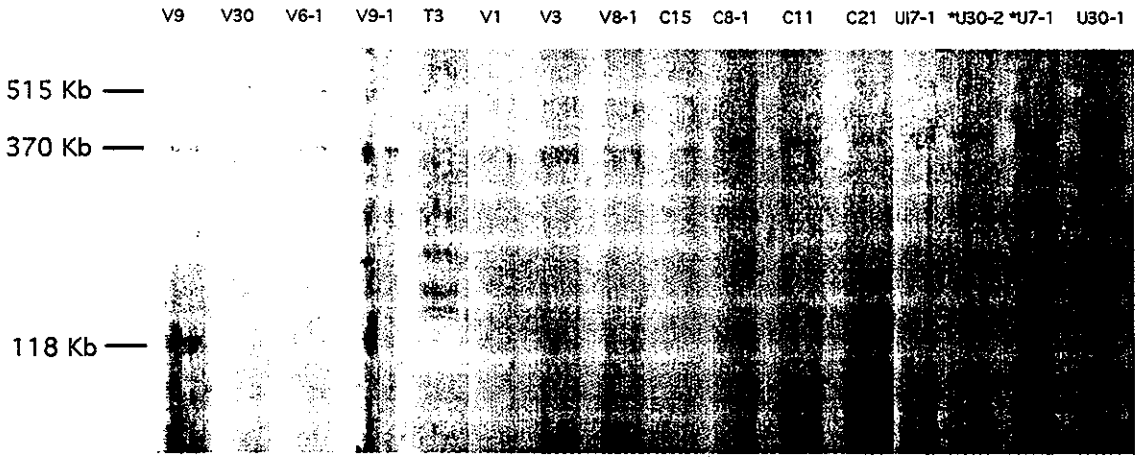

Figure 2: Plasmid profile of bacterial isolates.

Growth of the selected strains was studied in TY liquid medium in order to estimate their growth rates, at 30 °C, and the effect of salt on bacterial growth under these conditions (Table 1). All strains were "fast growers" with doubling times ranging between 43 (*C15) and approximately 78 min (C11). Three strains showed doubling times longer than 1 hour C11 (78 min), *U30-2 (65 min) and V8-1 (76 min). Strain V6-1 had a doubling time of approximately 56 min and all remaining strains had doubling times of 50 min or less.

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Table 1: Effect of salt on growth. Bacteria were cultivated TY medium containing 0.04 (control), 0.75, 1.0, 1.5, 1.75 or 2.0 M NaCl. Cultures were maintained in an orbital shaker at 30 °C. Isolates C, U and V were obtained from nodules of *Ammodendron conollyi*, *Astragalus unifoliolatus* and *Astragalus vilosissimus* respectively.

Isolate	Control	0.75 M		1.0 M		1.5 M		1.75 M		2.0 M	
	t_d (h)[a]	t_d (h)		t_d (h)		t_d (h)		t_d (h)		t_d (h)	
C8-1	0.82	1.29	(1.6)[b]	1.72	(2.1)	4.27	(5.2)	11.58	(14.1)	NG[c]	
C11	1.31	2.51	(1.9)	3.17	(2.4)	10.33	(7.9)	13.68	(10.4)	19.8	(15.1)
*C15	0.72	1.06	(1.5)	1.72	(2.4)	5.45	(7.6)	19.11	(26.5)	66.88	(92.9)
C21	0.68	1.00	(1.5)	1.51	(2.2)	5.12	(7.5)	10.38	(15.3)	NG	
*U7	0.84	1.29	(1.5)	2.01	(2.4)	15.57	(18.5)	NG		NG	
U17-1	0.84	1.46	(1.7)	2.08	(2.5)	8.85	(10.5)	33.44	(39.8)	NG	
*U30-1	0.77	1.08	(1.4)	2.52	(3.3)	11.92	(15.5)	42.13	(54.7)	NG	
*U30-2	1.08	1.66	(1.5)	2.6	(2.4)	12.04	(11.1)	45.60	(42.2)	NG	
V1	0.81	1.31	(1.6)	1.75	(2.2)	7.46	(9.2)	24.27	(30.0)	NG	
V3	0.76	1.44	(1.9)	2.94	(3.9)	20.19	(26.6)	NG		NG	
V6-1	0.93	1.64	(1.8)	2.32	(2.5)	7.04	(7.6)	11.04	(11.9)	NG	
V8-1	1.27	1.98	(1.6)	5.04	(4.0)	37.62	(29.6)	150.48	(118.5	NG	
V9	0.75	1.13	(1.5)	1.20	(1.6)	1.50	(2.0)			2.26	(3.0)
V9[e]	1.31					1.54	(1.2)	1.77	(1.4)	2.29	(1.7)
*V9-1	0.84	1.44	(1.7)	3.16	(3.8)	NG		NG		NG	
V30	0.81	1.29	(1.6)	2.12	(2.6)	NG		NG		NG	

[a] Doubling time.
[b] t_d : t_d of control.
[c] No detectable growth.

Doubling times increased with NaCl concentration in the medium. In the presence of 0.75 M NaCl, doubling times were 1.4 to 1.9 longer than in control medium, and 1.6 to 4 times longer than in the control in the presence of 1.0 M NaCl. At 1.5 M NaCl differences in the tolerance to salt became more marked: *V9-1 and V30 failed to grow, the doubling times of V3 an V8-1 were more than 26 times longer than those in control medium, while V9 was the isolate less susceptible to salt, with a doubling time only two-times longer than that in the control medium. In the presence of 1.75 M NaCl strains U7 and V3 failed to grow, and a further increase on the doubling times of all remaining strains was observed. Finally, only strains C11, *C15 and V9 grew at 2 M NaCl, with V9 showing a doubling time less than three times longer than that in the control medium; growth of strain C21 was extremely slow. As it is often reported in the literature, the upper limit of salt concentration at which a strain can grow was in many cases higher that in liquid medium.

Table 2: Relevant characteristics of the isolates described in Table 1.

Isolate	t_d[a] (h)	NaCl limit[b] (M)	Plasmid profile (kbp)	Antibiotic Resistance	Growth temp. (°C)	Growth pH	Melanin Production	Colony Type
C8-1	0.82	1.75	370, 515	Amp	8 - 40	4 – 11	-	I
C11	1.31	2.00	370, 515	Cm	8 - 40	6 – 10	weak	II
*C15	0.72	2.00	118, 370, 515	Amp	8 - 40	4 – 11	-	I
C21	0.68	1.75	370, 515	Amp	8 - 40	4 – 11	-	I
*U7	0.84	1.5	370, 515	Amp, Km, Str, Tc	8 - 40	4 – 11	+	I
U17-1	0.84	1.75	370, 515	Amp, Tc	8 - 40	4 – 11	+	I
*U30-1	0.77	1.75	370, 515	Amp, Km, Str, Tc	8 - 40	4 – 11	+++	III
*U30-2	1.08	1.75	370, 515	Amp, Tc	8 - 40	4 – 10	+	IV
V1	0.81	1.75	118, 370, 515	Amp, Tc	8 - 40	4 – 11	+	I
V3	0.76	1.50	118, 370, 515		8 - 40	4 – 11	-	II
V6-1	0.93	1.75	370, 515	Amp, Tc	8 - 40	4 – 11	+	I
V8-1	1.27	1.75	118, 370, 515	Amp, Cm	8 - 40	5 – 11	-	I
V9	0.75	2.00	118, 370, 515		12 - 45	4 – 11	-	V
*V9-1	0.84	1.0	118, 370, 515		8 - 40	5 – 11	-	IV
V30	0.81	1.0	370, 515	Amp, Cm, Km, Str	8 - 40	4 – 11	++	VI

[a] Doubling time in TY liquid medium at 30 °C.
[b] Highest concentration of NaCl in TY liquid medium at which growth was observed (see Table 1).

All isolates could grow in TY liquid medium at temperatures in the range of 12 - 40 °C, and V9 grew also at 45 °C. Except for V9, all isolates grew on agar plates at 8 °C (Table 2). When cultivated in TY liquid media adjusted to pH increments of one unit in the range of 4 to 11, most isolates could multiply. Exceptions were isolates V8-1 and *V9-1 which failed to grow at pH 4, C11 which grew only from pH 6, and *U-30-2 which grew up to pH 10 only (Table 2).

Strains of rhizobia differ in their natural resistance to various antibiotics, a characteristic which provides a means of identifying closely related strains. Thus, preliminary screening of antibiotic resistance was carried out by plating serial dilutions of cultures on TY medium containing with one of the following: ampicillin (10 µg/ml), chloramphenicol (20 µg/ml), kanamycin (30 µg/ml), streptomycin (50 µg/ml), or tetracycline (10 µg/ml). Although colony numbers may have decreased somewhat in the presence of chloramphenicol (and occasionally with other antibiotics) the resistance patterns presented on Table 3 were observed. Capacity to grow on plates supplemented with ampicillin was the most widely distributed, followed by that to grow in the presence of tetracycline. Isolates U7, U30-1 and V-30 grew in the presence of four of the antibiotics.

Production of melanin is a phenotypic characteristic which can also assist on identification of closely related rhizobia. Screening of the isolates for this property was by detection of the formation of a diffusible dark-brown pigment by cells grown on TY plates supplemented with tyrosine and copper (Cubo *et al.*, 1988). Rapid and abundant production of melanin was observed by isolate U30-1, closely followed by isolate V30; less abundant production was detected for isolates U7, U17, U30-2, V1 and V6. The remaining isolates failed to produce a diffusible dark pigment and were considered to be Mel^- (Table 2).

All isolates grew in Luris-Bertani. Inspection of the isolates on TY medium showed at least 6 different types of colonies (Table 2):
Type I: Medium-size, round, convex, shiny and white.

Type II: Young colonies as I, becoming yellow and umbonate later.
Type III: Young colonies as I, becoming beige later.
Type IV: As type I but smaller.
Type V: Medium, round, white, dull and curled.
Type VI: Young colonies as I, later becoming darker than III.

The above characterization studies confirmed that the selected isolates are salt-tolerant N_2-fixing nodulating bacteria with the ability to grow within a wide range of pH and temperature values. Furthermore, parameters allowing distinguishing the different isolates were defined.

Another set of bacterial isolates was obtained form nodules of alfafa growing in salinity affected soils in Uzbekistan. Their characterization is in progress, and data on their resistance to salt (when cultivated in solid pea broth-based medium) is summarized in Table 3. In addition, three new salt-resistant isolates (T1, T2 and T5) were obtained from nodules of *Onobrychis transcaucasica.*

2. Growth and inoculation of leguminous plants of the Kyzil-Kum desert

The next task was to define conditions favorable to plant growth and root nodulation. Direct inoculation of strains originally isolated from desert nodules on their host plant species was tested, as well as cross inoculation, i.e. inoculation on a plant species different of that from which the strain was isolated. Nodulation on the latter would give an indication of the potential of the isolates to serve as inoculants to different plant species.

2.1. *Ammodendron conollyi, Astragalus vilosissimus* and *Astragalus unifoliolatus*

First plants were studied for their capacity to grow under control conditions, i.e., in the absence of inoculum and salinity. A "light structure" potting mixture was used in which sand was the major component, and moisture was maintained within the range of 10-15%. Under these conditions, the *Astragalus* species developed better than *Ammodendron conollyi* (Table 4), although all plants grew several-fold faster than in their native habitat, the size of 2.5 months-old plants being similar to that of 1-2 years old plant growing in their natural habitat in Uzbekistan. This indicates that the growth potential of these species is only partially expressed under desert conditions, and that given sufficient water and mineral nutrients, faster growth is possible. Dry mass allocation to root and shoot was fairly equal for both, contrary to the native situation in which there is a preferential allocation of biomass to the root. Nodulation was limited to one single occasional nodule per plant.

Table 3: Tolerance to salt of bacteria isolated from nodules of alfalfa plants grown in salt-affected soils in Uzbekistan.

Strain	Control	NaCl concentration (M)							
		0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.5
1	+++	+++	+++	+++	+++	+	-	-	-
2	+++	+++	+++	+++	+++	+-	-	-	-
3	+++	++	+	-	-	+-	-	-	-
4	+++	+++	+++	++	+	-	-	-	-
5	+++	+++	+++	+++	+++	-	-	-	-
6	+++	+++	+++	+++	+++	-	-	-	-
7	+++	+++	+++	++	-	-	-	-	-
8	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	+++	+++	+++	+++	+++	-	-	-	+
10	+++	+++	+++	+++	+++	-	-	-	+
11	+++	+++	+++	+++	+++	-	-	-	-
12	+++	+++	+	-	-	-	-	-	-
13	+++	+++	++	+	+++	+++	+++	++	-
14	+++	+++	+++	+++	+++	+	+	+	-
15	+++	+++	+++	+++	+++	+++	+++	+++	+++
16	+++	+++	+++	-	++	-	-	-	-
17	+++	+++	+++	+++	+++	-	-	-	-
18	+++	+++	+++	+++	+++	-	-	-	-
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21	+++	+++	+++	+++	+++		-	-	-
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25	+++	+++	+++	+++	+++	-	-	-	-
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33	+++	+++	+++	+++	+++	+++	++	-	-
35	+++	+++	+++	+++	+++	+++	+	-	-
36	+++	+++	+++	+++	+++	+	-	-	-
37	+++	+++	++	-	-	-	-	-	-
38	+++	+++	+++	+++	+++	+	+	-	-
39	+++	+++	+++	+++	+++	+	-	-	-
40	+++	+++	++	-	-	-	-	-	-

Table 4: Development of 2.5-months old plants of *Ammodendron conollyi*, *Astragalus vilosissimus* and *Astragalus unifoliolatus*. Data are average of five replicate plants.

Plant species	Shoot biomass (mg plant^{-1})		Root biomass (mg plant^{-1})		Plant biomass (mg plant^{-1})		Plant height
	Fresh	Dry	Fresh	Dry	Fresh	Dry	(cm)
Ammodendron conollyi	630	329	587	318	1217	647	9
Astragalus vilosissimus	781	340	791	272	1572	612	20
Astragalus unifoliolatus	902	364	933	305	1835	669	22

Next the following inoculation experiments were carried out:

a) Seedlings of *Ammodendron conollyi* were inoculated with one of the 10 strains isolated from its nodules, or with one of the 10 strains isolated from nodules of *Astragalus vilosissimus*.

b) Seedlings of *Astragalus vilosissimus* were inoculated with one of the 10 strains isolated from its own nodules, with one of 5 strains isolated from nodules of *Ammodendron conollyi*, or with one of 5 strains isolated from nodules of *Astragalus unifoliolatus*.

c) Seedlings of *Astragalus unifoliolatus* were inoculated with its own 10 isolates and with 10 strains isolated from nodules of *Astragalus vilosissimus*.

The reason for the predominance of treatments with isolates from *Astragalus vilosissimsu* was that when collecting the material in the field these nodules were red, usually indicative of active N_2 fixation and the presence of leghaemoglobin. On the other hand, the nodules harvested from *Astragalus unifoliolatus* were white, and in *Ammodendron conollyi* only single dark-brown to black nodules were present. Bacteria for inoculation experiments, were grown to the end of the logarithmic phase in TY or pea broth liquid media, harvested, and resuspended in mineral medium to a concentration of approximately 10^9 cells ml^{-1}; 1 ml of this suspension was added to each plant container. The effect of inoculation was assessed by measuring the following plant parameters: root length, shoot length, fresh and dry weight, number and size of nodules, and acetylene reducing activity (ARA) of intact plants.

Nodulation was first tested in seedlings growing in glass tubes, on strips of filter paper moistened with plant growth medium. Plants showed good development during the first month, but further growth and nodulation were not observed.
Inoculation tests were then carried out in seedlings planted in a greenhouse, in one of the following potting substrates:

a) Sterile vermiculite.
b) A "light structure" substrate consisting of a sterilized 1:1:2 mixture of peat:vermiculite:sand.
c) A 3:1 sterile mixture of sand:vermiculite, a structure that was closer to natural field conditions.
d) Sede Boqer soil.

Seedlings grown in just vermiculite were larger than those growing in other test tubes. However, growth also stopped after 1-1.5 months and nodulation did not occur. This may have been due to the high moisture in the substrate in which the plants were growing.

Under natural environmental conditions in Uzbekistan deserts, moisture in the 0-30 cm soil horizon reaches only 7-15 % in early spring. The proportion of sand was higher in the 3rd type of potting substrate and moisture was maintained at 5-7 %. Under these conditions, plant growth was more vigorous than above and nodulation could be observed in all plants 2 months after planting (Tables 5-7).

Table 5: Effect of inoculation on growth and nodulation of two-months old plants of *Ammodendron conollyi*. Isolates as in Table 1. Results are average of 5 plants.

Isolate	Shoot biomass		Shoot	Root biomass		Root	Nodules	Nodule	Nodule	ARA	Relative	ARA
	Fresh	Dry	height	Fresh	Dry	length	plant^{-1}	size	mass		DW	nodule^{-1}
	(mg)	(mg)	(cm)	(mg)	(mg)	(cm)	(n°)	(mm)	(mg)	(units)	(%)	(units)
Control	**248**	**67**	**4.5**	**300**	**85**	**27**	-	-	-	-	**100**	
C8-1	285	96	4.5	820	233	28	2.2	1-5	12.5	6888	202	3131
C11	290	106	4.5	535	153	33	4.0	1-5	17.5	7031	151	1758
*C15	225	60	5.0	375	100	28	4.0	2-5	17.5	7446	109	1862
C21	270	80	4.5	320	88	31	5.0	2-5	27.5	12144	108	2429
C1-1	296	99	4.8	430	130	29	5.0	2-7	22.5	16692	132	3338
C2	240	73	4.9	480	143	28	5.2	1-6	22.5	13383	131	2574
C4-1	255	66	4.5	330	86	30	2.0	1-4	10.0	6416	107	3208
C12-1	310	96	5.0	723	212	37	5.0	1-6	20.0	16730	189	3346
C13-1	246	73	4.8	370	94	32	3.2	1-6	6.6	6076	112	1899
*C18-1	263	99	4.6	523	134	36	4.5	1-4	19.5	12153	143	2701
Avg. C	**266**	**83**	**4.7**	**473**	**133**	**31**	**4**		**17.6**	**10496**	**134.9**	**2624**
V1	343	103	5.0	366	96	28	4.0	1-3	15.0	11457	129	2864
V3	250	90	4.0	273	78	28	2.0	2-5	18.0	3040	95	1520
V6-1	256	89	5.5	520	140	32	4.0	1-4	14.0	9069	142	2267
V8-1	300	101	4.5	500	147	33	4.0	1-3	20.0	4587	146	1147
V9	290	90	4.7	490	122	28	1.0	1-3	4.0	2381	142	2381
* V9-1	346	113	4.0	650	149	29	3.0	1-4	15.0	3697	182	1232
V30	320	94	4.0	350	97	31	3.0	1-3	12.5	7776	122	2592
*V2	253	66	4.0	436	124	37	3.0	1-6	16.5	8011	126	2670
*V26-1	250	73	4.3	430	129	35	3.0	2-6	30.0	7300	124	2433
*V36-1	230	62	4.0	300	85	36	3.0	1-6	7.5	2377	97	792
Avg.V	**284**	**88**	**4.4**	**432**	**117**	**32**	**3.0**		**15.3**	**5970**	**130.5**	**1990**

Average biomass production was similar in all *Ammodendron conollyi* plants (Table 5) (Fresh C= 739, V= 716; Dry C= 114, V=120), as was shoot height and root length. Nodules were pink and large (up to 7 mm), and their number tended to be higher with C inoculants. Plants inoculated with C strains showed ARA twice higher than plants inoculated with V strains, suggesting a better symbiotic association between *Ammodendron conollyi* with C inoculants.

Table 6: Effect of inoculation on growth and nodulation of two-months old plants of *Astragalus vilosissimus*. Isolates as in Table 1. Data are average of 5 plants.

Isolate	Shoot biomass Fresh (mg)	Shoot biomass Dry (mg)	Shoot height (cm)	Root biomass Fresh (mg)	Root biomass Dry (mg)	Root length (cm)	Plant biomass Fresh (mg)	Plant biomass Dry (mg)	Nodules plant^{-1} (n°)	Nodule size (mm)	ARA plant^{-1} (units)	Relative DW (%)	ARA nodule^{-1} (units)
Control	**327**	**116**	**10.5**	**650**	**210**	**29**	**977**	**326**	**-**	**-**	**-**	**100**	
V1	428	143	9.5	803	179	32	1231	322	25.0	1-2.5	5965	126	239
V3	344	124	8.0	699	233	27	1043	357	10.0	1-2.5	1772	107	177
V6-1	371	115	10.5	713	174	26	1084	289	19.0	1-2.5	3972	111	209
V8-1	372	141	8.5	609	195	27	981	336	18.2	1-2.5	3805	100	209
V9	513	142	12.5	800	276	34	1313	418	18.2	1-2.5	6233	134	342
*V9-1	365	119	11.5	598	187	30	963	306	17.5	1-2.5	4623	99	264
V30	311	121	7.0	750	250	29	1061	371	17.0	1-2.5	4063	109	239
*V2	303	111	6.0	601	252	29	904	363	5.2	1-2.5	900	93	173
*V26-1	353	133	12.0	834	418	28	1187	551	20.0	1-2.5	7157	122	358
*V36-1	426	142	9.5	640	221	27	1066	363	11.0	1-2.5	890	109	81
Avg. V	**374**	**128**	**9.6**	**700**	**236**	**29**	**1074**	**364**	**16.1**		**3938**	**110**	**229**
*U7	330	110	9.0	652	233	28	982	343	10.0	1-2.5	750	100	75
U17-1	432	120	11.5	777	242	30	1209	362	12.0	1-2.5	1736	124	145
*U30-1	293	93	10.0	367	121	29	660	214	18.0	1-2.5	2454	68	136
*U30-2	301	90	10.5	353	108	27	654	198	12.0	1-2.5	1590	67	133
U3-1	354	141	11.0	503	204	34	857	345	15.0	1-2.5	843	88	56
Avg. U	**342**	**111**	**10.4**	**530**	**182**	**30**	**965**	**344**	**13.4**		**1475**	**89**	**109**
C8-1	416	149	10.0	1000	344	33	1416	493	15.5	1-2.5	12935	145	835
C11	400	133	10.0	850	291	31	1250	424	20.0	1-2.5	6634	128	332
*C15	435	132	15.0	334	129	33	769	261	29.0	1-2.5	12008	79	414
C21	510	155	12.0	1061	350	32	1571	505	21.0	1-2.5	7195	161	343
C1-1	357	119	13.0	543	173	28	900	292	6.2	1-2.5	1500	92	242
Avg. C	**423.6**	**138**	**12.0**	**758**	**257**	**31**	**1021**	**344**	**18.3**		**8054**	**121**	**433**

In *Astragalus vilosissimus* (Table 6) nodulation occurred with all inoculants and was absent in the non-inoculated controls. Nodules were more numerous and smaller (1-2.5 mm) than those of *Ammodendron conollyi,* and their colour varied from pink to pale green and white. The number of nodules formed per plant varied little with type of inoculum used. The highest ARA per plant and per nodule were observed with strains C. The lowest ARA per plant was observed with strains U, however this had little effect on the relative yield of plant biomass: plants inoculated with C had an average reductase activity double of that of plants inoculated V strains but showed only a 20% increase of biomass production.

Table 7: Effect of inoculation on growth and nodulation of two-months old plants of *Astragalu unifoliatus*. Isolates as in Table 1. Data are average of 5 plants.

Isolate	Shoot biomass Fresh (mg)	Shoot biomass Dry (mg)	Shoot height (cm)	Root biomass Fresh (mg)	Root biomass Dry (mg)	Root length (cm)	Root biomass Fresh (mg)	Root biomass Dry (mg)	Nodules plant^{-1} (n°)	Nodule size (mm)	ARA plant^{-1} (units)	Relative DW (%)	ARA nodule^{-1} (units)
Control	**450**	**112**	**6.0**	**642**	**206**	**30**	**1092**	**318**	-	-	-	**100.0**	-
U-7	300	73	5.0	835	193	28	1135	266	19.2	0.5-2.0	2624	65.1	137
U17-1	503	130	5.5	1030	253	35	1533	383	20.2	0.5-2.0	3772	116.0	187
*U30-1	590	170	6.0	990	330	29	1580	500	20.0	0.5-2.0	10811	151.7	541
*U30-2	670	136	6.0	1240	443	33	1910	579	21.0	0.5-2.0	2834	121.4	135
U3-1	498	116	7.0	1000	360	35	1498	476	41.0	0.5-2.0	7763	103.5	189
*U4	266	70	6.5	732	200	30	998	270	19.0	0.5-2.0	3557	62.5	187
*U20-1	465	123	11.0	980	366	33	1445	489	23.0	0.5-2.0	3648	109.8	159
*U23	627	153	9.5	990	230	38	1617	383	32.0	0.5-2.0	9783	136.6	306
*U2-1	261	70	8.0	646	170	25	907	240	19.0	0.5-2.0	1236	62.5	65
*U28	706	153	9.0	758	193	41	1464	346	24.0	0.5-2.0	4871	136.6	203
Avg. U	**489**	**119**	**7.4**	**920**	**274**	**33**	**1409**	**393**	**23.8**		**5090**	**106.6**	**211**
V1	660	170	8.5	726	270	40	1386	440	32.0	0.5-2.0	11502	151.7	359
V3	530	130	8.0	793	260	30	1323	390	27.0	0.5-2.0	7881	116.0	292
V6-1	650	173	7.5	770	293	28	1420	466	28.5	0.5-2.0	5473	154.4	192
V8-1	740	176	11.0	1.11	443	34	741.1	619	35.0	0.5-2.0	11258	157.1	322
V9	546	140	7.0	1.18	470	39	547.2	610	26.0	0.5-2.0	6960	125.0	268
*V9-1	650	163	8.0	780	280	33	1430	443	25.0	0.5-2.0	8073	145.5	323
V30	584	146	6.5	600	226	29	1184	372	23.5	0.5-2.0	8728	130.3	371
*V2	573	156	7.0	753	246	38	1326	402	35.0	0.5-2.0	7354	139.2	210
*V26-1	683	196	13.5	740	260	29	1423	456	28.7	0.5-2.0	9991	175.0	348
*V36	583	166	13.5	1240	390	37	1823	556	40.0	0.5-2.0	7401	148.2	185
Avg. V	**620**	**162**	**9.1**	**640**	**314**	**34**	**1260**	**475**	**30.1**		**8462**	**144.2**	**287**

Astragalus unifoliatus plants (Table 7) produced numerous nodules that varied in size from 0.5 to 2.0 mm, and were colored from pink to pale green and white. The number of nodules and the calculated ARA per nodule were higher with V strains. Production of biomass was also slightly higher in plants inoculated with V strains.

The above experiments were a preliminary screening of strains previously selected on the basis of their tolerance to salinity, and a first attempt to estimate the specificity of the host/bacteria interaction. Thus, the most important observation was that all strains caused nodulation in the three host species studied. In general, the number of nodules per plant was significantly lower in *Ammodendron conolly,* and plants of *Astragalus unifoliatus* produced the highest number of nodules. The observed lack of host specificity indicates that these salt-tolerant bacterial isolates are potential inoculants for different plant species under saline conditions, which are known to be adverse to a wide range of symbiotic nitrogen-fixing bacteria.

2.1.1 Drought tolerance

Inoculated seedlings of *Ammodendron conollyi, Astragalus vilosisssimus* and *Astragalus unifoliolatus* were planted in rows in a square plot in a greenhouse, in Sede Boqer (Israel). Plants were grown over a period of 2.5 months and the watering regime aimed at reaching

the desired levels of soil moisture, which within a week had reached 15-20 %. Further watering of the soil adjacent to the bed, was conducted along one side of the plot while the other was kept dry. Three waterings during the growth period allowed a fairly constant soil moisture gradient of approximately 4 to 7% (Table 8). Under similar experimental conditions, plants of *Ammodendron* grew slowly, while *Astragalus unifoliatus* plants grew much faster. Plant biomass production showed a strong response to changes in soil moisture content. The effect was more marked at the lower levels of moisture, and a 10 to 15-fold difference in biomass production was observed between the two extremes of the moisture gradient.

Table 8: Effect of soil moisture content on the development of 2.5 months-old plants of *Ammodendron conollyi, Astragalus vilosissimus* and *Astragalus unifoliolatus*.

Plant	Soil moisture	Shoot biomass (mg plant^{-1})		Dry weight	Plant height
	(%)	Fresh	Dry	(%)	(cm)
Ammodendron Conollyi.	3.8	203	73.3	36.1	4.5
	4.32	619	210	33.9	8
	4.86	1170	345	29.5	15
	5.48	1630	545	33.4	18
	6.41	2320	795	34.3	21
Astragalus Vilosissimus	3.8	160	63.3	39.6	8.5
	4.32	446	193	43.3	15
	4.86	683	286	41.9	22
	5.48	1446	696	48.1	31
	6.41	2416	980	40.6	30
Astragalus Unifoliolatus	3.8	366	105	28.7	9
	4.32	1030	293	28.4	14
	4.86	1616	433	26.8	16.5
	5.48	2426	733	30.2	18.8
	6.41	3596	1086	30.2	34

2.1.2. Effect of salinity on plant development and nodulation

Plants were exposed to salt by adding different concentrations of NaCl to the nutrient solution (0, 0.1, 0.2, 0.3 or 0.5 M), either in the absence or in the presence of 1 mM NH_4NO_3 (Table 9). Seedlings of *Ammodendron conollyi, Astragalus vilosissimus* and *Astragalus unifoliolatus* were inoculated with strains C8-1, V9 and U17-1 respectively.

All plant species showed a remarkable tolerance to salinity. Surviving plants exposed to 500 mM NaCl, a concentration at which known crops generally die, caused at the most 40% growth

Table 9: Effect of salinity, in the presence or absence of added N, on nodulation and development of plants of *Ammodendronconollyi*, *Astragalus vilosissimus* and *Astragalus unifoliolatus* inoculated with strains C8-1, V9 and U17-1 respectively.

N Supply	NaCl M	Shoot biomass (mg plant^{-1})		Shoot height (cm)	Root biomass (mg plant^{-1})		Root length (cm)	Plant biomass (mg plant^{-1})		DW inhib. (%)	Nodules plant^{-1} (n°)	Relative FW yield (%)	Survival (%)
		Fresh	Dry		Fresh	Dry		Root biomass					
Ammodendron conollyi													
-N	control	172	76	4	122	50	23	294	126	-	1.75	100	100
	0.1	206	78	4	120	48	19.5	326	126	0	1	111	100
	0.2	200	70	3.5	130	50	20	330	120	5	-	112	75
	0.3	165	62	3	114	38	18	279	100	21	-	95	50
	0.5	105	40	3.5	110	40	19	215	80	37	-	73	25
+N	control	290	80	5	122	40	24	412	120		2	100	100
	0.1	300	93	4.5	173	53	21	473	146	-22	2	115	100
	0.2	280	90	4	140	48	19.5	420	138	-15	-	102	100
	0.3	130	68	3	129	30	18	259	98	18	-	63	50
	0.5	100	44	3	130	33	19	230	77	36	-	56	50
Astragalus vilosissimus													
-N	control	463	100	9	623	100	38	1086	200		11	100	100
	0.1	315	57.5	4.5	370	50	34	685	107.5	46	4	63	46
	0.2	160	45	3.5	322	83	32	482	128	36	1	44	38
	0.3	152	40	3.6	240	57	21	392	97	52	-	36	8
	0.5	100	20	1	80	20	18	180	40	80	-	17	8
+N	control	397	90.9	8.5	524	76	39	921	166.9		9.5	100	100
	0.1	502	85	7	261	43	29	763	128	23	0.75	83	92
	0.2	307	57.5	5.5	260	40	28	567	97.5	42	0.25	62	38
	0.3	210	60	6	120	36	20	330	96	42	-	36	15
	0.5	300	60	3	80	30	19	380	90	46	-	41	8
Astragalus unifoliolatus													
-N	control	176	32	6	273	42	32	449	74		12.5	100	100
	0.1	286	45	3	246	46	30	532	91	-23	7.5	119	67
	0.2	222	36	3	273	43	30	495	79	-7	5	113	40
	0.3	158	32	2	112	28	28	270	60	19	4	100	20
	0.5	50	10	2	109	36	17	159	46	38	-	35	7
+N	control	247	50	7.5	307	42	29	554	92		8	100	100
	0.1	392	53	4	266	50	27	658	103	-12	3	119	73
	0.2	200	42.5	3	285	45	27	485	87.5	5	1	88	40
	0.3	150	35	2	113	33	25	263	68	26	-	48	20
	0.5	125	30	1	60	25	12	185	55	40	-	33	13

inhibition. Added N fertilization generally increased salt-tolerance, especially at the range 0.1-0.2 M NaCl, supporting the idea that one of the effects of salinity is to inhibit the uptake and assimilation of N ions from the soil. Added fertilization increases the capacity of the crop to survive and to keep growing in the presence of very high levels of salinity (0.5 M). Since non-inoculated controls were missing in this experiment, it is not known if

the inoculum increased the resistance of the plants to salt. This is being investigated in experiments in progress.

2.2. *Acacia saligna*

Acacia saligna was selected as a desert leguminous tree to test the range of hosts capable of using the salt and drought tolerant strains of N_2-fixers isolated.

Table 10: Effect inoculating *Acacia saligna* with bacteria isolated from nodules of desert plants bacteria. Results are average of six replicate in 45-days old plants.

Inoculum	Shoot length (cm)	Root length (cm)	Plant DW (mg)	N° Nodules plant^{-1}	Nodule size (mm)	ARA plant^{-1} (nmol)	Relative DW (%)
Control	9.0	12.8	14.1	-	-	-	100
C8-1	8.3	10.1	17.3	2.0	0.5-2.0	229	123
C11	9.1	10.6	15.6	-	-	-	111
*C15	7.4	11.5	14.8	-	-	-	105
C21	10.3	8.1	16.5	1.5	0.3-1.0	274	117
*U7	11.2	13.7	12.8	-	-	-	91
U17-1	10.1	9.5	14.4	-	-	-	102
*U30-1	6.8	12.1	14.7	-	-	-	104
V1	9.8	12.9	16.9	2.0	0.3-1.5	211	120
V3	10.5	12.3	13.7	-	-	-	97
V6-1	11.8	11.8	15.3	-	-	-	109
V8-1	8.4	13.7	16.8	1.7	0.3-2.0	123	119
V9	10.6	11.8	16.1	2.0	0.3-1.5	184	114
*V9-1	7.8	10.8	15.3	1.5	0.3-1.5	87	109
*V26-1	9.4	13.5	15.0	-	-	-	106
V30	9.5	15.1	15.3	-	-	-	109
*V36-1	8.7	14.3	11.7	-	-	-	83
T1	7.3	12.4	16.1	2.0	0.1-1.0	386	114
T2	10.7	8.6	14.2	1.5	0.1-0.8	115	101
T3	11.4	13.9	16.3	2.5	0.1-1.5	298	116

Germinated seeds of *Acacia saligna* were planted in 60 ml-sterile containers filled with vermiculite and 8-9 ml of mineral nutrient medium without nitrogen. Plants were grown for 45 days under greenhouse conditions. The effect of added mycorrhiza was also tested. Mycorrhiza are symbiotic fungi that interact with higher plant roots. Endomycorrhiza are capable of establishing symbiotic interactions with 70 % of the vascular plants, penetrating and growing within the plant root cells. The expected benefits of mycorrhiza are: a) concentrating and accumulating phosphorous and other essential mineral nutrients in poor soils; b) protection against pathogenic soil fungi; c) cellulase and pectinase activities which may play a role in the opening and loosening of the root surface thus facilitating the formation of nitrogen-fixing nodules on roots of non-legumes. Inoculation with mycorrhiza was carried out at the time of planting by placing at a depth of 1-2 cm 1 g of a commercial preparation of *Glomus mosseae* (VAMINOC, Great Britain).

Nodulation of *Acacia saligna* seedlings occurred in the presence of some of the bacterial isolates from nodules of *Ammodendron connollyi*, *Astragalus unifoliolatus*, *Astragalus vilosissimus* and *Onobrychis transcaucasica* (Table 10). In the presence of added mycorrhiza, nodulation occurred with all isolates and in many cases the effect of nodulation on biomass production was significant.

Table 11: Effect of inoculating *Acacia saligna* with bacteria isolated from nodules of desert plants bacteria in the presence of added mycorrhiza. Strains T were isolated from nodules of *Onobrychis transcaucasica*, other isolates as described in Table 1. Results are average of six 45-day old replicate plants.

Inoculum	Shoot length (cm)	Root length (cm)	Plant DW (mg)	N° Nodules (plant^{-1})	Nodule size (mm)	ARA (nmol plant^{-1})	Relative DW (%)
Control	10.6	13.1	14.6	-	-	-	100
C8-1	11.0	11.5	19.2	3.0	0.2-1.0	293	132
C11	11.8	14.2	20.0	2.7	0.5-1.5	290	137
*C15	9.7	14.7	16.0	1.5	0.2-1.0	89	110
C21	11.0	11.8	20.8	3.5	0.3-1.5	380	142
*U7	11.5	14.4	19.2	4.0	0.3-1.3	425	132
U17-1	11.2	11.6	18.0	2.5	0.5-1.0	350	123
*U30-1	10.8	12.8	15.8	1.7	0.3-1.1	285	108
V1	11.7	15.2	21.0	3.0	0.7-1.5	265	144
V3	11.0	10.0	12.3	1.5	0.2-0.5	105	84
V6-1	14.3	9.0	16.5	3.0	0.5-0.3	230	113
V8-1	9.0	12.2	17.0	1.7	0.2-2.0	215	116
V9	10.5	11.7	15.8	1.7	0.2-2.0	145	108
*V9-1	9.3	10.8	17.5	1.5	0.5-1.5	100	120
*V26-1	12.5	14.0	19.0	1.0	0.5-1.5	250	130
V30	11.8	15.3	18.5	2.5	0.8-1.5	135	127
*V36-1	11.5	12.0	19.8	2.3	0.5-1.0	285	136
T1	10.3	11.5	17.6	3.0	0.8-2.5	225	121
T2	12.3	13.0	15.5	1.3	0.5-1.0	150	106
T3	10.4	15.2	18.3	2.3	0.5-2.5	270	126

2.2. Alfafa

Alfalfa was the crop selected to test the host range of the N_2-fixing isolates. The alfafa varieties studied were the arid salt-tolerant "Khozem-2 (X-2), and the semi-arid "Tashkent-1728" (T-1728) and "Tashkent-2009". Germinated seeds were planted in 60 ml-sterile tubes, and plants were grown for 30 days under greenhouse conditions.

Bacteria isolated from *Ammodendron connollyi*, *Astragalus unifoliolatus* and *Astragalus vilosissimus* failed to produce N_2-fixing nodules on roots of alfafa plants, either in the presence or in the absence of added mycorrhiza. Pseudonodules were formed in the presence of V9, V3, *V26-1 and V2, and with U3-1 in the presence of mycorrhiza (results not shown). As might be expected, active nodules were formed in plants inoculation with strains that had been obtained from alfafa nodules, either in the presence or in the absence of added mycorrhiza, (Tables 12 and 13). With T-1728 plants and added mycorrhiza, the alfafa isolates were less effective in increasing biomass production than the commercially available *R. meliloti* inoculum, CXM1; in the presence of added mycorrhiza treatment with strain 24 resulted in the highest production of biomass (Table 12).

Table 12: Development of 30-days old T-1728 alfalfa plants inoculated with strains isolated from nodules of alfafa plants growing under saline conditions, and effect of mycorrhiza. Results are averages of 5 replicate plants

	Bacterial Inoculum	Salt-resistance (M NaCl)	Plant DW (mg)	N° nodules plant^{-1}	Nodule size (mm)	ARA (nmol plant^{-1})	Relative DW (%)
-M[a]	Control	-	5.8	-	-	-	100
	1	0.8	5.5	2.5	0.3-0.5	35.5	95
	3	0.2	7.0	2.6	0.3-1.5	131.3	121
	8	1.5	6.8	1.5	0.5	98.0	116
	11	0.8	6.5	2.0	0.5-1.5	83.0	112
	13	1.2	6.5	2.5	0.3-2.0	133.0	112
	20	1.0	6.8	2.6	0.5-1.0	87.0	117
	24	1.2	7.6	2.0	0.3-2.0	127.0	131
	28	0.8	8.0	3.0	0.3-1.5	163.0	138
	40	0.2	7.0	2.5	0.3-0.9	142.0	121
	CXM1[b]	0.4	8.1	3.0	0.3-2.0	159.0	141
+M[+] aaaa	Control	-	6.4	-	-	-	100
	1	0.8	5.5	2.0	0.3-0.5	42.0	86
	3	0.2	7.0	3.0	0.5-1.0	111.0	109
	8	1.5	6.8	2.0	0.5-1.5	131.0	105
	11	0.8	6.8	2.5	0.3-2.0	145.0	105
	13	1.2	7.5	3.5	0.5-1.5	154.0	117
	20	1.0	5.5	1.0	0.5	21.0	86
	24	1.2	8.1	3.5	0.5-1.5	184.0	127
	28	0.8	7.8	2.0	0.5-1.0	91.0	122
	40	0.2	7.2	2.5	0.3-1.5	112.0	113
	CXM1	0.4	7.6	2.5	0.3-1.0	161.0	119

[a] With or without mycorrhizal inoculation.
[b] CXM1 is a commercially available strain of *R. meliloti* resistant to 0.4 M NaCl.

A somewhat different picture emerged from the experiment with X-2 alfafa plants. In the absence of added mycorrhiza, similar production of biomass was observed in the treatments with CXM1 or with strains 12, 14, 24, 40; with mycorrhiza, the commercial rhyzobium inoculum brought about the highest production of biomass (Table 13).

Table 13: Development of 30-days old X-2 alfalfa plants inoculated with strains isolated from nodules of alfafa plants growing under saline conditions, and effect of mycorrhiza. Results are averages of 5 replicate plants.

	Bacterial Inoculum	Salt-resistance (M NaCl)	Plant DW (mg)	N° nodules (plant^{-1}	Nodule size (mm)	ARA (nmol plant^{-1})	Relative DW (%)
-M[a]	Control	-	4.7	-	-	-	100
	1	0.8	5.5	3.0	0.5-1.5	62.5	117
	3	0.2	5.8	3.5	0.5-1.0	93.8	123
	5	0.6	5.6	2.5	0.5-1.0	31.3	119
	8	1.5	6.2	3.8	0.3-1.5	135.0	132
	11	0.8	6.1	2.6	0.5-1.5	129.0	130
	12	0.2	6.7	2.5	0.3-2.0	130.0	143
	13	1.2	6.4	3.0	0.3-1.5	152.0	136
	14	0.8	6.8	4.0	0.5-1.0	150.0	145
	24	1.2	7.0	3.5	0.3-1.0	140.0	149
	40	0.2	7.0	6.0	0.3-1.5	170.0	149
	CXM1[b]	0.4	6.9	3.2	0.5-1.0	165.0	147
+M[a]	Control	-	6.2	-	-	-	100
	1	0.8	6.7	2.0	0.3-1.5	117.5	108
	3	0.2	6.5	3.5	0.5-1.0	102.5	105
	5	0.6	5.8	2.0	0.3-0.9	51.2	94
	8	1.5	7.2	3.5	0.5-1.0	77.0	116
	11	0.8	8.1	2.5	0.5-2.0	180.0	131
	12	0.2	7.0	4.0	1.0-1.5	150.0	113
	13	1.2	8.0	2.5	0.5-1.5	140.0	129
	14	0.8	7.0	6.0	0.3-2.0	165.0	113
	24	1.2	7.1	1.6	0.5-1.0	125.0	115
	40	0.2	8.0	2.5	0.5-2.0	200.0	129
	CXM1	0.4	8.6	3.9	0.5-1.2	141.8	139

[a] With or without mycorrhizal inoculation.
[b] CXM1 is a commercially available strain of *R. meliloti* resistant to 0.4 M NaCl.

References

Cubo M. T., Buendia-Claveria A. M., Beringer J. E. and Ruiz-Sainz J. E. 1988. Melanin production by *Rhyzobium* strains. Appl. Environ. Microbiol. 54: 1812-1817.

Maniatis T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbour, N.Y.

C) Scientific Impact of Collaboration

The Uzbek PIs have learned new and advanced molecular biology techniques used on the isolation of stress tolerant N_2-fixing strains. They have also been acquainted with a framework of planning and carrying out long-range research in a methodical way. They have been exposed to the use of modern laboratory instrumentation, both in molecular biology and on the use of modern computers, including literature searches through electronic libraries and the Internet. Their cooperation with Israeli colleagues has been developing very well.

D) Description of Project Impact

It is too early to assess the impact of this project to enhance the capacity to produce leguminous crops in desert areas affected by drought and salinity. Preliminary results are highly encouraging but further work is required, including field experiments that are now in preparation. The isolates of highly stress tolerant strains of N_2-fixers constitutes a very valuable collection which could be put to use within a relatively short time in Uzbekistan.

E) Strengthening of Developing Country Institutions

New equipment and training are the two major elements that this project has been providing to the Uzbek laboratory. The following equipment was purchased: two Hewlett Packard computers, one Astra 2400S scanner, two vortexes-shakers (Cole-Parmer), and one Orion pH meter. Our collaborators have greatly developed their capacity to prepare and present grants and to summarise and present experimental data.

F) Future Work

Work will now proceed along two parallel lines of work, one with the isolated strains of N_2-fixers and the second with the leguminous plants collected from the Kyzil-Kum desert area.

(a) To study the capacity of the isolates to form nodules with their traditional host plants from which they where originally isolated.

(b) To test the capacity of the selected N_2-fixers to make nodules with alternative wild and cultured leguminous plant species.

(c) To attempt the transfer of *nod* genes from peanut Rhizobium to our stress tolerant N_2-fixers and test their effect on peanuts growing in field conditions

(d) To study the stress tolerance of native plant species such as *Ammodendron connollyi* with and without association with N_2-fixers.

Section II

A) Managerial Issues

None

B) Budget

No special problems

C) Special Concerns

None

D) Collaboration, Travel, Training and Publications

Drs. Khakimov and Shakirov spent the period between May and October 2000 in Sede Boqer for work in the laboratories of Drs. Soares (Microbiology) and Lips (Plant Sciences). They will be in Sede Boqer once more from February 2002 for a period of 6 to 8 months. Publication of some of the results are in preparation while additional experimental work will be completed.

E) Request for American Embassy Tel Aviv or A.T.D. Actions

None