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Response of Fenugreek to Inoculation as Influenced by Salinity, Soil Texture, Chicken Manure and Nitrogen*

Hanan Abdelnabi Saeed Forawi and Elsiddig Ahmed Elmustafa Elsheikh

Department of Biochemistry and Soil Science, Faculty of Agriculture, University of Khartoum, Shambat, Sudan.

Abstract : Eight fenugreek (*Trigonella foenumgraecum*) cultivars and four *Rhizobium* strains were screened for their salt tolerance in three types of soils. *Rhizobium meliloti* strains were more salt-tolerant than fenugreek cultivars. All *Rhizobium* strains were able to form nodules on all fenugreek cultivars. Nodulation in 'Gerf' soil was significantly better than that in Shambat and Rawakieb soils. Several pot experiments were designed to study the effect of salinity, chicken manure and nitrogen on nodulation using indigenous or exotic *Rhizobium* strains. Dry matter production and nodulation of fenugreek plants inoculated with the exotic strain TAL 380 were slightly better than those inoculated with the local strain SHHI. Application of chicken manure significantly increased nodulation, dry matter production and plant nitrogen content. Salinity significantly reduced all measured parameters. N-fertilization significantly increased dry matter production and plant nitrogen content. Effect of salinity on *Rhizobium* inoculated plants was slightly more than N-fertilized ones.

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INTRODUCTION

Large areas of the Sudan were reported to be saline (Mustafa, 1986). Previous work in the Sudan showed that fenugreek could be successfully grown at Soba, south of Khartoum, an area of salt-affected soils; and the yield of the crop was increased by the addition of chicken manure or nitrogen, but the increment was not significant (Yousif, 1982). Work outside the Sudan indicated that inoculation of fenugreek plants with eight *Rhizobium meliloti* strains increased the nodulation, dry matter accumulation, plant nitrogen content and seed yields (Poi *et al.*, 1991). Although fenugreek is frequently grown in northern Sudan, no inoculation or N-fixation programmes were adopted (Mahdi, 1993). The objective of this work was to study the response of fenugreek to inoculation in the presence of salinity, N-fertilization and chicken manure.

MATERIALS AND METHODS

Strains, cultivars and soils

Three introduced *Rhizobium meliloti* strains, namely TAL 380, TAL 1372 and TAL 1373 which were kindly supplied by the NifTAL Project (USA), and a local strain (SHHI) isolated by the first author from a 'Gerf' soil, were used in this study. Seeds of eight fenugreek cultivars namely, Abu Hamad, Berber, Damar I, Damar II, Dongola, Habashy, Hindy and Rubatab, were kindly supplied by the New Halfa Research Station. Surface soil samples (0-30 cm) from three different sites in Khartoum State, namely Gerf, Shambat and Rawakieb, were used (Table 1).

Pot experiments (general)

In all pot experiments, seven surface sterilized seeds were sown in plastic pots (20.0 cm in diam.) and thinned to three plants pot^{-1} after two weeks from sowing. Irrigation was carried out either with tap water or with tap water containing NaCl + $CaCl_2$ salts (1:1 w/w) to give salinity of different electrical conductivities. Irrigation with saline water started after

Table Some physical and chemical properties of the soil samples used in this study*

		Particle size distribution %			pH	EC	Soluble cations meq/1				SAR	Total N %
	Depth (cm)	Sand	Silt	Clay	Paste	dS/m	K	Na	Ca	Mg		
Shambat	0-30	17	20	63	8.4	.29	0.25	11.96	6.25	.75	5.98	0.047
Gerf	0-30	30	30	40	7.8	1.44	0.52	1.23	9.49	1.96	0.51	0.110
Rawakieb	0-30	47	18	35	7.8	0.05	0.20	3.30	1.60	2.50	2.31	0.020

*EC = Electrical conductivity of the saturation extract at 25°C.
SAR = Sodium adsorption ratio of the saturation extract.

two weeks from sowing. In all pot experiments, inoculation of seedlings with the appropriate *Rhizobium* strain was carried out after one week from emergence. Seedlings were inoculated with 5 ml yeast extract mannitol (YEMA) (Vincent, 1970) containing about $1X10^9$ cfu ml^{-1}. Five pot experiments were conducted as follows :

Experiment [1] : Screening *Rhizobium* strains and fenugreek cultivars for salt tolerance.

NaCl was added to YEMA medium (Vincent, 1970) before autoclaving to give the following levels of salinity : 0, 4, 8, 16, 32 or 64 dS/m. The medium was dispensed as 20 ml aliquots into test tubes (22 mm in diam) then each tube was inoculated with one loopful of the inoculum. Test tubes were incubated at 28°C and checked daily for growth.

In a pot experiment, the eight fenugreek cultivars were screened for salt tolerance. Irrigation was carried out either with tap water or with saline water of EC = 16.0 dS/m. The soil used was 'Gerf' soil and each treatment was replicated three times. Plants were harvested after four weeks from sowing. Shoot and root dry weights were determined.

Experiment [2] : Nodulation tests.

(a) In seedling agar: Each of the eight fenugreek cultivars were inoculated with the four *Rhizobium* strains separately. The seedling agar (Vincent, 1970) was dispensed into test tubes. One seedling was selected and planted into each agar slant. One ml of *Rhizobium* culture was dispensed over the roots of seedling in the agar slants after roots establishment was achieved. Aluminum foil was wrapped around the lower part of the tubes to protect the roots from light. The tubes were kept in an incubator for proper seedlings development at 22-25°C. Plants were harvested after five weeks and roots were checked for the presence of nodules.

(b) In three types of soils : Three types of soils, namely Shambat, 'Gerf' and Rawakeib, were used. Plants were either uninoculated or inoculated with *Rhizobium* strain TAL 380. Salinity levels used were : 0.26, 2.0, 4.0 or 6.0 dS/m. Plants were harvested after eight weeks from sowing.

Experiment [3] : Exotic versus indigenous *Rhizobium* strains.

Fenugreek cultivar Berber was either inoculated with the indigenous (local) strain SHHI or the exotic strain TAL 380, in sterile 'Gerf' soil. Irrigation was carried out either with tap water (0.26 dS/m) or with saline water of EC = 3.0 dS/m. Plants were harvested after eight weeks from sowing.

Experiment [4] : Effect of salinity and chicken manure on growth and nodulation.

All treatments were inoculated with the indigenous strain SHHI. Levels of chicken manure used were zero or 2.5 tons/fed. All pots were irrigated frequently for 15 days prior to sowing (Abdalla, 1989). Irrigation was carried out either with tap water or saline water of EC = 4.0 dS/m. Plants were harvested after eight weeks from sowing.

Experiment [5] : Inoculation versus N-fertilization

Treatments were either uninoculated, *Rhizobium* inoculated or N-fertilized fenugreek plants. The cultivar Berber and the strain TAL 380 were used in 'Gerf' soil. Application of urea as N-fertilizer was carried out after emergence at the rate of 0N or 2N (86 kg N/ha). Irrigation was carried out either with tap water or saline water of EC = 6.0 dS/m. Plants were harvested after eight weeks from sowing.

RESULTS

Experiment [1]

All *Rhizobium* strains tested were able to grow at similar rates in media containing salinity up to 32.0 dS/m, in 24 hours, but they failed to grow in media containing 64.0 dS/m. Salinity significantly decreased shoot and root dry weights of all fenugreek cultivars but the dry weights of

cultivar Berber were significantly higher than those of the other cultivars (Table 2).

Experiment [2]

All the tested fenugreek cultivars showed pink and brown nodules on their roots with each of the four *Rhizobium meliloti* strains, indicating the infectivity of these strains and the compatibility between them and the host. The presence of nodules on the roots of uninoculated fenugreek plants grown in 'Gerf' and Shambat soils and the absence in Rawakieb soil indicates the existence and absence of indigenous rhizobia in these soils, respectively (Table 3). Inoculation significantly increased nodules number per plant in all types of soils, whereas salinity significantly reduced them. Nodulation in 'Gerf' soil was significantly better than that in Shambat and Rawakieb soils.

Experiment [3]

Inoculation significantly increased the dry weights of shoots and roots and the number of nodules per plant (Table 4). Shoot and root dry weights of plants inoculated with the exotic strain TAL 380 were slightly better than those inoculated with the local strain SHHI, however, the differences were not significant. Salinity significantly reduced shoot and root dry weights of all treatments.

Experiment [4]

Application of chicken manure significantly increased nodulation, dry matter production and the plant nitrogen content (Table 5). Salinity significantly reduced all measured parameters.

Experiment [5]

Inoculation with *Rhizobium meliloti* strain TAL 380 or N-fertilization significantly increased nodulation, shoot and root dry weights and N-content/plant (Table 6) of fenugreek cultivar Berber, while salinity significantly decreased these attributes in inoculated or N-fertilized plants. However, N-content of N-fertilized plants was not-significantly higher than inoculated ones under saline conditions.

Table 2. Salinity tolerance of fenugreek cultivars.

Cultivars of fenugreek	Salinity tolerance %
Abu Hamad	9.5
Berber	30.6
Damar I	7.3
Damar II	16.6
Dongola	22.7
Habashy	10.0
Hindy	7.5
Rubatab	20.5

Table 3. Effect of salinity (dS/m), inoculation and soil type on number of nodules per plant of fenugreek cultivar Berber.

Soil type	Salinity			
	0.26	2.0	4.0	6.0
No inoculation				
Rawakieb	0.00	0.00	0.00	
'Gerf'	17.04	16.01	10.01	1.32
Shambat	2.00	1.00	0.00	0.00
***Rhizobium* TAL 380**				
Rawakeib	16.01	16.00	9.87	1.01
'Gerf'	70.09	69.02	29.02	
Shambat	41.01	39.14	20.00	.01
SE +	1.95	1.95	1.95	.95

Table 4. Effect of salinity and inoculation with exotic TAL 380 or local SHHI *Rhizobium* strain on shoot and root dry weight (g/plant) and nodule number per plant after eight weeks from sowing.

Treatment	Shoot	Root	Nodule number
No salt			
No inoculation	0.71	0.17	0.00
Rhizobium TAL 380	1.50	0.32	180.03
Rhizobium SHHI	1.46	0.29	174.01
Salinity (3.0 dS/m)			
No inoculation	0.30	0.09	0.00
Rhizobium TAL 380	0.93	0.22	135.12
Rhizobium SHHI	0.91	0.21	128.21
SE ±	0.07	0.04	9.10

Table 5. Effect of salinity and chicken manure on shoot and root dry weight (g/plant), number of nodules per plant and N-content (mg/plant) of inoculated fenugreek plants, after eight weeks from sowing.

Treatment	Shoot	Root	No. of Nodules	N-Content
No salt				
Control	2.00	0.76	93.1	70.66
2.5 ton/fed	3.24	1.38	151.0	141.37
Salinity (4.0 dS/m)				
Control	1.09	0.43	45.1	34.34
2.5 ton/fed	2.52	1.05	109.0	104.71
SE ±	0.50	0.25	7.30	7.00

Table 6. Effect of salinity on shoot and root dry weight (g/plant), number of nodules per plant and nitrogen content (mg/plant) of inoculated or N-fertilized fenugreek plants, after eight weeks from sowing.

Treatment	Shoot dry weight	Root dry weight	No. of Nodules	Plant N Content
No salt				
Control	0.72	0.44	60.02	
Rhizobium (TAL 380)	0.95	0.57	160.00	
N-fertilized	0.98	0.55	6.52	
Salinity (6.0 dS/m)				
Control	0.15	0.08	6.05	
Rhizobium (TAL 380)	0.40	0.28	27.11	
N-fertilized	0.50	0.32	0.00	
SE ±	0.05	0.04	7.80	4.4

DISCUSSION

Rhizobium meliloti strains tolerated very high levels of salinity (up to 32 dS/m), and this finding agrees with the previous reports that rhizobia are generally more able to cope with salinity than their host legumes (Elsheikh, 1992; Hafeez *et al.*, 1988). Increasing levels of salinity (3, 4, 6 and 16 dS\m) significantly reduced shoot and root dry weights and nitrogen fixation. These harmful effects of salinity on nodulation and growth of leguminous crops were shown by many research workers e.g. Elsheikh (1992), Hafeez *et al.* (1988) and Elsheikh and Wood (1990).

All fenugreek cultivars tested in this investigation showed nodules on their roots with the four *Rhizobium meliloti* strains. This indicates that there is compatibility between these two partners (Poi *et al.*, 1991). The presence of indigenous *Rhizobium meliloti* strain in 'Gerf' and Shambat soils and the absence of these rhizobia in Rawakieb soil, could be attributed to the continuous cultivation of lucerne, in Shambat and Gerf soils, which is a member of cross inoculation group of these bacteria. Nodulation of fenugreek was significantly better in 'Gerf' soil which proved to be an ideal soil for growing fenugreek. This may be because 'Gerf' soil is light loamy soil, well drained and very rich in various plant nutrients required for nodulation.

Application of chicken manure significantly increased nodulation and dry matter production. This may be due to the fact that manures are known to provide plant nutrients and improve soil physical properties (Abdalla, 1989). Inoculation of fenugreek cultivar Berber with the exotic or indigenous *Rhizobium meliloti* strains significantly increased dry matter production and nodulation.

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مجلة جامعه الخرطو للعلو الزراعية المجلد الثالث العدد الثانى يوليو ١٩٩٥

استجابه الحلبه للتلقيح بالريروبيو تحت تأثير الملوحه وقوام التربة ومحلفات الدواجن وسماد النتروجين

حنان السى سعيد فوراو
الصديق أحمد المصطفى الشيخ

قسم الكيمياء الحيويه والتربه
كلية الزراعه جامعه الخرطو
سمبات السودان

موجز البحث تمت دراسة مدى تحمل ثمانية أصناف الحلبه أربع سلالات من بكتريا العقد الجذريه للملو أظهر النتائج أن سلالات البكتريا أكثر تحملا للملوحه من أصناف الحلبه أن العقد المكونة فى تربة الجر أكثر المكونة فى تربتى الر اكنب وسمبات

عدة تجارب فى أصص لدراسة أثر الملو محلفات الدواجن سماد النتر جين على تكوين العقد الجذرية باستخدام سلالات بكتيريه محليه ومستوردة أدت اضافة محلفات الدواجن إلى زيادة فى الوزن الجاف للنباتات ومحتوى النبات النتر جين العقد الجذرية فى النبات مقارنة بالشاهد بينما أدت الملو إلى نقص معنوى فى الخصائص أدت اضافة السماد النتر جينى إلى زيادة معنويه فى الوزن الجاف للنباتات محتوى النتر جين مقارنة بالشاهد تدل النتائج على أن نبات الحلبة معاو للملو كما أنه يستجيب للتلقيح بمختلف سلالات بكتريا العقد الجذرية