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Response of Legume-*Rhizobium* Symbiosis to Salinity in the Sudan : a Review

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Abstract : Research on the effect of salinity on *Rhizobium* and its symbiotic properties with legumes in the Sudan was reviewed. The review indicated that (i) rhizobia are more salt tolerant than their host legumes; (ii) the fast growing rhizobial strains are more salt tolerant than the slow growing bradyrhizobia; (iii) salinity significantly reduces plant growth, nodulation and nitrogen fixation; (iv) the response to *Rhizobium* inoculation under saline conditions, with different soil textures, varies from no response to a highly significant response; (v) under saline conditions, application of N, P, S, mycorrhiza and chicken manure gives comparable results to that of *Rhizobium* ; and (vi) *Rhizobium* inoculation has a great potential for improving fertility in saline soils.

INTRODUCTION

Salt-affected soils in the Sudan occur in the desert and semi-desert climatic zones, e.g. the high terrace of the River Nile and its tributaries, and in the arid regions, e.g. central clay plain-north Gezira (Abdalla 1986). Most of the salt-affected soils in the Sudan have a relatively low nutrient status and contain 0.01 - 0.02% organic nitrogen. The impact of salinity on agriculture is now being felt in irrigated areas in which soil- and water-borne salts are accumulating during repeated cycles of water use. Non-saline soils could easily be damaged and degraded by secondary salinization through irrigation with water from the Blue Nile, White Nile and River Nile (Abdalla 1986; Mustafa 1986). These problems will become more serious as increasing population leads to more intensive use of land and water, and as presently unused or marginally used resources are pressed into service. The entire physical and biological systems involved in saline agriculture must be understood and carefully managed if increased production is to be achieved without exacerbating the existing problems.

Biofertilization, as compared to the use of chemical fertilizers, is steadily receiving increased attention and recognition from scientists because the microbial inoculants (including *Rhizobium* and mycorrhizal inoculants) introduced into soil or plant culture will directly or indirectly enhance plant productivity. Biofertilizers must receive more attention in countries, like the Sudan, with a predominantly low-input agricultural system of production where chemical fertilizers, if available, may not be economically affordable (Mahdi 1993).

Salt tolerance of rhizobia

Rhizobia and bradyrhizobia strains vary in their tolerance to salinity. It has been reported that strains of *Rhizobium* and/or *Bradyrhizobium* species nodulating the same leguminous plant differ in their tolerance to salinity (Adlan 1995, Elsheikh and Wood 1995). Elsheikh (1998) reported that fast-growing rhizobia are more salt tolerant than slow-growing bradyrhizobia and that salt tolerance in rhizobia depends on the carbon and the energy source used in the growth medium, incubation temperature, pH value and type of salt used. According to Forawi (1994), the four tested *Rhizobium mellilotí* strains tolerated salinity up to 64 dS/m in solution culture. However, very little work was carried out in the Sudan to screen different strains of rhizobia and bradyrhizobia for salt tolerance. This could be attributed to the well documented fact that rhizobia are more salt tolerant than their host leguminous crops (Elsheikh 1992).

Despite the numerous experiments conducted, worldwide, on the effect of salinity on the growth and survival of rhizobia and bradyrhizobia strains, most of the reports confound the effects of salt and pH and express the concentration of salts in percentage regardless of electric conductivity (EC), ionic species and molecular weight (Elsheikh and Wood 1990; Elsheikh 1998), and ignore the interactions between salt, temperature and pH value. Salts may inhibit growth because of the abnormally high intracellular solute concentration that results rather than any effect they might have on the osmolarity in the cytoplasm. It has been shown that some mineral ions affect growth because they inhibit the activity of specific enzymes (Elsheikh 1998).

Effect of salinity on legumes crops

Leguminous crops differ in their tolerance to salinity. Elsheikh (1992) found that chickpea is more salt sensitive than the *Rhizobium* strain, and N-fixation is more sensitive than plant growth. Salinity decreases dry matter production, nodule number, nodule weight, N and K content and increases Na content in chickpea (Adlan 1995; M. Ismail, personal communication) and faba bean (Osman 1993). According to Ahmed (1996), faba bean cultivar Basabeer was more tolerant than cultivars Agabat, Silaim and Shambat 616. Among eight fenugreek cultivars; namely, Abu Hamad, Berber, Damar I , Damar II, Dongola, Habashy, Hindy and Rubatab, Forawi and Elsheikh (1995) found that cultivar Berber is the most salt tolerant and cultivar Damar I is the most sensitive to salinity. These variations among cultivars could be attributed to (i) the variation in salt resistance among legumes, (ii) the exclusion of sodium and chloride ions from the leaves, and (iii) the accumulation of toxic ions in various parts of the plant.

Effect of salinity on the process of nitrogen fixation

The amount of nitrogen fixed by a particular plant-*Rhizobium* association may vary according to the growing conditions of the plant. Factors such as salinity, temperature, water supply, pH, mineral nutrition and combined nitrogen are of great importance to the symbiosis process. Nodule initiation in the legume-rhizobia symbiosis involves complex interactions between host root, rhizobial strain and environment. The step of survival and competition of rhizobia and the attachment process of rhizobia to the root surface followed by formation of the infection thread and the nodule function may all be sensitive to salinity. Salinity usually has a negative impact on plant growth, photosynthesis and demand for nitrogen. These effects lead to decrease in the density of the root of the leguminous crop and consequently decrease in nodule number and function. Increasing salinity level delays germination and decreases its percentage, decreases leghemoglobin content, number and weight of nodules/plant and nodule activity, and restricts outward movement of fixed nitrogen from nodules (Elsheikh and Wood 1990; Elsheikh 1992, Adlan 1995).

Leguminous crops are essential components of the cropping systems throughout the world. Legumes, which are also an important source of protein in human diet, can biologically fix a considerable amount of atmospheric nitrogen. Research on legume-*Rhizobium* symbiosis under saline conditions, conducted in the soils of the Sudan (Table 1), could be summarized as follows :-

(a) Chickpea

Chickpea cultivars, in general, are very sensitive to salinity; however, they vary in their sensitivity depending on the environmental conditions. Among 33 cultivars of chickpea, only six survived at 50 mM NaCl (Elsheikh 1989). Poor germination under saline conditions resulted in poor establishment and consequently poor crop growth. According to Elsheikh (1989; 1992), shoots, roots and nodulation of chickpea are significantly inhibited by salinity. However, *Rhizobium* inoculation improved the shoots, roots and nodulation by about 50% (Table 2). Similarly, Elsheikh (1992) found 40% reduction in nodulation of chickpea with only 1.0 dS/m, whereas *Rhizobium* inoculation improved the nitrogen fixed by more than three folds compared to uninoculated non-saline control. Significant reductions in nodule dry weight (59.8%) and nitrogen fixation (63.5%) were evident even at the lowest salinity level used (3 dS/m) (Adlan 1995).

(b) Soybean

Soybean cultivars, in general, are classified as moderately tolerant to salinity. According to Elsheikh (1989), salinity significantly reduces the shoots and roots dry weight of soybean, whereas inoculation significantly improves the shoots, roots and nodule number, and in most instances the absolute figures were superior than the untreated controls. Similarly, Elsheikh and Wood (1995) proved that *Rhizobium* strain selection for salt tolerance is important, and that the performance of a salt tolerant strain is superior in nodulation and N fixation compared to a salt-sensitive strain and to the untreated control.

Table 1 Some of the physical and chemical properties of the soils used (Forawi 1994; Ahmed 1996).

	Particle size distribution (%)			pH	EC	Soluble cations (meq/1)				SAR	
	Sand	Silt	Clay	paste	dS/m	K	Na	Ca	Mg		N%
Shambat	17	20	63	8.4	1.29	0.25	11.9	6.25	1.75	5.98	0.047
Gerf	30	30	40	7.8	1.44	0.52	1.23	9.49	1.96	0.51	0.110
Elrwakeeb	47	18	35	7.8	0.05	0.20	3.30	1.60	2.50	2.31	0.020

Table 2 Effect of salinity (dS/m) on shoot and root dry weight and number of nodules per plant of different leguminous crops either uninoculated or inoculated with *Rhizobium* strains.

Crop	Cultivar	Presence of *Rhizobium*	EC = 0			EC = 4			% Reduction from the control			Reference
			I	II	III[1]	I	II	III	I	II	III	
Faba bean	Basabeer	- *Rhizobium*	1.40	1.10	5.0	0.97	0.70	1.0	30.7	36.0	80.00	Ahmed (1996)
		+ *Rhizobium*	1.85	1.26	26.0	1.30	0.85	16.0	7.1	23.0	+220[2]	Ahmed (1996)
Faba bean	Agabat	- *Rhizobium*	1.54	0.63	70.2	1.31	0.53	42.2	14.9	16.0	28.80	Osman (1993)
		+ *Rhizobium*	2.86	0.87	128.3	1.69	0.62	70.0	+9.7	1.6	0.20	Osman (1993)
Fenugreek	Berber	- *Rhizobium*	0.71	0.17	0.0	0.30	0.09	0.0	57.7	47.0	0.00	Forawi and Elsheikh (1995)
		+ *Rhizobium*	1.50	0.32	16.0	0.93	0.22	9.0	+31.0	+29.0	+ ∝	Forawi and Elsheikh (1995)
Chickpea	NEC 1101	- *Rhizobium*	0.81	0.71	53.0	0.54	0.50	31.0	33.0	30.0	41.50	Ismail (unpublished)
		+ *Rhizobium*	1.55	0.93	82.0	0.92	0.60	55.0	+13.6	15.0	+3.80	Ismail (unpublished)
Chickpea	ILC 482	- *Rhizobium*	0.80	0.55	0.0	0.40	0.23	0.0	50.0	58.2	0.00	Elsheikh (1989)
		+ *Rhizobium*	0.90	0.59	80.0	0.59	0.31	27.0	26.3	43.6	+ ∝	Elsheikh (1989)
Soybean	814-49E	- *Rhizobium*	3.50	1.20	0.0	2.50	1.00	0.0	28.6	16.7	0.00	Elsheikh (1989)
		+ *Rhizobium*	5.50	2.40	85.0	5.00	2.20	87.0	+42.9	+83.3	+ ∝	Elsheikh (1989)

[1] I,II and III indicate shoot, root and number of nodules per plant, respectively,
[2] The positive sign indicates that the performance in saline treatment is better than the untreated non-saline control

(c) Fenugreek

Fenugreek cultivars proved their tolerance to salinity with some differences encountered among cultivars. The nodulation process was affected by salinity and soil texture (Forawi 1994). Moreover, Forawi and Elsheikh (1995) found that nodulation increases nine folds due to inoculation in saline soil compared to untreated control. The shoot and root dry weights of fenugreek cultivar Berber increased by 31 and 29%, respectively, in *Rhizobium* inoculated plants under salt stress compared to untreated plants (Forawi and Elsheikh 1995).

(d) Faba bean

Faba bean genotypes vary greatly in their tolerance to high salinity levels. The variation in the efficiency of rhizobial strains with different faba bean cultivars under salt stress may be attributed to the variation in compatibility between the cultivar and the *Rhizobium* strain (Ahmed 1996). *Rhizobium* inoculation significantly increased the shoots and roots weight and N fixation of faba bean under saline and non-saline conditions, which indicates the efficiency and compatibility between the *Rhizobium* strain and the cultivar used (Ahmed and Elsheikh 1998). Moreover, Ahmed (1996) and Osman (1993) concluded that the harmful effect of salinity on faba bean could be reduced or alleviated by using efficient *Rhizobium* strains.

(e) Other legumes

The deleterious effect of salinity on nodulation, growth and nitrogen fixation under saline conditions was reported for *Phaseolus vulgaris* in both pot and field experiments (Osman *et al.* 1996). Similar results were also reported for alfalfa (Musa 1976). Seedlings of tree legumes such as *Leucaena leucocephala* (Abdel Magid *et al.* 1985; Ahmed 1986), *Acacia senegal* and *Acacia albida* (El Hussien 1996) were also reported to be greatly affected by salinity. *Rhizobium* inoculation significantly improved the shoots and roots dry weight as well as nodulation and nitrogen fixation of these forest trees (Ahmed 1986; El Hussien 1996). Although groundnut is an important leguminous crop in the Sudan, there is no published work on its tolerance and symbiotic activity under salt stress.

Effect of soil texture

Forawi and Elsheikh (1995) examined the effect of salinity on nodulation of fenugreek in three different types of soil texture (Table 3). they noticed the absence of indigenous rhizobia in Elrwakeeb sandy soil and their presence in the *Gerf* and Shambat soils. They attributed this to the continuous cultivation of alfalfa in *Gerf* and Shambat soils, which is a member of the cross inoculation group of these bacteria. They also found that nodulation of fenugreek was significantly better in *Gerf* soil which proved to be an ideal soil for growing fenugreek. They attributed this to the fact that *Gerf* soil is a light loamy soil, well drained and very rich in various plant nutrients required for nodulation. Similar results were obtained for nodulation of chickpea under *Gerf* and Shambat soils (M. Ismail, personal communication).

Effect of fertilizers and/or amendments

Table 4 summarizes the effect of salinity on nodule number of different leguminous crops as influenced by different fertilizers. According to Ahmed and Elsheikh (1998), the application of superphosphate significantly increases fresh and dry weights of shoots, roots and number of nodules of faba bean plants under saline and non-saline conditions. They concluded that P fertilizers could be used under salt stress to improve the performance and symbiotic properties of faba bean.

Interest in dual symbiosis of legumes with vesicular arbuscular mycorrhiza (VAM) and *Rhizobium* has been explained by the fact that the process of nitrogen fixation increases plant demand for P, and that most common nutritional disorders in legumes are related to P deficiency. Ahmed and Elsheikh (1998) demonstrated that dual inoculation of faba bean with both *Rhizobium* and VAM significantly increases and enhances nodulation, nitrogen fixation and dry matter production under saline conditions.

The process of nitrogen fixation is more sensitive than nitrogen assimilation by nitrogen fertilized plants. Nitrogen fixing plants differ from the nitrogen fertilized plants in that : (i) they must form nodules and fix atmospheric N, and (ii) they have different biochemical mechanisms for

Table 3. Effect of salinity and soil type on number of nodules per plant of fenugreek cultivar Berber*.

	Salinity (dS/m			
	0.26	2.0	4.0	6.0
No. inoculation				
Elrwakeeb	0.00	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				
Elrwakeeb	16.01	16.00	9.87	1.01
Gerf	70.09	69.02	29.02	5.43
Shambat	41.01	39.14	20.00	1.01
SE ±	1.95	1.95	1.95	1.95

*Source : Forawi and Elsheikh, 1995.

Table 4. Effect of salinity (dS/m) on number of nodules per plant of different leguminous crops as influenced by fertilizers in the absence of *Rhizobium* inoculation..

Crop	Fertilizer treatment	EC 0	EC 4	Recovery %	Reference
Faba bean	Control	23	10	56.6	Ahmed and Elsheikh (1998)
	180 kg P	38	20	13.0	Ahmed and Elsheikh (1998)
Faba bean	Control	23	10	56.5	Ahmed and Elsheikh (1998)
	VA mycorrhiza	29	14	39.1	Ahmed and Elsheikh (1998)
Faba bean	Control	5	1	80.0	Ahmed (1996)
	50 kg N + 160 kg P	16	8	+60.0	Ahmed (1996)
Fenugreek	Control	93.1	45	51.7	Forawi and Elsheikh (1995)
	2.5 t manure/fed	151	109	+17.1	Forawi and Elsheikh (1995)
Fenugreek	Control	60	6.1	89.8	Forawi (1994)
	86 kg N/ha	160	27.1	54.8	Forawi (1994)

[1] I, II and III indicate shoot, root and number of nodules per plant, respectively,
[2] The positive sign indicates that the performance in saline treatment is better than the untreated non-saline control.

nitrogen assimilation. In some instances, the process of nitrogen fixation needs a starter dose of fertilizer nitrogen.

According to Forawi and Elsheikh (1995), the application of chicken manure significantly increases nodulation and dry matter production of fenugreek plants in saline and non-saline conditions. They attributed this to the fact that manure are known to provide plant nutrients and improve soil physical properties.

Characteristics of rhizobia for successful inoculation

The indigenous rhizobial populations in saline soils were reported to be very poor (Elsheikh 1992). Therefore, to obtain maximum benefit from nitrogen fixation by legumes in these soils, which often have low nitrogen content, it is necessary to inoculate legumes with improved strains of rhizobia. Furthermore, for successful inoculation, the introduced rhizobia should have the following characteristics : (i) tolerate high levels of salts, (ii) survive as free-living organisms in such soils, (iii) tolerate high temperatures and high pH values, (iv) compete successfully with the indigenous rhizobia, and (v) fix nitrogen effectively.

CONCLUSIONS

The reclamation of salt-affected soils should take into consideration the poor indigenous population of rhizobia as well as inhibitory salt effect on the introduced rhizobia and bradyrhizobia.

Research on genetically engineered rhizobia should be addressed.

Statistical models relating the effect of *Rhizobium* and/or other fertilizers and amendments, under different irrigation regimes, should be developed.

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استجابة العلاقة التكافلية بين البقوليات وبكتيريا العقد الجذرية للملوحة

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موجز البحث : تمت مراجعة الأبحاث العلمية التى أجريت فى السودان عن استجابة العلاقة التكافلية بين البقوليات وبكتيريا العقد الجذرية ، واتضح أن البكتيريا عموماً أكثر تحملاً للملوحة من البقوليات . وأشارت النتائج إلى أن الملوحة تؤدى لنقص معنوى فى نمو النبات وأعداد العقد الجذرية وعملية تثبيت النيتروجين الجوى . وتختلف الاستجابة للقاح الرايزوبيوم فى وجود الملوحة حسب بناء التربة . كما أن إضافة النيتروجين والفسفور والكبريت وفضلات الدجاج والميكروهيز تعطى نتائج شبيهة بالنتائج التى يتم الحصول عليها باللقاح البكتيرى . تشير هذه الدراسات إلى أن للقاح الرايزوبيوم أهمية كبيرة فى استصلاح وزيادة خصوبة الترب المالحة .