INFLUENCE OF SALINITY ON GROWTH, YIELD, NUTRIENT UPTAKE AND BIOLOGICAL NITROGEN FIXATION IN GUAR

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Abstract

This study reports the effect of salinity and inoculation on growth, ion uptake and nitrogen fixation by guar (Cyamopsis tetragonoloba (L) Taub.) A soil of EC_e level 8.3 dS/m was quite detrimental, causing about 60% decline in dry matter and grain yield of guar plants whereas a soil of EC_e level 10.0 dS/m was almost toxic. In contrast, most of the studied strains of Rhizobium were salt tolerant. Nevertheless, nodulation, nitrogen fixation and total nitrogen concentration in the plant were drastically affected at high salt concentration. A noticeable decline in acetylene reduction activity occurred when salinity level increased to 8.3 dS/m.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba (L) Taub.*) is an important pulse crop in Egypt. Being a legume it could produce food rich in protein without addition of nitrogen fertilizers. Therefore, this crop is especially suitable in developing countries like Egypt where availability of protein is insufficient and fertilizers are expensive.

Nodulation in some legumes under field condition is very poor (Idris et al., 1990, Ramaswamy and Nair 1965, and El-Sayed 1989). It may be due to absence of Rhizobium in such soils. Alternatively other environmental factors such as salinity (Abdel-Ghaffar et al., 1982, and Lauter et al., 1981), high temperature and drought (Marshall 1964, Mass and Hoffman 1977, and Vincent et al., 1962) may affect the

nodulation and nitrogen fixation of leguminous plants. For example a few studies (Bernstein and Ogata 1966, and Wilson 1970) show that the effect of salinity on nitrogen fixation ability of soybean and alfalfa could be very detrimental. In fact, pulse crops are reported not to nodulate on salt-affected land even though native rhizobia are known to be present (Bhardwaj 1974, and El-Etriby 1990). The major point to investigate is whether symbiotic nitrogen fixation is more sensitive to salinity than host plant growth.

This paper reports the effect of salinity on the growth, nitrogen fixation, yield and nutrient uptake in guar. In addition, in vitro salt tolerance of five strains of Rhizobium spp. was also assessed.

MATERIALS AND METHODS

The seeds of guar (*Cyamopsis tetragonoloba* (*L*) *Taub*.) were obtained from the Agricultural Research Centre Ministry of Agriculture, Giza, Egypt. Three experiments were conducted in this study. The effect of different salt concentrations on the host (Guar) was studied in the first and the second experiments. The endophyte (Rhizobium) was studied in the third experiment.

The first experiment

This experiment was conducted in sterilized sand flushed with nutrient solution contained in Leonard Jars. Seeds were inoculated with locally prepared carrier (gamma irradiated, filter mud amended with clay loam and sucrose) based Rhizobium inoclum at a dose of 15 g/100 g seeds. Inoculum was a mixture of five stains [ARC. 800 G, ARC. 801 G, ARC. 802 G, (ARC. 800 G + ARC. 801 G), and (ARC. 802 G + ARC. 801 G)]. The seeds were successively coated with gum arabica, Rhizobium inoculum and superphosphate for ensuring firm association of Rhizobium to the seeds. The number of viable cells at sowing time were 1.5 x 10¹⁰ /g of inoculum and 6 x 10⁶/seed. Sand cultures were flushed daily with 1/4 strength nitrogen free Hoagland solution. At first leaf stage, thinning was done to leave uniform seedlings in each jar. At this stage NaCL, CaCl₂, Na₂SO₄ and MgCl₂ were added in ratio of 5:6:1:1 to the flushing nutrient solution to produce the salt concentrations of 1.8, 6. 0, 8.3 and 10.0 dS/m. Each treatment was replicated seven times. Plants were grown for 35 days. At harvesting, shoot and root dry weight were recorded and roots were studied for nodulation and nitrogen fixation as estimated by acetylene

reduction technique (Hardy *et al* 1968). Nitrogenase activity was measured by incubating excised nodulated root systms in 250-ml plastic bottles tightly closed with screwed caps fitted with suba seals. A 10:90 acetylene air atmosphere was created inside the bottle. After incubation for 1 hour at room temperature the gas samples (100 UL) were analysed on a gas chromatography (carlo-Erba Mode 180) fitted with a 2 m x 3 mm steel column filled with porapak R (85-105 mesh) and a H_2 flame ionization detector. Nitrogen was used as a carrier gas at a flow rate of 30 ml/min. Two controls, one bottle with C_2H_2 but without nodules and the other with nodules but without addition of acetylene, were also included during each assay to check indigenous production of ethylene.

The Second experiment

This experiment was conducted in soil in 9 kg capacity plastic pots. The soil used in the experiment was loamy sand, non-saline (ECe = 1.3 dS/m; pH 7.9) and had available N and P of 0.032 and 1 ppm respectively. Available nitrogen in the soil was determined according to Bremner (1965). Available phosphorus in the soil was assayed according to Olsen methods (Watanabe and Olsen 1965, Jackson 1973, and Cottenie 1980). Artificial salinization of the soil was achieved with NaCL, CaCl₂, Na₂SO₄ and MgCl₂ mixed in ratio of 5:6:11:1 to produce salinity levels of ECe of 1.8, 6.0, 8.3 and 10.0 dS/m (Qureshi *et al.*, 1977). Ammonium sulphate (8 kg/Feddan) and potassium phosphate (25 kg/Feddan) were applied at rates of 0.15 g and 1.5 g per pot respectively. Fifteen seeds were sown in each plastic pot. The pots were not drained and water was given as the first experiment. Each treatment was replicated fifteen times. At first leaf stage the seedling were thinned out to four uniform plants, in each pot.

Plants were sampled for nodulation and acetylene reduction assay at preflowering, 38 days after sowing and flowering (48 days after sowing) stages and for yield at 68 days after sowing. Oven dried plant samples as well as air dried grains were ground and thoroughly homogenized. Such samples were used for nitrogen, phosphorus, potassium and sodium assay. For the estimation of total nitrogen concentration in plant, samples were digested in concentrated H₂SO₄ and measured by micro-kjeldahl method (Bremner 1965, and Black 1965). For phosphorus, sodium and potassium assay, plant material was digested in a mixture of HNO₃ and HClO₄ (Richards *et al* 1968, and FAO 1980). Phosphorus concentration in plant was determined colorimetrically (Jackson 1973 and Evenhuis and De Waard 1978). Potassium and sodium concentrations were measured by flamephotometry. Each value was the

mean of four replicates.

The third experiment

The salt tolerance of five Rhizobium strains was studied in yeast mannitol (Vincent 1970, and Bremner and Mulvaney 1982) cultures containing 1.7, 5, 10, 25, 50, 100, 150 and 200 mol/m³ NaCL. The strains were chosen on the basis of their efficiency to nodulate guar. Cells were grown for three days in the dark at 29°C in shaken Erlenmeyer flask containing 50 ml medium. The initial cell density was 10⁶ viable cell/ml. The five Rhizobium strains were obtained from the Agricultural Microbiology Research Department, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Viable cells were counted by using standard serial dilution and plated by spread plate count method (Vincent 1970, and Blakemore *et al* 1981).

Statistical procedure

Analysis of variance of the measured traits was used according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Germination and plant survival

The germination was 100 percent in both soil and sand cultures at all salinity levels; only a delay of four days in germination was noted in the case of 8.3 and 10.0 dS/m. In fact, in most plant species, moderate levels of salinity delay germination and not the germination percentage (Salim et al 1979, and El-Sayed 1989). In the second experiment, the survival of plants was decreased with increasing salinity levels. The survival was 69% at 8.3 dS/m, at an ECe level of 10.0 dS/m the survival of plants decreased from 80% to 58% at flowering stage and all plants died by maturity. At high salt concentration, death of seedlings after germination has also been reported in case of Sesbania aculeata (Salim et al 1979) and Leucaena Leucocepleala (Niazi et al., 1985).

Plant growth and yield

Dry matter yield per plant (Tables 1and 2) significantly decreased with increase in salinity levels regardless of the stages of plant growth. Data also showed

Table 1. Effect of salinity and inoculation on dry matter yield of guar at different stages of growth (the second experiment).

EC _e of soil	Dry weight (g/plant)*					
	Preflowering	Flowering	Maturity			
1.8 Uninoc.	0.72	1.89 b	2.16 ab			
1.8 Inco.	0.99	2.43 a	2.79 a			
6.0 Uninoc.	0.54	0.90 d	1.71 bc			
6.0 Inco.	0.90	1.44 c	2.25 ab			
8.3 Uninoc.	0.36	0.54 e	0.90 d			
8.3 Inco.	0.54	0.99 d	1.17 cd			
10.0 Uninoc.	0.27	0.27 e				
10.0 Inco.	0.27 NS	0.36 e				

Mean followed by same letter are not significantly different at 5% probability level.

Uninoc. = Uninoculated. Inoc. = Inoculated.

NS: Non-significant

* Values are mean of four readings

Table 2. Effect of salinity and inoculation on dry matter yield and nodulation on guar grown in nutrient solution*. Plants were harvested after 35 days of growth (the first experiment).

EC _e of soil dS/m	No. of no- dule / plant	Frequency of nodulation (%)	Dry weight of nodules (mg/ plant)	U mole C ₂ H ₄ dry nodule/h	Dry matter mg/plant (whole plant)
Uninoc.					
1.8	0	0	0	0	678±30
Inoc.		1 1 10g 1911 C	nam te mezer la		A NIEGO ME
1.8	6	77	16±0.2	20±2.0	811±15
6.0	3	36	7±0.1	16±2.3	659±12
8.3	er be p elusion	9	3±0.4	2±1.4	375±11
10.0	0	0	0	0	69±5
	make of testing	Spingston and with	est acresimle		squeroser i li

Uninoc. = Uninoculated.

Inoc. = Inoculated.

^{*} Values are mean of four readings

that soil salinity of 8.3 dS/m was quite detrimental to cause a 50-60% decline in dry matter yield of guar plant, whereas, the soil EC_e level of 10.0 dS/m was almost toxic. Reduction in plant growth and dry matter accumulation were observed at moderate salinity levels in guar. This is in agreement with salt sensitivity reported in other leguminous plant species: *Vicia Faba* and *Phaseolus vulgaris* grown at the same salinity levels (Abdel-Ghaffar et al., 1982) and *Glycine wightii* (Wilson, 1970).

An inverse relation was found between salinity and grain weight. The higher the soil salinity the lower was the weight of grains (Table 3). However, the number of pods per plant and grains per pod were not significantly affected up to a salinity level of 8.3 dS/m. The grain yield of guar was bout 60% lower at salinity levels of 6 dS/m and 8.3 dS/m as compared to 1.8 dS/m while it was completely depressed at 10 dS/m. Similar results have been reported on other leguminous plant species: *Vicia, Phaseolus, Glycine* and *Medicago* (Abdel-Rahman and Abdel-Hadi 1984, Bernstein and Ogata 1966, Wilson 1970, and El-Sayed 1995a).

Nodulation and Nitrogen Fixation

The response of guar to inoculation is shown in Tables 2 and 4. In the first experiment, uninoculated plants showed no nodules and had a 16% lower dry matter yield than the inoculated plants at salinity level of ECe 1.8 dS/m. In case of the second experiment, not a single nodule was observed in uninoculated plants, therefore, soil is either devoid of Rhizobium or indicates the ineffectiveness of the two mixture srains to infect guar roots. Similar results were obtained by Idris et al. (1990). The frequency of nodulation on guar at flowering stage was 88%. In this experiment, inoculation with a mixture of five Rhizobium strains clearly benefitted guar as indicated with increased nodulation, nitrogen fixation, dry matter production and grain weight per plant. Howerver, the effect of inoculation was not significant on the number of pods per plant and number of grains per pod as compared to controls.

The effect of salinity on nodulation and nitrogen fixation in guar is presented in Tables 3 and 4. Nodulation and nitrogen fixation of inoculated plants were highly sensitive to salt. Frequency of nodulation, weight of nodules and nitrogen fixation decreased by increasing salinization. Nodulation was reduced to about half at salinity level of EC $_{\rm e}$ 6.0 dS/m as compared to 1.8 dS/m. The nodulation was completely depressed at EC $_{\rm e}$ 10.0 dS/m regardless of plant growth stages. The effect of salinity was more severe on the number of nodules per plant than on the specific nitrogenase

activity. A noteable decline in acetylene reduction activity occurred when salinity level increased to 8.3 dS/m. These results corroborate the earlier findings on *Glycine, Macroptilium, Neonotonia, Medicago, Vicia* and *Phaseolus* species (Abdel-Ghaffar *et al.*, 1982, Bernstein and Ogata 1966, Lakshmi *et al.*, 1974, Wilson 1970, 1985, and El-Sayed 1995b).

Table 3. Effect of salinity and inoculation on some components of grain yield of guar grown in soil (the second experiment).

EC _e of soil	Yield components*						
dS/m	No of pod/ plant	No of grain/pod	Wt. of grain/ plant (mg)	Wt. of 1000 grain (g)			
	2.43	3.69	455 a	41.13 a			
1.8 Uninoc.	3.60	4.23	734 b	39.06 a			
1.8 Inco.	2.07	3.33	193 d	22.68 b			
6.0 Uninoc.	3.33	3.87	311 c	19.53 b			
6.0 Inco.	1.53	4.77	176 d	19.44 b			
8.3 Uninoc. 8.3 Inco.	2.07	4.77	297 с	24.30 b			
	NS	NS					

Mean followed by same letter are not significantly different at 5% probability level.

Uninoc. = Uninoculated. Inoc. = Inoculated.

NS: Non-significant

* Values are mean of four readings

Nutrient uptake

The concentration of nitrogen in plant tissue as affected by soil salinity was in harmony with those of dry matter at maturity stage (Table 5). With the rise of salinity the total nitrogen concentration in pod grain and whole plant significantly decreased. The concentration of nitrogen significatnly increased in grains and whole plant at all levels of salinity when incoculated with Rhizobium. The results are in agreement with those reported earlier on *Phaseolus vulgaris*. Inoculation of *Phaseolus velgaris* markedly enhanced nodulation, N2-fixation, plant dry matter, N content and final yield (Abdel-Ghaffar *et al.*, 1982, El-Sayed 1989, and El-Keiy and El-Sayed 1991).

In general K⁺ concentration was similar in different plant parts at preflowering and flowereing stages of growth of guar grown at various salinity levels. In con-

Table 4. Effect of salinity and inoculation* on nodulation and nitrogen fixation of guar grown in soil (The second experiment).**

EC _e of soil	No. of nodulation/plant	ation/plant	Frequency of nodulation	nodulation	Dry weight nodule	nodule	U mole C2H4/g dry	4/g dry
dS/m	er W		(%)	7	(mg)	0	nodule/h	le/h
1 58	Preflowering Flowering Flowering Preflowering Preflowering Flowering Flowering	Flowering	Preflowering	Flowering	Preflowering	Flowering	Preflowering	Flowering
1.8	12	18	59	62	11±1.8	14±4.9	12	18
0.9	က	œ	30	34	7±1.9	10±2.1	! m	2 00
8.3	17 C	1	30	7	3±1.0	4±1.8	elu:	M_
10.0	0	0	0	O				. c

* Data are given only for inoculated plants where uninoculated plants had no nodules. ** Values are mean of four readings

Table 5. Effect of salinity and inoculation on total nitrogen concentration* at maturity (The second experiment).

ECe of soil dS/m	Pod shell	Grains	Whole plant
1.8 Uninoc.	10.00 ab	25.11 b	45.63 bc
1.8 Inoc.	12.24 a	29.61 a	53.46 a
6.0 Uninoc.	7.29 bc	22.23 cd	41.04 cd
6.0 Inoc.	8.28 abc	23.76 bc	47.70 ab
8.3 Uninoc.	4.23 c	20.70 d	39.69 bcd
8.3 Inoc.	4.50 c	23.67 bc	45.09 d

Mean followed by same letter are not significantly different at 5% probability level

Uninoc. = Uninoculated.

Inoc. = Inoculated.

Table 6. Effect of salinity on concentration of Na⁺ and K⁺ in guar grown in soil*.

Plants were harvested at different stages of growth (The second experiment)..

EC _e of soil dS/m	Flowering		Maturity						
Consiste and	Na ⁺ K ⁺		Pod shell		Grain		Shoot		
SHILL IN SHE TONING		Na ⁺	K ⁺	Na ⁺	K+	Na ⁺	K ⁺		
1.8	21.51	77.13	4.32	69.66	3.24	59.76	25.65	75.42	
6.0	20.70	77.67	5.22	52.74	4.10	-	38.70	77.76	
8.3	45.00	76.50	6.84	67.86	5.31	60.30	54.90	79.20	
10.0	68.04	68.76	-	-	-	-	-	-	

^{*} meq/100 dry matter, values are mean of four readings

^{*} mg N/g dry matter, values are mean of four readings

trast, the higher the EC_e level in the soil, the higher was the concentration on Na⁺ in the shoot and plant as a whole at both preflowering and flowereing stages. However, there was only a slight increase in concentration of Na⁺ in pods and grains with the increase in EC_e level of the soil (Table 6).

The third experiment

In this experiment five *Bradyrhizobium* strains were checked for their relative salt tolerance. There was no significant difference in the growth of all the strains tested as even 200 mol/m³ NaCl did not affect the growth. Strains mixture (ARC. 800 G + ARC. 801 G) however showed a slight decrease of 13% in its cell numbers at 200 mol/m³ NaCL. Similar results showing relatively high salt tolerance of Rhizobium strains have also been reported by other workers while working with *Rhizobium meliloti* (Douka *et al* 1984, Kassem *et al.*, 1985, Singleton *et al.*, 1982, and El-Sayed 1995b).

This study suggests that salinity has an indirect effect on biological nitrogen fixation in guar. The rhizobia are generally more capable to cope with salinity than their host legumes. However, the effect of salinity was more pronounced on the number and weight of nodules per plant than on their specific nitrogenase activity. It was clear from Table 4 that at preflowering stage a reduction of 15% in nodule dry weight due to increase in salinity was estimated as compared to only 40% in case of nitrogenase activity. These observations indicate that in addition to the indirect effects, salinity affected nodule formation. Howerver, it seems that when a nodule was formed, then subsequently there was a little influence of salinity on its function provided that the plant can maintain a reasonable photosynthetic activity as indicated from the biomass yields at different salinity levels and the physiological age of the plant.

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تأثير الأملاح على النمو والمحصول وإمتصاص العناصر الغذائية والتثبيت الحيوى للنيتروجين في الجوار

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الغرض من هذا البحث هو دراسة تأثير الملوحة والتقليح البكتيرى على النمو وإمتصاص الأيونات والتثبيت الحيوى للنيتروجين بواسطة محصول الجوار.

عندما وصل التوصيل الكهربائي للتربة المستخدمة في التجربة الي ٨,٣ ديسي سيمين / م (dS/m) لوحظ أن درجة الملوحة بهذه التربة كانت ضارة إلى حد بعيد حيث أدت إلى إنخفاض المحصول بمقدار ٦٠٪ في كل من المادة الجافة ، ومحصول الحبوب لنباتات الجوار، وعندما كان مستوى التوصيل الكهربائي للتربة ١٠ ديسي سيمين / م (dS/m) أحدث تسمماً لنباتات الجوار . كانت سلالات الرايزوبيوم مقاومة للأملاح. وبالرغم من ذلك فإن العقد البكتيرية، والتثبيت الحيوى للنيترجين والتركيز الكلى لعنصر النيتروجين في نباتات الجوار تأثرت تأثراً كبيراً عند التركيزات المرتفعة من الأملاح وكان ملفتا للنظر حدوث إنخفاض كبير في نشاط وحيوية وفعالية الإستيلين المختزل عندما وصل مستوى الأملاح عند ٨,٢ ديسي سيمين / م (dS/m).