IMPACT OF SALINITY ON GROWTH AND SYMBIOTIC PROPERTIES OF SOME GUAR RHIZOBIA

BY

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ABSTRACT:

The effect of salinity (Ece:0, 4, 8, 12 and 16 dSm^{-1}) on growth of 3 *Bradyhizodium* strains, an imported strain TAL 169 and two local isolates: ENRRI 16A and ENRRI 16C was studied in the laboratory. Rhizobial growth was measured in Yeast Extract Mannitol (YEM) broth after incubation at 28C for 5,10,15, and 20 days using a spectrophotometer. Growth of rhizobia was also estimated by the plate count method after 5 days of incubation. Two of the *Bradyrhizobium* strains, TAL 169 and ENRRI 16A, were then used to inoculate guar (Cyamposis tetragonoloba L.) seeds grown in sandy loam soil in pots and irrigated with saline water having different electrical conductivity levels of 0, 2, 4 and dSm^{-1}. Plant samples were taken 40 days after sowing and number of nodules, dry weight of nodules, shoot dry weight and shoot nitrogen content were determined.

Salinity of > 4 dSm^{-1} significantly reduced growth of the three rhizobia. However, average number of viable cells/ml tended to be higher in TAL 169 up to EC 8 than in the local strains. The pot experiment showed that salinity level of 6 dSM^{-1} significantly reduced nodulation, nodule dry weight and shoot nitorgen content of guar plants. However, in association with Guar, strain ENRRI 16 A was found to be more tolerant to salinity compared with strain TAL 169.

Keywords: *Cyamopsis tetragonoloba*, guar, inoculation, *Rhizobium*, salinity.

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ملخص:

تمت دراسة تأثير الملوحة على نمو 3 سلالات من بكتيريا البراديرايزوبيم منها سلالة مستجلبة (TAL 169) وسلالتان معزولتان محليا (ENRRI 16C, ENNRI 16A). تم تحديد نمو البكتيريا في وسط مستخلص الخميرة والمانيتول بعد التحضين عند 28 درجة مئوية لمدة 5 و 10 و 15 و 20 يوماً باستخدام جهاز المطياف الضوئي. كما تم أيضاً تحديد نمو الرايزوبيا في وسط أجار مستخلص الخميرة والمانيتول بعد التحضين عند 28 درجة مئوية لمدة 5 أيام بطريقة عد الأطباق. تم اختيار سلالتين من البراديرازوبيم لتلقيح نباتات القوار في أصص تحتوي على تربة رملية لومية وتم ري النباتات بماء مالح (ملوحة 0 و 2 و 4 و 6 dSm^{-1}) لدراسة تأثير الملوحة على العلاقة التكافلية بين الرايزوبيا والقوار.

نتج عن الملوحة بمستوى أكبر من 4 dSm^{-1} نقصا معنويا في نمو الرايزوبيا وكانت السلالة ENRRI 16A أكثر السلالات مقاومة للملوحة بينما كانت السلالة ENRRI 16C أقلها مقاومة. وأدت الملوحة بمستوى dSm^{-1} إلى نقص معنوي في عدد وزن العقد الجذرية ومحتوى نبات القوار من النيتروجين وكانت السلالة ENRRI 16A أكثر السلالات مقاومة للملوحة مقارنة مع السلالة TAL 169 من السلالة حيث كفاءة العلاقة التكافلية مع القوار تحت ظروف الملوحة.

INTRODUCTION:

Root-nodule bacteria (rhizobia), which fix nitrogen in symbiotic relation with leguminous plants, are affected by high concentrations of salts, and their sensitivity to salinity differs with different species and differs with different species and different strains of the same species [1]. The deleterious effects of salinity on *Rhizobium* growth are also well documented by a number of research workers [2,3]. A particular strain may grow well at a certain level of salinity but its effectiveness in nitorgen fixation might be drastically affected at this level. Moreover, it is not necessary that a positive correlation should always exist between salt tolerance of the microsymbionts gorwn *in vitro* and their effectiveness when they are associated with plants grown under salt-affected conditions [4]. It is commonly believed that the salinity tolerance of the host is lower than that of the rhizobia themesleves [3]. Hence, selection of salt tolerant rhizobia is best tested in the field when they are associated with their host legumes. Saline soils are known to limit plant growth and nitrogen fixation by hindering water uptake, causing nutritional imbalances, inducing toxicity by specificions [5]. Further more, sodicity affects the physical properties of the soil [6]. It was previously [7] shown that guar germination was not affected by salinity level up to 16 dSm^{-1}. However, increase in salinity level reduced shoot dry matter and nodulation of guar plants drastically [8].

This study was conducted to study the effect of different salinity levels on growth of 3 rhizobial isolates and on the *Rhizobium*-guar symbiotic properties.

MATERIALS AND METHODS:

LABORATORY EXPERIMENTS:

Three *Bradyrhizobium* strains were used in this experiment: TAL 169 which is an intorduced strain from Niftal project, Hawaii, USA, and two local isolates, ENRRI 16A and ENRRI 16C isolated from guar plants provided by the Biofertilization Department of the Environment and Natural Resources Research Institute, Khartoum. NaCl and $CaCl_2$ salts were used to prepare different salinity concentrations in order to achieve different electrical conductivities (EC of 0, 4, 8, 12 and 16 dSm^{-1}) in yeast extract mannitol broth [9]. Each strain was used to inoculate six sets of tubes, replicated three times, each se containing YEM broth with one of the diffeent above-mentioned salinity levels. Rhizobial growth was estimated turbidmetrically using a spectrophotometer at 450 nm, 5, 10, 15 and 20 ays after incubation at 28°C. The optical density was taken as an index for estimating growth.

5 days after incubation, the plate count method was used to determine the average number of viable cells per ml of broth at different salinity levels.

POT EXPERIMENT:

A sandy loam soil (EC = 27 dSm^{-1}) was used to grow guar (*Cyamopsis tetragonoloba L.*) seeds cultivar GAF 53. Guar seeds were either noninoculated or inoculated with *Bradyrhizobium* strains TAL 169 or ENRRI 169A. Plants were irrigated with water of salinity 0, 2, 4, and 6dSm^{-1} prepared by the use of NaCl and $CaCl_2$ salts. The pots were arranged in a completely randomized design with 3 replicates. Samples were taken at 40 days after sowing, where the number of nodules, dry weight of nodules and shoot dry matter were determined. The average nitrogen content of shoot was determined by the Kjeldahl method. Analysis of variance was performed to determine the effect of treatments on the measured parameters and the Least Significant Difference test (LSD,$P\leq0.05$) was used to separate between means.

RESULTS AND DISCUSSION:

LABORATORY EXPERIMENTS

Growth of rhizobia increased with incubation up to 15 days after which it started to decline under all salinity levels (Figure 1). Growth of Rhizobium strain TAL 169 tended to be the highest at 0 dSm-1 with *Rhizobium* strain ENRR1 16A performing better at higher salinity levels, while strained ENRRI 16C tended to be the least tolerant (Figure1).These differences were, however, statistically not significant ($P\leq0.05$). The increase in salinity level to EC>4 dSm^{-1} significatly ($P\leq0.05$) reduced growth of rhizobia. However, there was no significant difference between the effects of 8, 12 and 16 dSm^{-1} on growth.

The plate count results showed a similar trend to those of the spectrophotometer results where the growth of all rhizobial strains started to decline when salinity was increased to > 4 dSm^{-1}, with drastic reduction of growth observed when salinity level reached 8 dSm^{-1}. Unlike the in vitro laboratory results, viable TAL 169 cells seemed to exceed those of the two local strains up to salinity of 8 dSm^{-1}. It was previously reported that turbidmetrically estimated rhizobial growth in liquid cultures were highly correlated with estimates by plate count [10].

POT EXPERIMENT:

Table 1 shows that the roots of non inoculated guar plants were found to contain nodules which indicated the presence of indigenous guar rhizobia in the soil under investigation. However, inoculation significantly ($P \leq 0.05$) increased inodulation of guar plants (Table 1), whereas salinity significantly ($P \leq 0.05$) reduced nodulation. Increasing salinity of irrigation water from 0 to 6 dSm^{-1} reduced nodulation by 18.4%. This is constant with previous findings where the number of nodules was approximately halved in *Vicia faba* under salt stress [11]. Strain TAL 169 produced significantly higher nodule formation That ENRRI 16A at water salinity level of 0 dSm^{-1}. This trend reversed with increasing salinity, where ENRRI 16A produced significantly hgier nodulation than TAL 169, indicating that the latter strain is more tolerant to salinity than strain TAL 169 with respect to nodule formation. A similar result was previously reported when the same strains were used for inoulating guar plants grown in a heavy clay soil [12]. These results confirm that rhizobial strains differ in heir adaptation to salinity [1].

Inoculation significantly ($P \leq 0.05$) increased nodule dry weight of guar plants (Table 1), which indicates that the indigenous rhizobia are less effective in nitrogen fixation than any of the three introduced rhizobia. It is known that nitrogen fixation since this is strongly correlated to nodules weight [13]. Table 1 shows that nodule dry weight was significantly reduced average nodule dry weight by 39%. The effect of salinity was more profound on nodules formed by TAL 169 than those formed by ENRRI 16A. An observed decline in nodule dry matter of soybean plants under salt sress was previously reproted and explained by the inadequate supply of photosynthates resulting from a decline in shoot growth under these conditions [14].

Shoot dry matter of guar plants was insignificantly reduced by increasing salinity up to 6 dSm^{-1} (Table2) which indicates that salinity had little effect on growth of these plants [7]. However, plants inoculated with strain ENRRI 16A tended to be more tolerant to salinity than those inoculated with TAL 169. It was previously reported that shoot dry matter decreased with inceasing salinity from 1.3 to 12.4 dSm^{-1} in field -grown guar plants [8].

Shoot nitrogen content of guar plants was significantly ($P \leq 0.05$) decreased with increasing salinity (Table 2) which might be attributed to the negative effect of salts on rhizobia (Figures 1 and 2) and hence on nitrogen fixation rather than on plants growth. High salt stress was previously found to decrease shoot nigrogen percentage of inoculated chick pea plants [15] although it had no effect on nitrogen content of faba bean shoots [16]. Plants inoculated with ENRRI 16A were more tolerant to salinity levels of 4 and 6 dSm^{-1} than those inoculated with TAL 169 with regard to shoot nitorgen content. This again indicates that ENRRI 16A is more effective in nitrogen fixation under saline conditions than strain TAL 169.

It can be generally concluded that, based on the results discussed above, the locally isolated guar rhizobia ENRRI 16A is more tolerant to salinity than the other two strains, and thus an effective symbiosis between the guar plant and these rhizobia can be achieved under the saline conditions investigated. Accordingly, rhizobia of strain ENRRI 16A is tentatively recommended to be used for preparation of guar inoculant particularly under saline conditions.

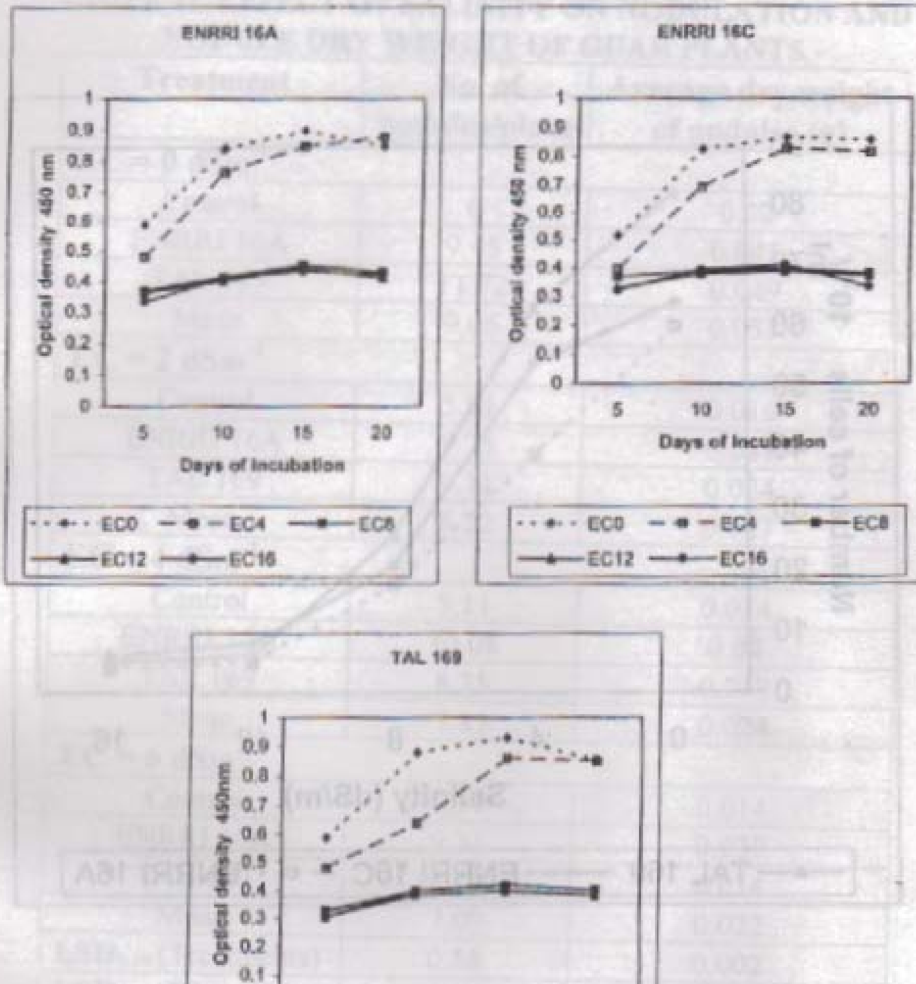

Figure 1: Effect of salinity (dSm^{-1}) level on *Rhizobium* growth in YEM broth

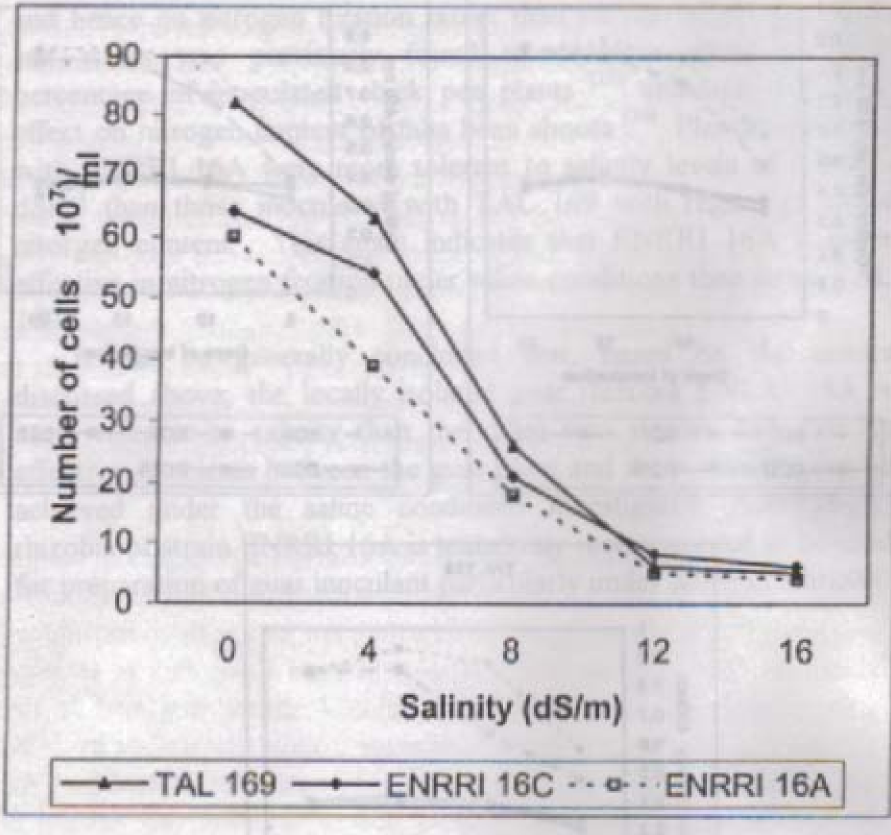

Figure 2: Growth of rhizobia under different salinity levels as measured by the plate count method (5 days after incubation)

TABLE 1: EFFECT OF SALINITY ON NODULATION AND NODULE DRY WEIGHT OF GUAR PLANTS

Treatment	No. of nodules/plant	Average dry weight of nodules (g)
EC = 0 dSm^{-1}		
Control	6	0.02
ENRRI 16A	9.45	0.041
TAL 169	11.72	0.049
Mean	9.06	0.037
EC = 2 dSm^{-1}		
Control	5.81	0.016
ENRRI 16A	9.75	0.041
TAL 169	9.11	0.024
Mean	8.22	0.027
EC = 4 dSm^{-1}		
Control	5.11	0.014
ENRRI 16A	10.08	0.04
TAL 169	8.25	0.019
Mean	7.81	0.024
EC = 6 dSm^{-1}		
Control	5.17	0.014
ENRRI 16A	9.31	0.039
TAL 169	6.78	0.014
Mean	7.09	0.022
$LSD_{0.05}$ (Treatments)	0.58	0.002
$LSD_{0.05}$ (Means)	0.50	0.001

TABLE 2: EFFECT OF SALINITY ON SHOOT DRY WEIGHT (G) AND SHOOT NITROGEN CONTENT OF GUAR PLANTS

Treatment	Shoot dry weight (g)	Shoot nitrogen content (%)
EC = 0 dSm^{-1}		
Control	0.464	3.43
ENRRI 16A	0.595	3.64
TAL 169	0.768	3.57
Mean	0.609	3.55
EC = 2 dSm^{-1}		
Control	0.494	3.15
ENRRI 16A	0.522	3.15
TAL 169	0.691	3.22
Mean	0.576	2.96
EC = 4 dSm^{-1}		
Control	0.551	3.01
ENRRI 16A	0.543	3.01
TAL 169	0.634	2.87
Mean	0.576	2.96
EC = 6 dSm^{-1}		
Control	0571	2.87
ENRRI 16A	0.568	3.08
TAL 169	0.484	2.66
Mean	0.541	2.87
$LSD_{0.05}$ (Treatments)	0.363	0.14
$LSD_{0.05}$ (Means)	0.316	0.13

REFERENCES:

1- **Craig, G.F; Bell. D.T. and Atkins, C.A. (1990).** Response to salt and water logging stress of ten taxa of Acacia selected from naturally saline areas of Western Australia. Australian Journal of Botany 38:619-630.

2- **Elsheikh, E.A. E. and Wood, M. (1989).** Salt effects on the survival and multiplication of chickpea and soybean rhizobia. Soil Biology and Biochemistry 22:343-347.

3- **Faituri, M. Y.; El-Mahi, Y.E. and El-hassan, G.A. (2001).** Effects of some salts and sodicity on the growth of a Rhizobium leguminosarum bv. Viceae strain isolated from a salt-affected soil. Canadian Journal of Microbiology 47:807-812.

4- **Mass, E.V. (1986).** Salt tolerance of plants. Applied Agricultural research 1: 12-26.

5- **Elsheikh, E.A.E. (1998).** Effects of salts on rhizobia and bradyrhizobia: a review. Annals of Applied Biology 132:507-524.

6- **Reddell, P.(1993).** Soil constraints to the growth of nitrogen-fixing trees in tropical environments. In: Symbiosis in Nitrogen-Fixing Trees (ed. N.S. Subba Rao and C. Rodriguez-Barrueco) pp. 65-83. IBH Publishing Co, New Delhi. Oxford.

7- **Kumar, A.; Bahadur, B. and Sharma, B.K. (1988).** Effects of salinity germination, seedling growth and some quantitiative characters of Cyamopsis tetragonoloba (L.) Taub. New Botanist 15(1): 23-27.

8- **Francois, L. E.; Donovan, T. J. and Mass, E.V. (1990).** Salinity effects on emergence, vegetative growth and seed yield of guar. Agronomy Journal 82(3): 587-592.

9- **Somasegaran, P. and Hoben, H.J. (1994).** Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer-Verlag. New York. 450 pp.

10- **Correa, O. S. and Barneix, A.J. (1997).** Cellular mechanisms of pH tolerance in Rhizobium loti. World Journal of Microbiology and Biotechnology 13:153-157.

11- **Sprent, J.R. and Zahran, H.H. (1988).** Infection, deelopment and functioningof nodules under drought and salinity. In: Nitorgen Fixtion by Legumes in Mediterranean Agriculture (ed. D.P. Beck and L.A. Materon). Martinus Nijhoff Publishers, the Netherlands.

12- **Elsheikh, E.A.E. and ibrahim, K.A. (1999).** The effect of Braddrhiobium inoculation on yield and seed quality of guar (Cyamopsis tetragonoloba L.) Food Chemistry 65: 183-187.

13- **Ali, M., Horiuchi, T. and Miyagawa, S. (1997).** Nodulation, nitrogen fixation and growth of soybean plants (Glycine max Merr.) in soil supplemented with chitin and chitosan. Japanese Journal of Crop Science 66(1): 100-107.

14- **Yadav, H.D.; Yadav, O.P.; Dhankar, O.P. and Oswal, M.c. (1989).** Effect of chloride salinity and boron on germination, growth and mineral composition of chick pea (Cicer arietinum L.) Annals of Arid Zone 28 (1-2): 63-67.

15- **Cordovilla, M.; Ligero, F. amd ::icj. C. (1999).** Effect of salinity on growth, nodulation and nitrogen assimilation in nodules of faba bean (Vicia faba L.). Applied Soil Ecology 11:1-7.