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Modeling the Effect of Salt-stress on Growth, Nodulation and Nitrogen Fixation of Cowpea (*Vigna unguiculata* L.)

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Abstract: The effect of salinity on *Bradyrhizobium* strains and growth and symbiotic performance of cowpea (*Vigna unguiculata* L.) was investigated in laboratory and pot experiments. *Bradyrhizobium* strains were more salt-tolerant than cowpea plants. Salinity significantly ($P \leq 0.05$) reduced the shoot and root dry weights, number and dry weight of nodules and nitrogen content of cowpea. Inoculation with *Bradyrhizobium* strain ENRRI-16C significantly ($P \leq 0.05$) increased all the parameters measured under normal and saline conditions. The results were also expressed in statistical models, which indicated positive relationships between plant weight and nodules number and negative relationships between salinity and nodules number. These statistical models are good tools to predict the status of nodulation or plant weight or both, grown under saline conditions without uprooting the plant.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is grown for its leaves, green pods, grain and stover. It is an extremely resilient crop, and is cultivated under some of the most extreme agricultural conditions in the world. The crop is sensitive to salinity during the vegetative stage and becomes less sensitive during the flowering and pod-filling stages. The response of cowpea to inoculation in Sudan was investigated by many workers. Their results showed that the crop is usually well nodulated and is active nitrogen fixer, but it still responds to inoculation with competent rhizobial strains (Mahdi

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and Habish 1975; Habish and Mahdi 1976). Moreover, inoculation significantly enhances nodulation, fresh and dry matter yields and N P K content (Galal El Din 1993; Osman *et al.* 2002; Singh and Usha 2003).

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. Most of the salt-affected soils in the Sudan have a relatively low nutrient content. According to Egeh and Zamora (1992), irrigation using saline water (7.0 dS/m) significantly reduced the plant height, root length and dry matter production of cowpea. Moreover, salinity is known to decrease nodulation and nitrogen fixation through its harmful effect on nodule activity (Elsheikh 1992).

It has been reported that saline conditions may limit the legume-*Bradyrhizobium* symbiosis by (i) affecting survival and proliferation of rhizobia in the soil and rhizosphere, (ii) inhibiting the infection process, (iii) directly affecting root nodule function, and (iv) reducing plant growth, photosynthesis and demand for nitrogen (Elsheikh 1998). Moreover, salinity reduces dry weight of cowpea (Egeh and Zamora 1992), faba bean (Ahmed 1996), chick pea (Elsheikh 1992), dry bean (Cachorro *et al*. 1993) and fenugreek (Forawi 1994). Research on the effect of salinity on *Bradyrhizobium* and its symbiotic properties with legumes, in the Sudan, indicates that nodulation and growth of legumes can be significantly enhanced by *Bradyrhizobium* inoculation (Elsheikh 1998).

Generally, the number of nodules per plant could only be determined by uprooting the plant from the soil. Hence, the objective of this work was (a) to determine the effect of salinity on *Bradyrhizobium*-cowpea symbiosis (b) to derive a model, if possible, for the relationship between salinity and symbiotic activity, and (c) to predict the status of nodulation at any salinity level without uprooting the plant.

MATERIALS AND METHODS

Source of seeds, soil and *Bradyrhizobium* strain

Seeds of cowpea (*Vigna unguiculata* L.) cultivar "Black Eye" were obtained from Elobeid Agricultural Research Station, Sudan. A "Gerf" soil with the following characteristics was used: 35% sand, 35% silt and 30% clay; pH = 7.7; EC = 0.94 dS/m, SAR = 0.5; Na, Ca, Mg, K, Cl and SO_4 were 0.1, 3.0, 1.5, 0.1, 1.5 and 1.2 (meq/l), respectively; the total P was 2.0 μg/g and N was 0.02%. *Bradyrhizobium* strain ENRRI-16C was supplied by the National Centre for Research, Sudan. The basal medium used was a defined medium (Wood and Cooper 1988).

Effects of EC and SAR on Bradyrhizobium strain

This experiment was conducted to study the growth and survival of *Bradyrhizobium* strain on different levels of electrical conductivity (EC) or sodium adsorption ratio (SAR). Defined medium with different levels of salinity was dispensed as 75 ml aliquots in 200 ml sterile medical bottles. Calcium chloride and sodium chloride were added to the defined medium before autoclaving to give a final electrical conductivity of 0.07, 10.0, 20.0, 30.0 or 40.0 dS/m or a final sodium adsorption ratio of 0.0, 15.0, 30.0, 45.0 or 60.0 $(meq/l)^{1/2}$. The media were inoculated with a four-day old culture and shaken at 90 rpm, with three replicates. Optical densities at wavelength 540 nm were measured immediately after inoculation, and then every five days for 15 days.

Pot experiments

Pot experiments were carried out at the glass-house, Faculty of Agriculture, University of Khartoum, in a randomized complete block design with three replicates. Five sterile seeds were sown in each pot, and the seedlings were thinned to two per pot, 15 days later. Five-ml culture of *Bradyrhizobium* strain ENRRI-16C (10^9 cfu ml^{-1}) was added where appropriate. Irrigation with tap water continued for three weeks after sowing, and then the pots were irrigated with saline water as desired. Calcium chloride and sodium chloride were used for preparing mixed-salt solutions of different electrical conductivity (EC) and different sodium adsorption ratio (SAR), according to the following equations:

$$EC = Na^{+} + Ca^{++} \quad (1)$$

$$SAR = \frac{Na}{\sqrt{\frac{Ca^{++}}{2}}} \quad (2)$$

where EC = electrical conductivity (dS/m); Na^{+} = sodium concentration (meq/l); Ca^{++} = calcium concentration (meq/l) ; SAR = sodium adsorption ratio $(meq/l)^{1/2}$.

The number of nodules and fresh weights of shoots and roots were determined immediately after harvesting. Shoot, root and nodules dry weights were determined after oven drying at 80°C for 48 hours. Nitrogen content was determined by the micro–Kjeldahl method (Chapman and Pratt 1961). The data were analyzed statistically using the analysis of variance and the linear, logarithmic, exponential, polynomial and power regression models .

(a) Experiment I: This experiment was carried out to study the effect of salinity (dS/m) and inoculation with *Bradyrhizobium* on growth and symbiotic properties of cowpea. The treatments were

1- plants irrigated with 0.26 dS/m,
2- plants irrigated with 2.00 dS/m,
3- plants irrigated with 3.00 dS/m,
4- plants irrigated with 4.00 dS/m,
5- plants irrigated with 5.00 dS/m, and
6- plants irrigated with 6.00 dS/m.

Each of these treatments was either uninoculated or inoculated with *Bradyrhizobium* strain ENRRI-16C. The plants were harvested eight weeks after sowing.

(b) Experiment II: This experiment was conducted to study the effect of sodium adsorption ratio (SAR) and inoculation with *Bradyrhizobium* on growth and symbiotic properties of cowpea .The treatments were

1- plants irrigated with 0.0 $(meq/l)^{1/2}$,
2- plants irrigated with 5.00 $(meq/l)^{1/2}$,
3- plants irrigated with 10.0 $(meq/l)^{1/2}$,
4- plants irrigated with 15.0 $(meq/l)^{1/2}$, and
5- plants irrigated with 20.0 $(meq/l)^{1/2}$.

Each of these treatments was either uninoculated or inoculated with *Bradyrhizobium* strain ENRRI-16C. The plants were harvested eight weeks after sowing.

RESULTS

Both EC and SAR significantly ($P \leq 0.05$) decreased the growth of *Bradyrhizobium* strain ENRRI–16C. The growth (turbidity in terms of optical density) decreased with increased salinity levels and increased with incubation period (Figs.1 and 2).

In the first pot experiment, salinity significantly ($P \leq 0.05$) reduced the shoot and root dry weights and number and dry weight of nodules of cowpea plants (Table 1). *Bradyrhizobium* inoculation significantly increased nodule number and shoot, root and nodule dry weights under normal and saline conditions. Inoculation with *Bradyrhizobium* strain ENRRI-16C significantly ($P \leq 0.05$) increased the total nitrogen content of the shoot of cowpea in all salinity levels (dS/m) compared to their homologous uninoculated plants, whereas salinity significantly ($P \leq 0.05$) decreased the total plant nitrogen in all treatments (Table 1).

In the second pot experiment, sodicity (SAR) significantly ($P \leq 0.05$) decreased the shoot and root dry weights and number and dry weight of nodules. Inoculation with *Bradyrhizobium* significantly ($P \leq 0.05$) increased the shoot, root and nodule dry weights and nodule number compared to the uninoculated control plants (Table 2).

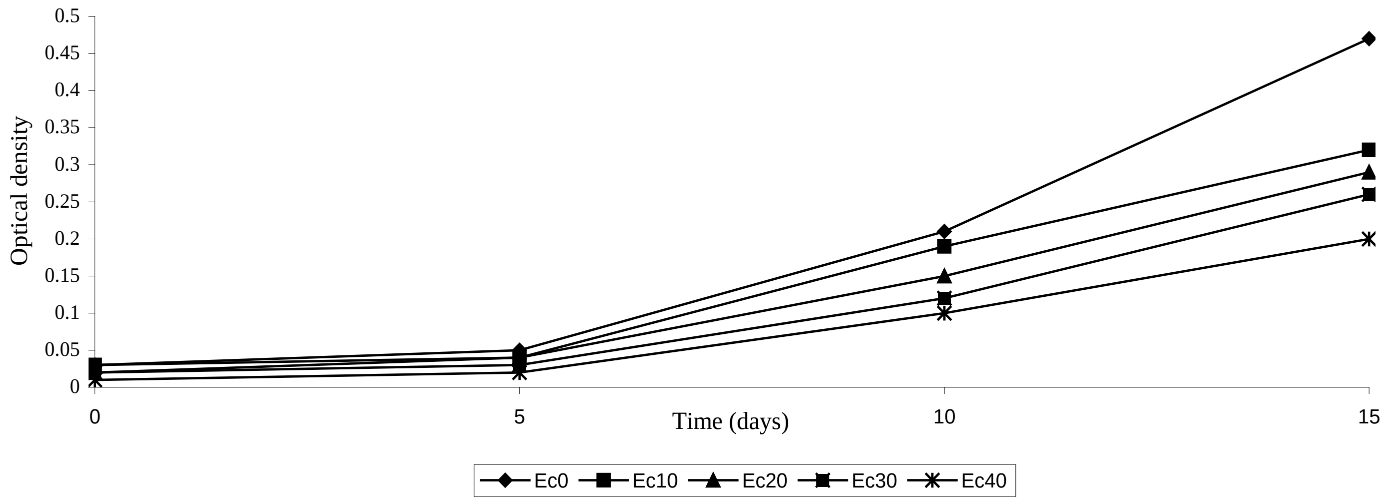

Fig.1. Effect of salinity (dS/m) and incubation period on turbidity (optical density) as index of growth of strain ENRRI-16C

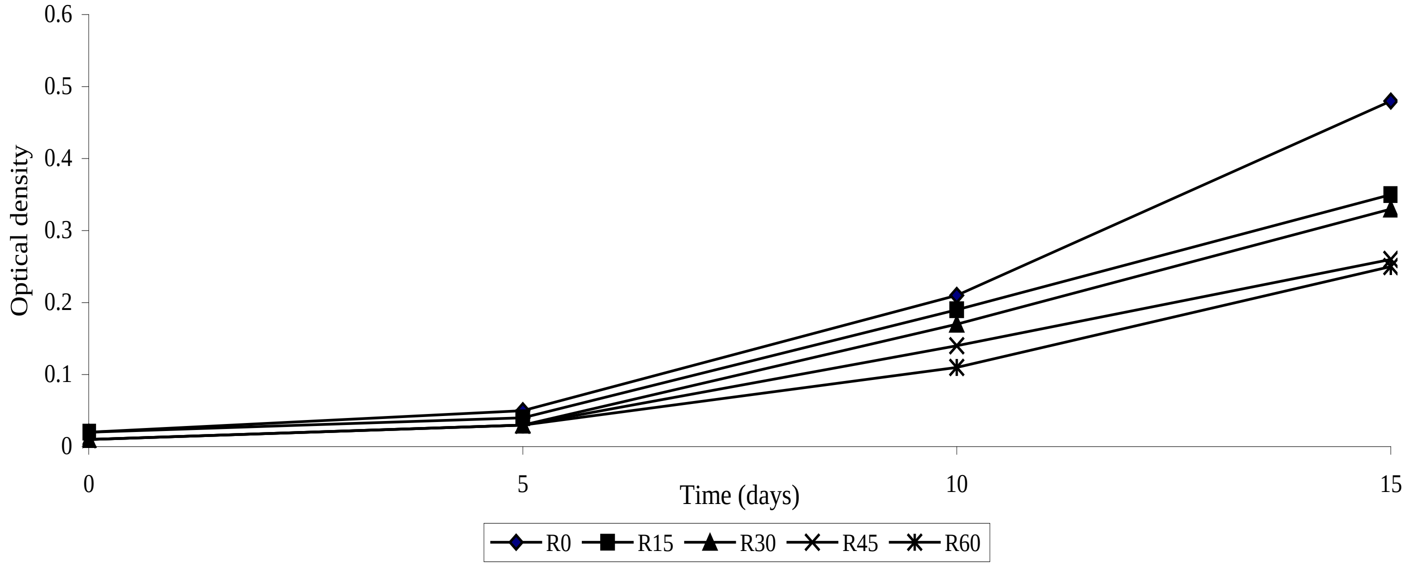

Fig.2. Effect of sodium absorption (meq/l) and incubation period on turbidity (optical density) as index of growth of strain ENRRI-16C

Table 1. Effect of salinity (dS/m) and *Bradyrhizobium* inoculation on shoot dry weight, root dry weight, nodules number, nodules dry weight and nitrogen content of cowpea after eight weeks from sowing

Parameter	Salinity level (dS/m)					
	0.26	2.0	3.0	4.0	5.0	6.0
			Shoot dry weight (g/plant)			
Uninoculated	3.25	3.01	2.89	2.00	1.75	1.28
Inoculated	4.86	4.12	3.83	3.05	2.62	2.10
			Root dry weight (g/plant)			
Uninoculated	0.52	0.48	0.39	0.30	0.25	0.20
Inoculated	0.67	0.59	0.45	0.38	0.31	0.26
			Nodules number (per plant)			
Uninoculated	12.0	10.0	9.0	7.0	5.0	4.0
Inoculated	28.0	25.0	23.0	20.0	19.0	16.0
Uninoculated	53.0	43.0	38.0	29.0	20.0	15.0
Inoculated	83.0	72.0	63.0	51.0	43.0	31.0
			Nitrogen content (%)			
Uninoculated	3.17	2.73	2.63	2.44	2.23	2.12
Inoculated	3.65	2.94	2.89	2.82	2.71	2.50

SE for shoots = ± 0.005; SE for roots = ± 0.006; SE for nodule No. = ± 0.58
SE for nodule dry weight = ± 0.58; SE for N content = ± 0.02

Table 2. Effect of sodium adsorption ratio $(meq/l)^{1/2}$ and *Bradyrhizobium* inoculation on shoot dry weight, root dry weight, nodules number, nodules dry weight and nitrogen content of cowpea after eight weeks from sowing

Parameter	Sodicity level $(meq/l)^{1/2}$				
	0	5	10	15	20
	Shoot dry weight (g/plant)				
Uninoculated	2.36	1.92	1.56	1.43	1.33
Inoculated	2.64	2.23	2.03	1.64	1.47
	Root dry weight (g/plant)				
Uninoculated	0.43	0.42	0.38	0.36	0.32
Inoculated	0.53	0.44	0.42	0.37	0.34
	Nodules number (per plant)				
Uninoculated	11.0	9.0	7.0 b	6.0	5.0
Inoculated	25.0	20.0	19.0	16.0	12.0
	Nodules dry weight (mg/plant)				
Uninoculated	55.0	49.0	36.0	30.0	25.0
Inoculated	85.0	72.0	68.0	59.0	40.0
	Nitrogen content (%)				
Uninoculated	3.16	3.00	2.88	2.81	2.74
Inoculated	3.63	3.46	2.95	2.36	2.83

SE for shoots = ± 0.06; SE for roots = ± 0.02; SE for nodule No. = ± 0.47
SE for nodule dry weight = ± 0.58; SE for N content = ± 0.053

Table 3. Regression models of experiment 1

	Linear		Exponential		Log		Power		Polynomial	
	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*
Nodules number versus initial salinity										
a_0	12.75	28.86	14.22	29.68	9.85	24.46	9.44	24.46	12.35	28.52
a_1	-1.46	-2.08	-0.20	-0.09	-2.31	-3.35	-0.30	-0.14	-1.04	-1.73
a_2									-1.07	-0.05
r	0.99	0.99	0.97	0.99	0.87	0.89	0.80	0.85	0.99	1.00
Nodules number versus final salinity										
a_0	13.27	29.68	14.86	30.60	11.64	27.40	11.72	27.43	11.83	27.89
A_1	-0.67	-0.96	-0.09	-0.04	-2.13	-3.12	-0.27	-0.14	0.18	-0.08
a_2									-0.07	-0.09
r	0.94	0.89	0.89	0.93	0.83	0.83	0.72	0.78	0.99	1.00
Plant weight versus initial salinity										
a_0	4.19	5.78	4.58	6.17	3.30	4.67	3.30	4.51	3.92	5.68
a_1	-0.43	-0.57	-0.17	-0.15	-0.65	-0.91	-0.24	-0.23	-0.16	-0.46
a_2									-0.04	-0.018
r	0.97	0.99	0.95	0.98	0.81	0.89	0.76	0.83	0.98	1.00
Plant weight versus final salinity										
a_0	4.30	5.99	4.70	6.42	3.78	5.37	3.80	5.39	5.49	5.49
a_1	-0.19	-0.26	-0.73	-0.07	-0.59	-0.84	-0.22	-0.21	0.15	0.04
a_2									-0.03	-0.02
r	0.89	0.95	0.85	0.91	0.72	0.82	0.67	0.76	0.98	0.99
Nodules number versus plant weight										
a_0	0.37	-2.03	1.02	0.77	-1.57	-13.84	0.46	0.03	-0.19	-1.86
a_1	0.29	0.27	0.11	0.07	2.16	5.79	0.86	1.56	0.46	0.25
a_2									-0.01	0.003
r	0.98	0.99	0.97	0.98	0.98	0.99	0.98	0.99	0.98	0.99

a_o , a_1 , a_2 are constant parameters

In the first pot experiment, the relationship between the nodules number and the initial salinity (salinity$_I$; the salinity of the irrigation water) nearly fits in all calculated correlation models. The relationship was negative, which was indicated by the negative sign in front of the slope (a_1) values. That means nodule number decreases as salinity increases. Table 3 shows the regression models that relate the total number of nodules per plant to the final salinity (salinity $_f$) of the soil extract at harvest. The data fit well in most models and are in harmony with that of nodules number versus the initial salinity.

The regression models showed a negative correlation between the initial salinity and the total plant weight. The relationship was significant in all correlation models. The data of total plant weight and their corresponding final salinity followed a trend similar to that of initial salinity (Table 3). A positive correlation between the total number of nodules per plant and total plant weight was obtained, and the data fit well in most models.

In the second pot experiment, although the data of nodules number per plant fit with initial SAR (SAR_i; the SAR of the irrigation water) values in most models, the polynomial model was the most suitable for all treatments (Table 4). The r values of the power models were insignificant indicating a weak relationship. The correlation between the total nodules number per plant and the final SAR (SAR_f) values at harvest showed a trend similar to that of the nodules number and the SAR_i value. The relationship between the plant weight and the SAR_f at harvest followed a trend similar to that of the initial SAR of irrigation water. A highly positive correlation between the total number of nodules per plant and the total plant weight was obtained, and the data fit in most of the models (Table 4).

Table 4. Regression models of experiment

	Linear		Exponential		Log		Power		Polynomial	
	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*	Control	*Bradyrh-izobium*
Nodules number versus initial salinity										
a_0	10.80	24.40	11.15	25.07	14.05	29.72	18.87	36.91	10.94	24.04
a_1	-0.30	-0.60	-0.04	-0.03	-2.93	-5.39	-0.42	-0.34		-0.60
a_2									0.002	9.91
r	0.99	0.98	0.99	0.98	0.97	0.90	0.95	0.88	0.99	0.98
Nodules number versus final salinity										
a_0	11.67	26.18	12.44	27.57	13.29	29.47	14.97	32.31	11.50	25.52
a_1	-0.26	-0.52	-0.03	-0.02	-2.27	-4.57	-028	-0.24	-0.2	-0.38
a_2									-0.001	-0.005
r	0.99	0.98	0.97	0.96	0.93	0.92	0.88	0.87	0.99	0.98
Plant weight versus initial salinity										
a_0	2.67	3.10	2.69	3.14	3.12	3.76	3.48	4.37	2.79	3.15
a_1	-0.06	-0.07	-0.03	-0.03	-0.49	-0.63	-0.25	-0.28	-0.11	-0.08
a_2									0.002	0.001
r	0.97	0.99	0.98	0.99	0.99	0.96	0.99	0.96	0.99	0.99
Plant weight versus final salinity										
a_o	2.85	3.30	2.91	3.40	3.22	3.68	3.41	3.93	2.97	3.30
a_1	-0.05	-0.06	-0.02	-0.02	-0.46	-0.52	-0.20	-0.20	-0.07	-0.06
a_2									0.001	0.00002
r	0.98	0.99	0.99	0.98	0.97	0.93	0.96	0.91	1.00	0.99
Nodules number versus plant weight										
a_0	0.64	0.40	1.05	1.03	-0.68	-3.06	0.56	0.39	0.89	1.09
a_1	0.19	0.11	0.08	0.05	1.38	1.90	0.64	0.80	-0.15	0.03
a_2									0.021	0.002
r	0.97	0.98	0.98	0.99	0.94	0.96	0.96	0.98	0.99	0.99

a_o, a_1, a_2 are constant parameters

DISCUSSION

In this study, *Bradyrhizobium* strain ENRRI-16C tolerated salinity up to 40 dS/m and sodium adsorption ratio up to 60 $(meq/l)^{1/2}$, whereas the cowpea plants tolerated salinity up to 6.0 dS/m and sodium adsorption ratio up to 20 $(meq/l)^{1/2}$. According to Forawi (1994), the four tested *Rhizobium meliloti* strains tolerated salinity up to 64 dS/m in solution cultures. It is well documented that the rhizobia and bradyrhizobia or both are generally more able to cope with salinity than their host legumes. Moreover, the harmful effect of salinity on rhizobia or bradyrhizobia may be due to direct specific ion effects or to the indirect effect of salinity by raising the pH value and decreasing osmotic potential (Elsheikh 1998).

Salinity significantly reduced the shoot and root dry weights, nodules number, nodules dry weight and nitrogen content of cowpea. The nodule number was more affected by salinity than plant growth. Elsheikh (1998) found that salt reduces the development of new nodules by depressing the nodules activity.

All uninoculated cowpea plants showed few nodules in their roots which indicated the presence of indigenous *Bradyrhizobium* in “Gerf” soil. The presence of *Bradyrhizobium* strains in “Gerf” soil could be attributed to the continuous cultivation of cowpea and other crops of the same cross inoculation group of these bacteria along the banks of the Blue Nile and the White Nile (Hadad and Loynachan 1985). Inoculation of cowpea with *Bradyrhizobium* strain ENRRI-16C significantly increased shoot and root dry weights, number and dry weight of nodules and nitrogen content.

There was a negative relationship between salinity levels (initial and final) and nodules number, indicating that the nodules number decreased gradually with increasing salinity level. The regression models also showed a negative correlation between plant weight and salinity, indicating a reduction in plant weight by increasing salinity level. As expected, there was a positive relationship between nodules number and plant weight.

Modeling of the data is an efficient way by which a reliable relationship between the factor(s) and the parameter(s) measured could be produced. The data are usually fitted in different equation(s) using different models. These models could be used as standards and yardsticks to give information to be generated by any real or hypothetical value(s) of that particular parameter(s), and could be used as good tools to predict the number of nodules or plant weight, or both, grown under saline conditions without uprooting the plant. More pots and field experiments need to be carried out to verify these models.

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تأثير الملوحة على نمو اللوبيا وقدرتها على تكوين العقد الجذرية وتثبيت النيتروجين الجوى

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موجز البحث: أجريت تجارب معملية وأخرى فى أصص لدراسة تأثير الملوحة على بكتيريا العقد الجذرية *Bradyrhizobium* سلالة ENRRI-16C وعلى نمو نبات اللوبيا وأدائها التكافلى. كانت سلالة العقد الجذرية أكثر تحملاً من نبات اللوبيا ، وأدت الملوحة إلى إنخفاض معنوى فى كل من الوزن الرطب والجاف لكل من المجموع الخضرى والجذرى وعدد العقد الجذرية ووزنها الجاف ومحتوى نبات اللوبيا من النيتروجين. دلت الدراسة على أن تلقيح نبات اللوبيا بسلالة بكتيريا العقد أدى إلى زيادة كل المعايير التى تم قياسها سواء تحت الظروف العادية أو تحت ظروف الملوحة.

بينت معادلة أنموذج الانحدار أن العلاقة بين عدد العقد الجذرية لنبات اللوبيا والملوحة علاقة عكسية بينما أوضحت أن العلاقة بين وزن النبات وعدد العقد الجذرية علاقة طردية. كذلك أوضحت الدراسة أن هذه المعادلات يمكن أن تكون أداة جيدة للتنبؤ بمعدل تكوين العقد الجذرية ووزن النبات تحت ظروف الملوحة دون الحاجة لاقتلاع النبات.

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