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EFFECT OF SALINITY ON GROWTH OF *RHIZOBIUM* SPP., NODULATION AND HEIGHT OF *PROSOPIS* SPECIES

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Abstract

The effect of salinity on the growth of *Rhizobium* spp., isolated from *Prosopis glandulosa* and *P. juliflora* was studied *in vitro*. Growth of *Rhizobium* isolated from both *Prosopis* species increased at all salinity levels as compared with the control (0.01%) except at 1% NaCl level. *Rhizobium* sp., isolated from *P. glandulosa* showed a gradual decline as salinity increased from 0.3 to 1.0%; that isolated from *P. juliflora* showed a decline as salinity increased from 0.2 to 1.0%. Increasing salinity caused a gradual reduction in seed germination, height of the plant, number of nodules per plant and nodule size.

Introduction

Increasing soil salinity has greatly reduced agricultural productivity in various parts of the world. The effect of salinity on leguminous crops appear to be greater presumably because of a direct influence on the nitrogen-fixing ability of root nodule bacteria (Munns *et al.*, 1977). Increasing levels of NaCl have been found to cause reduction both in nodulation and nitrogen-fixing ability in peas and mungbeans (Steinborn & Roughley, 1975). Mesquite (*Prosopis* spp.), a nitrogen-fixing tree legume, grows luxuriantly under various salinity and aridity regimes. The plant has been found to survive and even flourish in soil made saline with 12,800 mg/l NaCl, and its roots can extract water from soil that is salinized with more than 17,900 mg/l (Jarrell & Virginia, 1984). *Prosopis* seeds collected from widely divergent saline areas of Africa, Argentina, Mexico, and the United States have been grown in sand culture up to 3.3% NaCl concentration (Rhodes & Felker, 1988). Nodulation has been reported in 12 *Prosopis* species with different rhizobial strains (Felker & Clark, 1980).

The present work was carried out to study the effects of different levels of NaCl salinity on the growth of *Rhizobium* species isolated from *P. glandulosa* and *P. juliflora*. The study also investigated the effects of different NaCl concentrations on seed germination, height of the plant, and the number and size of nodules of *P. juliflora* after inoculation with *Rhizobium* sp.

Materials and Methods

Isolation of Rhizobium spp.: *P. glandulosa* and *P. juliflora* roots collected from fields of Sujawal, Pakistan, were used. After washing in running tap water, the nodules were separated and surface sterilized in 0.01% HgCl for 5 minutes, followed by washing in sterilized distilled water. The nodules were dipped in 70% ethyl alcohol for 3 minutes, and after washing in sterilized distilled water, were crushed with a sterilized glass rod in a Petri dish and streaked on YEM agar containing Congo red (Subba Rao, 1977).

Effect of Salinity on growth of Rhizobia in vitro: Yeast extract mannitol broth (yeast extract, 1.0 g; mannitol, 10 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; distilled water, 1 L) was transferred @ 25 ml/flask and sterilized at 15 psi for 15 min. NaCl was used to adjust the salinity of the YEM broth to 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, and 1.0% concentrations. Flasks with 0.01% NaCl served as the control. Three-day-old rhizobial cultures isolated from *Prosopis* sp., were inoculated in YEM broth @ 1 ml/flask. Five replicates of each treatment were incubated for 72 h at room temperature (30 - 35 °C) on a rotary shaker. The growth of rhizobia in culture was determined at every transfer of suspension by determining the number of colony-forming units (cfu) of the broth culture. Using serial dilution of the broth culture, one ml from 10^{-6} to 10^{-9} dilutions was transferred aseptically in sterile Petri dishes, and was then mixed with 15 ml melted YEMA. Each of these treatments was replicated 3 times. Petri dishes were incubated at 30 °C for 3 days, and the number of colonies (cfu) in each plate was then counted (Somasegaran & Hoben, 1985). The population of rhizobia was estimated using the formula:

Colony-forming unit (cfu) of rhizobia = No. of colonies x dilution factor

Effect of Rhizobial Inoculation Under Saline Conditions on Seed Germination, Height of Plant, and Nodulation: Seeds of *P. juliflora* collected from Sujawal, were used. Rhizobial strains isolated from nodules of *P. juliflora* were grown on yeast extract mannitol agar. The rhizobia were transferred serially from the culture of lower to higher salt concentration and maintained at 0.01, 0.2, 0.4, 0.6, 0.8, and 1.0% NaCl salts. The seeds were scarified in concentrated H_2SO_4 and thoroughly washed with distilled water, then inoculated with the appropriate suspension of *Rhizobium* using 1% gum arabic as sticker. Loamy sand soil collected from Karachi University experimental plots was autoclaved at 15 psi and transferred into 8 cm diameter plastic pots without any drainage, 250 g in each. The soil was moistened to field capacity with respective saline solutions to give an EC_e equivalent to 0.2 to 1% NaCl solutions. Seeds inoculated with *Rhizobium* from the different salinity treatments were sown in the pots in soil with the same adjusted salinity of 0, 0.2, 0.4, 0.6, 0.8 and 1.0% NaCl. In a second set of experiments, uninoculated seeds were grown in sterilized soil. When the seedlings were 10 days old, the soil was drenched with rhizobial suspension of acclimatized salinities. In a third set of experiments, seed inoculation treatment, was followed by soil drench. There were three replicates of each treatment and the pots were randomized on a greenhouse bench. The soil was kept at 50% WHC by daily watering the plants. The plants were harvested after 6 week and height of the plant and number and size of root nodules were recorded.

Results

Effect of Salinity on Growth of Rhizobium in vitro: The growth of *Rhizobium* sp., isolated from *P. glandulosa* showed an increase from 32 x 10^6 to 252 x 10^6 cfu at 0.1

and 0.2% NaCl salinity, respectively, followed by a decline to 125 x 10^6 cfu at 0.3% and 29 x 10^6 cfu at 1% salinity, as compared with the control at 0.01% salinity (Fig. 1a). *Rhizobium sp.*, isolated from *P. juliflora* (Fig. 1a) showed a gradual decline from 208 x 10^6 cfu at 0.1% NaCl salinity to 35 x 10^6 cfu at 1% salinity as compared with the control. *Rhizobium* grown at different salinity levels showed no change in cell size and morphology.

Effect of Rhizobial Inoculation Under Saline Conditions on Seed Germination, Height of Plant, and Nodulation: Seeds of *P. juliflora* inoculated with *Rhizobium* acclimatized at different salinity levels and sown in soil adjusted to different NaCl salinity levels showed 62, 25, 20, 16 and 6.6% germination, respectively, in 0.2, 0.4, 0.6, 0.8 and 1.0% salinity, as compared with 84% germination in the control. With the gradual increase in soil salinity, there was a gradual reduction in height of the plants as compared with the control. There was a significant ($P < 0.05$) decrease in number of nodules at 1.0% NaCl salinity as compared with the control (Table 1). With an increase in salinity, a significant decrease ($P < 0.05$) in diameter of nodules was observed. When seedlings of *P. juliflora* were drenched with rhizobial suspension, a gradual reduction in size of plant, number of nodules/plant, and size of nodules was observed in soil adjusted at 0.2 to 1.0% NaCl salinity as compared with the control. The plants were taller and nodule more numerous than those grown in salinized soil. In the third set of experiments, where seed inoculation was applied followed by a soil drench with *Rhizobium*, no significant difference in height of the plants was observed. A significanct ($P < 0.05$) increase in number and size of nodules was observed at 0.2% NaCl salinity; however, a gradual decrease in height of plants, number of nodules per plant and size of nodules was recorded from 0.4 to 1.0% NaCl treatment as compared with the control (Table 1).

Discussion

The growth and multiplication of the *Rhizobium* isolated from *P. glandulosa* and *P. juliflora* declined rapidly as the NaCl concentration increased from 0.2 to 1.0%. A wide variation in salt tolerance has been reported among *Rhizobium* species. *Rhizobium japonicum* isolated from *Glycine javanica* was found tolerant to 0.5% (Wilson & Norris, 1970); *R. trifolii* isolated from *Trifolium* sp., to 0.50-0.72% (Pillai & Sen, 1966); and *R. meliloti* isolated from *Melilotus* sp., to 3% NaCl salinity (Graham & Parker, 1964; Subba Rao *et al.*, 1972). The growth and multiplication of rhizobia from soybean declined rapidly when the NaCl concentration was increased from 0.2 to 0.8% (Tu, 1981). Similarly the growth of *R. leguminosarum* declined rapidly as the salinity increased from 0.01-2.0% (Rai, 1983).

Salinity reduced seed germination in *P. juliflora*. Inoculation with *Rhizobium* did not appear to be advantageous at the germination stage. Higher soil salinity has been reported to delay seed germination in wheat (Ansari *et al.*, 1980) and in tropical legumes (Keating *et al.*, 1986). In the present work, the presence of a high NaCl concentration in the soil decreased the growth and multiplication of rhizobia and reduced the number and size of the nodules of *P. juliflora*. Similar observations have been made with other legumes (Tu, 1981; Haq & Larher, 1983). Conditions that

Table 1. Effects of inoculation of *Prorops juliflora* with *Rhizobium* sp., at different levels of NaCl salinity in soil on seed germination plant height, size and number of nodules.

NaCl Concentration	Seed Germination (%)	Seed inoculation			Soil drench			Seed inoculation + Soil drench		
		No. of Nodules per plant	Diameter of nodules (mm)	Plant Height (cm)	No. of Nodules per plant	Diameter of nodules (mm)	Plant Height (cm)	No. of Nodules per plant	Diameter of nodules (mm)	Plant Height (cm)
Control	84.0	5.8	4.4	19.8	14.0	5.0	24.2	4.4	3.6	19.0
(EC: 1.7 $dS.m^{-1}$)	±3.4	±1.5	±0.5	±1.5	±1.7	±0.7	±2.2	±0.7	±0.3	±0.5
0.2%	62.0	7.6	3.0	14.4	10.2	3.8	16.2	9.2	5.4	19.2
(EC: 3.3 $dS.m^{-1}$)	±6.2	±1.4	±0.7	±0.6	±1.3	±0.4	±2.3	±3.4	±0.2	±1.0
0.4%	25.0	3.0	2.6	12.4	9.6	2.6	14.4	2.2	1.8	14.0
(EC: 6.6 $dS.m^{-1}$)	±2.5	±0.5	±0.2	±0.4	±2.3	±0.9	±0.3	±0.7	±0.5	±0.7
0.6%	20.0	5.6	2.6	11.0	6.6	3.8	13.8	1.8	1.9	12.0
(EC: 9.8 $dS.m^{-1}$)	±2.9	±1.0	±0.2	±0.3	±1.0	±0.9	±0.3	±0.3	±0.6	±1.1
0.8%	16.0	5.2	3.2	11.2	4.2	2.2	12.2	3.2	1.3	13.0
(EC: 12.9 $dS.m^{-1}$)	±3.4	±0.3	±0.1	±0.5	±0.8	±0.7	±0.7	±0.8	±0.2	±1.0
1.0%	6.60	2.6	2.2	9.8	3.8	2.8	13.4	1.2	2.8	10.8
(EC: 16.0 $dS.m^{-1}$)	±2.9	±1.6	±0.3	±0.3	±0.3	±0.6	±0.6	±0.3	±0.7	±0.7
F-Value	65.5	2.4	3.0	21.7	7.5	1.8	9.4	3.6	8.8	16.5
$LSD_{0.05}$	10.9	3.5	1.2	2.2	4.1	2.2	4.1	4.4	1.4	2.5

promote growth of root hairs, rhizobial growth and multiplication are considered favourable for nodulation (Nutman, 1958). This would suggest that high salinity adversely affects nodulation. Of the three methods used for inoculation, drenching of soil with Rhizobial inoculum appear to be the most suitable method of inducing nodule formation. Better growth performance of the plant was also observed.

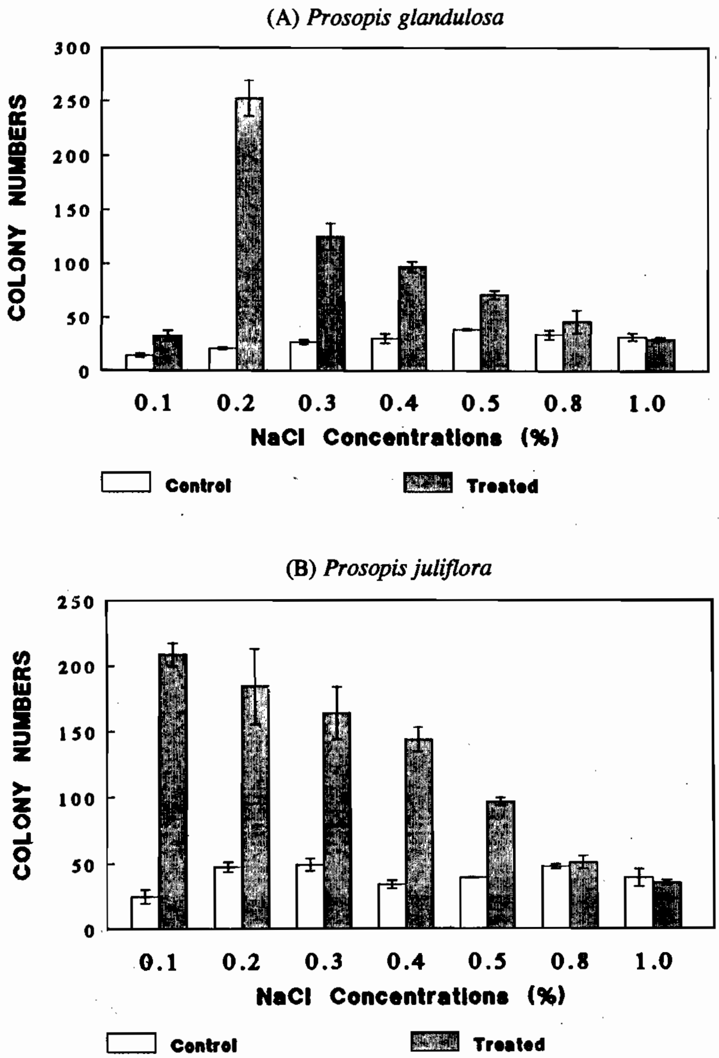

Fig.1. Effects of different salinity regimes on multiplication of *Rhizobium* isolated from *P. glandulosa* (a) and *P. juliflora* (b) on yeast mannitol broth with varying level of NaCl.

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