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# Selection of salt-tolerant *Rhizobium* isolates of *Acacia nilotica*

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**Among 35 *Rhizobium* isolates of *Acacia nilotica*, from different agro-climatic zones, two, ANG4 and ANG5, tolerated up to 850 mM NaCl and one, ANG3, was sensitive to NaCl above 250 mM. Nodulation and nitrogenase activity of the three isolates decreased with increasing concentration of salt up to 150 mM. Nodulation by ANG3 was 15% at 75 mM NaCl and nil at 100 mM. With ANG4 and ANG5, nodulation was only slightly decreased at 150 mM NaCl. Nitrogenase activity associated with plants inoculated with ANG3 was halved at 25 mM NaCl compared with salt-free controls, whereas isolates ANG4 and ANG5 retained 25% and 15% activity, respectively, even at 100 mM NaCl. Salt-tolerant *Rhizobium* isolates can therefore nodulate and fix $N_2$ in saline soils.**

*Key words: Acacia nilotica*, nitrogenase activity, *Rhizobium*, salt-tolerant.

Of the World's 220 × $10^6$ ha of irrigated soils, 40 × $10^6$ ha are affected by salinity and nearly 40% of the World's land surface can be categorized as having potential salinity problems. The fertility of these saline soils can be reclaimed by afforestation with tree legumes. Successful afforestation with leguminous trees can be achieved by a salt-tolerant legume/*Rhizobium* combination although high salinities can affect *Rhizobium* activities. The adverse effects of salts on nodulation, plant growth and $N_2$ fixation have been reported for *Acacia* (Craig *et al.* 1991). Salinity affects the infection process by inhibiting root hair growth and by decreasing the number of nodules per plant and the amount of $N_2$ fixed per unit weight of nodules. Thus, in saline soils the yield of leguminous crops is decreased due to the lack of successful symbiosis (Hafeez *et al.* 1988). The aim of the present study was to select salt-tolerant *Rhizobium* for afforestation with *Acacia nilotica* in saline areas.

## Materials and Methods

*Organism and Source*

Thirty-five *Rhizobium* cultures were isolated from the root nodules of *Acacia nilotica* collected from different agro-climatic regions, including saline and saline calcareous soils, by the methods of Vincent (1970). All isolates were purified, characterized and identified as belonging to the genus *Bradyrhizobium* (Krieg & Holt 1984). The purified isolates were then screened for their nodulation and $N_2$-fixing potential in Leonard jars under sterile and non-sterile soil conditions. Leonard jars were incubated in light (2000 lux) for 16 h at 28°C. The 35 isolates were tested in pure cultures for their tolerance to salt at 30°C and 200 rev/min.

*Growth Conditions*

Growth of selected isolates was studied in yeast extract/mannitol (YEM) broth (Vincent 1970) containing 0 to 900 mM NaCl (pH 7.0) with two replicates in a gyratory shaker at 200 rev/min and 30°C. Cells grown in YEM broth for 18 h were used as an inoculum (2% v/v, $10^7$ c.f.u./ml). Growth was followed turbidimetrically at 550 nm.

Salt-tolerant genotype seeds of *Acacia* collected from Maqdulpur Forest Research Centre (a potential saline area) of northern India were inoculated ($10^7$ cells/seed) and then sown in 22 × 15 cm earthen pots (in six replicates), each containing 3 kg sterilized garden soil (with an electrical conductivity of 0.36 mS/cm in 1:5 ratio). The soil in these pots was saturated by watering with saline water (1000 ml of saline water per pot) containing 0 to 200 mM NaCl. Uninoculated plants were also maintained on water with varying NaCl concentration as controls. Plants were grown at 28°C with 16 h light (2000 Lux). The plants were irrigated with 300 ml sterilized water/pot/day. After 2 months the plants were harvested and nitrogenase activity determined. Nodule number, fresh wt, dry wt and plant dry wt were also recorded along with the electrical conductivity of the soil at the start (after adding salt) and end of the experiment.

*Nitrogen Fixation*

Nitrogenase activity was measured in root nodules immediately

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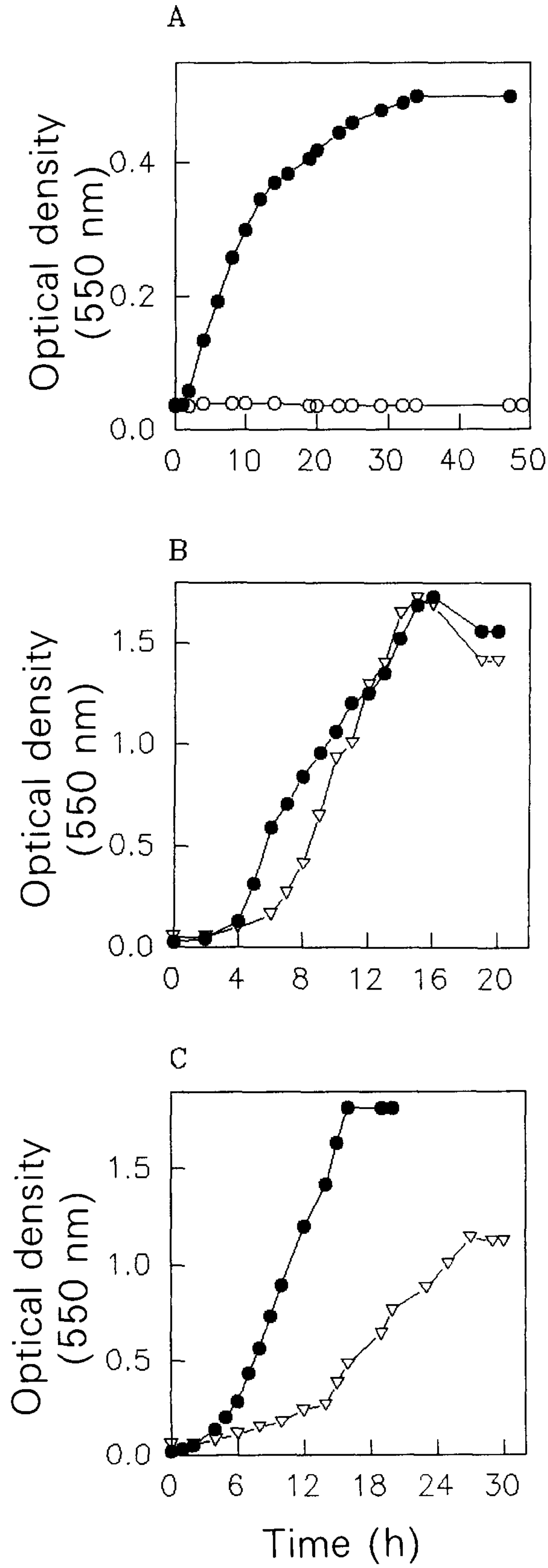


**Figure 1.** Growth of the salt-sensitive-*Rhizobium* isolate ANG3 (A) and the salt-tolerant isolates ANG4 (B) and ANG5 (C) on standard YEM (●) and YEM with 300 (○) or 850 (▽) mM NaCl.

after harvest of plants. Nodules, with root system, were placed in 300-ml flasks and 10% (v/v) acetylene added. Samples (1 ml) of gas were removed after 30 and 60 min using a gas-tight syringe and the ethylene produced was determined by GC.

## Results and Discussion

*Growth Characteristics*

Out of 35 isolates screened, two isolates, ANG4 and ANG5, were highly salt-tolerant (850 mM NaCl) and one isolate, ANG3, was the most sensitive to NaCl (300 mM). The growth curves of the salt-sensitive (ANG3) and salt tolerant isolates (ANG4 and ANG5) are shown in Figure 1. Strain ANG3 could not grow with above 250 mM NaCl. With ANG5, growth decreased as salt concentration increased, but with ANG4 maximum growth still occurred at the highest salt level tested. Hua *et al.* (1982), with *Prosopis*-specific rhizobia, and Kassem *et al.* (1985), with three *R. meliloti* strains, also observed growth at 500 to 600 mM NaCl. Further, the pattern of growth rates observed in this study was similar to that observed by Lauter *et al.* (1981) using rhizobia specific for chickpeas.

*Nitrogen Fixation, Nodule Number and Nodule Dry Weight*

The infectivity (measured by the nodule number) of the different strains of *Rhizobium* sp. differed according to salt concentration (Table 1). The number of nodules was directly related to the plant dry wt which was decreased by 80% in plants inoculated with ANG3 and given 100 mM NaCl. In plants inoculated with ANG4 and ANG5 there was only a 32 to 36% decrease in dry wt at the same concentration of NaCl.

The ability of *Rhizobium* sp. to fix $N_2$ in nodules of *A. nilotica* declined with increasing salt concentrations (Table 2). This was reflected in total plant nitrogen (data not shown). Strain ANG3 had no nitrogenase activity in plants treated with 50 mM NaCl. Although strains ANG4 and ANG5 retained nitrogenase activity up to 100 mM, it was lost at 150 mM. As shown in Table 1, nodulation occurred with ANG3 even at 50 and 75 mM NaCl but no nitrogenase activity was observed. Thus, in the present study with increasing NaCl concentrations, nitrogenase activity was more severely affected than the nodulation process itself. These results differ from those of Singleton & Bohlool (1984) and Hafeez *et al.* (1988), who showed that nodule initiation rather than nodule activity was the most sensitive process in an effective *Rhizobium* symbiosis under saline conditions. In conclusion, while rhizobia can grow in pure culture at salt concentrations up to 850 mM they cannot establish symbiosis with *Acacia* at these high salt concentrations.

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**Table 1. Effect of salt concentrations on nodulation of *A. nilotica* by three rhizobial strains: ANG3 (salt sensitive) and ANG4 and ANG5 (salt tolerant).***

| Salt (mM) | ANG3 | | ANG4 | | ANG5 | |
|---|---|---|---|---|---|---|
| | (nodules/plant) | (mg dry wt/plant) | (nodules/plant) | (mg dry wt/plant) | (nodules/plant) | (mg dry wt/plant) |
| 0 | 82 ± 9.2 | 160 ± 9.36 | 92 ± 5.17 | 156 ± 8.72 | 102 ± 5.62 | 207 ± 6.34 |
| 25 | 39 ± 2.19 | 51 ± 5.0 | 66 ± 3.44 | 107 ± 2.63 | 69 ± 2.48 | 187 ± 5.09 |
| 50 | 15 ± 1.63 | 15 ± 2.0 | 45 ± 3.72 | 88 ± 4.32 | 39 ± 4.53 | 95 ± 4.11 |
| 75 | 7 ± 1.16 | 7 ± 0.63 | 25 ± 5.38 | 52 ± 1.75 | 16 ± 1.75 | 36 ± 4.35 |
| 100 | ND | ND | 14 ± 3.55 | 27 ± 5.13 | 15 ± 4.22 | 20 ± 2.18 |
| 125 | ND | ND | 10 ± 1.67 | 12 ± 2.58 | 12 ± 2.28 | 18 ± 1.41 |
| 150 | ND | ND | 7 ± 0.75 | 7 ± 0.75 | 8 ± 1.0 | 8 ± 0.63 |
| 200 | ND | ND | ND | ND | ND | ND |

* Values are means of six replicates ± standard deviations.
ND—Not detected.

**Table 2. Effect of salt concentrations on nitrogenase activity and plant dry wt of *A. nilotica* after inoculation with three rhizobial isolates.***

| Salt (mM) | ANG3 | | ANG4 | | ANG5 | |
|---|---|---|---|---|---|---|
| | $N_2$-ase activity (μM ethylene/g dry nodule/h) | Plant dry wt (g/plant) | $N_2$-ase activity (μM ethylene/g dry nodule/h) | Plant dry wt (g/plant) | $N_2$-ase activity (μM ethylene/g dry nodule/h) | Plant dry wt (g/plant) |
| 0 | 0.92 ± 0.04 | 1.46 ± 0.12 | 1.75 ± 0.03 | 1.52 ± 0.10 | 2.69 ± 0.10 | 1.66 ± 0.05 |
| 25 | 0.45 ± 0.07 | 1.25 ± 0.06 | 1.40 ± 0.05 | 1.41 ± 0.12 | 2.38 ± 0.15 | 1.55 ± 0.09 |
| 50 | ND | 0.79 ± 0.17 | 0.41 ± 0.03 | 1.20 ± 0.05 | 1.52 ± 0.03 | 1.30 ± 0.03 |
| 75 | ND | 0.46 ± 0.08 | 0.41 ± 0.003 | 1.10 ± 0.00 | 0.32 ± 0.05 | 1.18 ± 0.03 |
| 100 | ND | 0.28 ± 0.05 | 0.33 ± 0.03 | 1.03 ± 0.04 | 0.29 ± 0.04 | 1.05 ± 0.03 |
| 125 | ND | 0.20 ± 0.01 | 0.05 ± 0.01 | 0.70 ± 0 | 0.10 ± 0.02 | 0.84 ± 0.04 |
| 150 | ND | 0.16 ± 0.01 | 0.011 ± 0.008 | 0.58 ± 0.02 | 0.019 ± 0 | 0.65 ± 0.03 |
| 200 | ND | ND | ND | ND | ND | ND |

* Values are means of six replicates ± standard deviations.
ND—Not detected.

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