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The study salt and drought tolerance of *Sinorhizobium* bacteria to the adaptation to alkaline condition

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Abstract: Salinity, alkalinity and drought stress are the major abiotic stresses hindering the productivity of alfalfa (*Medicago Sativa*) in arid and semi-arid regions. Therefore an ecological survey was conducted to characterize 600 *Sinirhizobium* strains of diverse geographical origin, isolated from the root nodules of alfalfa (*Medicago Sativa*). Rhizobial isolates were studied under free living conditions. *Shinorhizobium* sp. Tolerated yeast extract mannitol broth (YEB) containing 0, 1%, 2.5% and 4.5% salt (NaCl, wt/vol) for up 18 h of incubation at 30^0C. Growth of *Shinorhizobium* sp. at water potential 0, -1, -2 and -3.5 MPa (with PEG_{6000}, wt/vol) for up 18 h of incubation at 30^0C was identical. The results showed that salinity and drought tolerance among isolates was significantly different. All isolates were grouped in two clusters: sensitive and tolerant based on their growth rate in YEB media containing different concentrations of NaCl and PEG_{6000}. To obtain strains resistant to alkaline conditions (high pH range between 7-9) the medium was buffered with AMPD buffer, while for the low pH range between 4 and 5 it was buffered with 25 mM HOMOPIPES A positive correlation was found between the salt tolerance and the adaptation to alkaline pH.

Key words: Alfalfa, *Sinorhizobium*, Salinity, Water potential, pH

INTRODUCTION

In agriculture, leguminous biological nitrogen fixation is used to improve infertile soils, especially those affected by salinity (Brockwell *et al.,* 1995; Zhang *et al.,* 1991). Rhizobial strains are very sensitive to soil environmental factors like high salt, water potential, pH, and temperature stresses, which affect their dinitrogen fixation capacity and hence the productivity of legumes (Abdelmoumen *et al.,* 1999; Feiker *et al.,* 1981).

Rhizobia growing in alkaline soils in India during summer season are subjected to high temperature, pH, and salt stress (Surange *et al.,* 1997). An understanding of the growth of *Rhizobium* isolated from the root nodules of legumes is likely only when the physiology of these organisms has been carefully studied under these suboptimal conditions. *Rhizobium* with the genetic potential for increased tolerance to high salt, water potential, pH and temperature stresses could enhance production of food and forage legumes in semiarid and arid regions the world (Brockwell *et al.,* 1995; Zahran, 1999).

Salinity stress is one of the most serious factors limiting the productivity of agriculture. The detrimental effects of salt on plants are a consequence of both a water deficit, resulting in osmotic stress, and effects of excess sodium ions on critical biochemical processes (Apse *et al.,* 1999). Salt may affect symbiosis by its effects on the growth and survival of rhizobia in soil, restriction on root colonization, inhibition of processes of infection and nodule development, or impairment of active nodule functioning. The presence of high sodium chloride (NaCl) concentration has been reported to cause a reduction in the number of *Rhizobium* in legume inoculants. Thus, tolerance to salt stress in an important part of saporophytic competence and competitiveness in *Rhizobium* (Yap *et al.,* 1983). These effects may be mediated through an effect of salt on the host or through a specific effect on the micro-symbiont itself (Abdelgadir and Alexander, 1997). Leguminous plants growing in highly saline environments require both the free-living rhizobia and the host to be tolerant to salt. Generally, the rhizobia are more tolerant to salt stress than their leguminous hosts, although some tree species, like Acadia and Prosopis, and, among agricultural crops, *Medicago* and *Sesbania* are known to be salt tolerant [7]. Consequently, symbiosis is more sensitive to salt stress than free-living rhizobia (Zahran, 1999). An efficient *Rhizobium*–legume symbiosis under salt stress requires also the selection of salt-tolerant rhizobia (Zahran, 1999). The effects of salt or drought stress on nitrogen fixation have been reported in several studies (Kulkarni *et al.,* 2000; Zahran, 1999). Several authors have discussed the inhibitory effect of salt stress on root-

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nodule activity in legumes (Vealgaleti and Marsh, 1989). It is well known that the host plant inoculation by native strains with high efficiency has a positive effect on plant yield and biological nitrogen fixation process (Jebera *et al.,* 2000; Rehman *et al.,* 2002; Sadiki and Rabih, 2001; Shamseldin, 2005; Shamseldin, 2004).

MATERIAL AND METHODS

In the present survey, 600 *Sinorhizobim* sp. strains were screened from individual root nodules of alfalfa (*Medicago Sativa*) growing in neutral soils. *Rhizobium* strains were isolated from the nodules (15 nodules per plant) of alfalfa.

Nodules were disinfected and individually crushed in a small amount of sterile saline (0.85% wt/vol NaCl), and the suspension was streaked on yeast extract mannitol agar solid medium (YEM) as described earlier (Surange *et al.,* 1997). *Rhizobium* strains, unless otherwise stated, were grown and maintained on YEM that contained (per liter): 10 g mannitol, 1 g yeast extract, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$ and 0.1 g NaCl with 1.5% (wt/vol) agar and 0.025% (wt/vol) congo red.

The tolerance of rhizobia to salt was measured by their grown on YEM plates 0, 1, 2.5, and 4.5% NaCl (wt/vol) in triplicate. The tolerance of rhizobia to water stress was measured by their grown on YEM plates 0, -1, -2, and -3.5% MPa (with PEG_{6000}, wt/vol) in triplicate. Growth was visually assessed after the plates were incubated at 30^0C for 3 days. All the 600 *Rhizobium* sp. strains grew on the congo red-incorporated YEM plates (0% salt) as white, translucent, glistening, and elevated colonies whit entire margins (Vincent and Manual, 1970).

The strains growing on 1, 2.5, and 4.5 % NaCl-supplemented YEM plates were scored accordingly, as tolerant to salt. The ability of strains to grow in broth was tested with YEM medium without agar (YEB) in 150 ml Erlenmeyer flasks containing 50 ml YEB, with the initial inoculation of a bout 10^7 CFU/ml. The pH of medium was adjusted to 7, and the control cultures were grown at 30^0C. The flasks were incubated on refrigerated incubator shaker at 180 rpm. The growth *Rhizobium* sp. strains were recorded as optical density (OD) with three replicates. To obtain strains resistant to alkaline conditions (pH>7–9) the medium was buffered with AMPD buffer, while for the low pH range between 4 and 5 it was buffered with 25 mM HOMOPIPES. To adjust the medium pH from 5.5 to 7, 20 mM MES was added as described by Priefer et al. (Priefer *et al.,* 2001).

These experiments were set up in completely randomized design. Each treatment was replicated four times. Analysis of variance was performed on the data, and significant differences among treatment means were calculated by Duncan's multiple range test (α=0.05).

RESULTS AND DISCUSSION

Symbiotic nitrogen fixation is commonly limited by soil infertility conditions, including salinity. Optimization of the benefits of legume inoculation with *Rhizobium* depends on the survival of rhizobia in soil. The introduction and persistence ability of a strain are affected by a number of biotic factors like high salt, high water potential, high pH, and high temperature (Johri *et al.,* 1999; Surange *et al.,* 1997). Therefore, tolerance to high salt and water stresses may be important in the survival, multiplication, and spread of *Sinohizobium* isolated from the root nodules of legumes growing under such stressed conditions is likely when the physiology of these organisms has been carefully studied under these suboptimal conditions (Surange *et al.,* 1997).

Such rhizobial strains could be used on stressed sites as an inoculum to promote leguminous plant growth in the tropics and sub tropics. Soil degradation due to Stalinization or drought is one of the most serious problems affecting the fertility of soils, especially in arid and semi-arid areas. Ten percent of the total degraded soils in the world are high-saline or high-pH soils (Surange *et al.,* 1997). Consequently, we aimed to test new isolates of *Sinorhizobium* as regards their tolerance to high salt and drought to select strains resistant to these environmental conditions. The results shown in Table 1 demonstrate that among eight strains we found a high degree of diversity. Two strains were highly tolerant to a salt concentration up to 4% NaCl (SK 36 and 27) .This strain could grow at 4% NaCl only when the incubation time was prolonged to 3 days (data not shown). Two strains were moderately tolerant up to 2% NaCl (SK 50 and 53). Strains SK 13, 21, 56 and SK 64 were the most sensitive to salt concentration. These data are consistent with the results obtained by Graham and Parker (Graham and Parker, 1964), and Shamseldin and Werner (Shamseldin and Werner, 2004) Amarger *et al.* (1997) noted that tolerance to salinity, acidity, and alkalinity was more strain-specific than species-specific. Similar results have been reported by Nogales *et al.* (2002).

Strains tested in this study also seemed to be well adapted to high or low water potential. At low Water potential, strains SK 27 and SK 36 were better adapted than strains SK 13, 21 and 56. All strains tolerating salt concentrations from 2.5% to 4.5% NaCl were highly resistant to water potential conditions (Table 1). A significant positive correlation was found between the salt tolerance and the adaptation of rhizobial strains in drought conditions. Strains tested in this study also seemed to be well adapted to high or low pH. At low pH, strains SK 21 and 36 were better adapted than strains SK 13, 27, 50, 53, 50 and 56. At high pH, strains SK 13, 21, 50 and 56 were less tolerant than strains SK 27, 36, 53 and 64. All strains tolerating salt concentrations from 2.5% to 4.5% NaCl were highly resistant to alkaline conditions (Table 1). A significant positive correlation was found between the salt tolerance and the adaptation of rhizobial strains in alkaline conditions. These findings confirm previous reports with *Rhizobium* strains from other areas (Kulkarni *et al.,* 2000).

Strain SK 36 (salt-tolerant) and strain SK 64 (salt-sensitive) were selected to study the interaction between salinity and alkalinity. The interaction between high salt and high pH on the viability of *Sinorhizobium* strain SK 64 was evaluated after 3 days. Strain SK 64 was able to survive at different levels of pH from 7 to 9 (Fig.1a), compared with pH 7 without salt. At 2.5% NaCl there was no growth at all levels (Fig. 2a) in SK 64. The salt-resistant strain SK 36 survived well (Fig. 2b, 3b) with 2.5% and 4.5% NaCl at different pH levels. The results are in agreement with those obtained by Kulkarni *et al.* (2000). Salt-tolerant strain (SK 36) was unable to grow at 4.5% NaCl at different pH levels, although they could survive at 4.5% NaCl without the stress of alkalinity (Table 1).

Table 1: Phenotypic characteristics of *Sinorhizobium* strains nodulating *Medicago Sativas* grown under environmental stresses

Strain	NaCl % inhibiting the growth	Water potential with PEG_{6000} (MPa)	Low pH values tolerated	High pH values tolerated
SK 13	1.0	-1.0	5.5	8.5
SK 21	1.0	-1.0	5.0	8.5
SK 27	4.5	-3.5	5.5	9.0
SK 36	4.5	-3.5	5.0	9.0
SK 50	2.5	-2.0	5.5	8.5
SK 53	2.5	-2.0	5.5	9.0
SK 56	1.0	-1.0	5.5	8.5
SK 64	1.0	-1.0	5.5	9.0

SK, Sinorhizobium Kerman

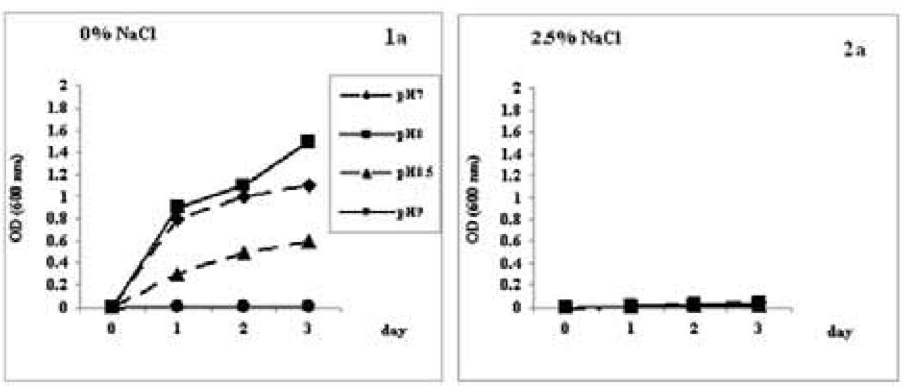

Fig 1: Growth curves of *Sinohizobium* SK 64 at different pHs and salinity levels. Add±standard error bar whith each value

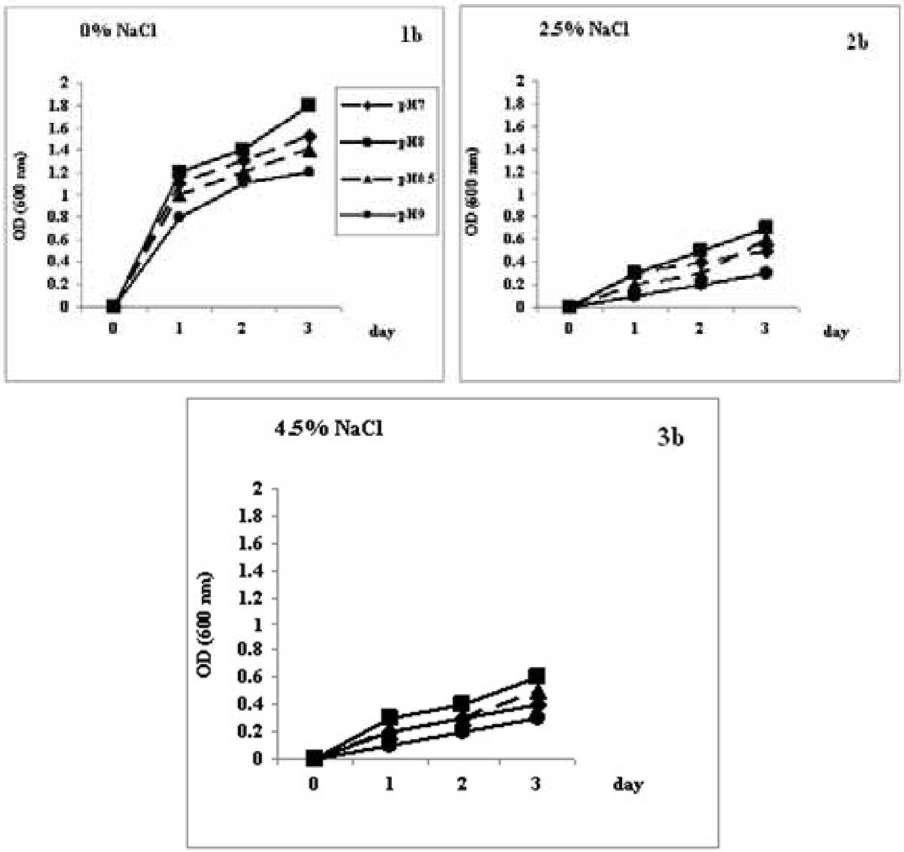

Fig 2: Growth curves of *Sinohizobium* SK 36 at different pHs and salinity levels. Add±standard error bar whith each value

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REFRENCES

Abdelgadir, A.H., M. Alexander, 1997. Procedure to enhance heat resistance of *Rhizobium*, Plant Soil, 188: 93-100.

Abdelmoumen, H., A. Filali-Maltout, M. Neyra, A. Belabed, M.M. El Idrissi, 1999. Effect of high salts concentrations on the growth of rhizobia and responses to added osmotic, J Appl Microbiol., 86: 889-898.

Amarger, N., V. Macheret, G. Laguerre, 1997. Rhizobium gallicum sp. nov. And Rhizobium giardinii sp. nov., from *Phaseolus vulgaris* nodules, Int J Syst Bacteriol., 47: 996-1006.

Apse, M.P., G.S. Asharon, W.A. Snedden, E. Blumwald, 1999. Salt tolerance conferred by overxpression of vacuolar Na^+/H^+ airport in *Arabidopsis*, Science, 285: 1256-1258.

Brockwell, J., P.J. Bottomly, J.A. Thies, 1995. Manipulation of rhizobia micro flora for improving legume productivity and soil fertility. A critical assessment, Plant Soil, 174: 143-180.

Felker, P., P.R. Clark, A.E. Laag, P.F. Pratt, 1981. Salinity tolerance of the tree legumes. Mesquite (prosopis glandulosa var. torreyana, P. velutina and *P. and P. articulate*), algarrobo (*P. chilensis*), kiawa (*P. pallida*) and tamarugo (*P. amarugo*) grown in sand culture on nitrogen-free media, Plant Soil, 61: 1228-1236.

Fouge, F. Are, D. Le Rudulier, J.G. Streeter, 1991. Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids and cytosol of alfalfa (*Medicago sativa* L), Plant Physiol., 96 : 1228-1236.

Graham, P.H., C.A. Parker, 1964. Diagnostic features in the characterization of the root nodule bacteria of legumes, Plant Soil, 20: 383-396.

Jebera, M., M. Elarbi, A. Ridha Mhamfi, R. Ghrir, M. Mars, 2000. Effect of salt on *Sinorhizobium* sp. isolates from Tunisia in vitro or in association with *Medicago* sp., Agricul., 9(2): 99-102.

Johri, J.K., S. Surange, C.S. Nautiyal, 1999. Occurrence of salt, pH and temperature-tolerant, phosphate-solubilizing bacteria in alkaline soils, Curr Microbiol., 39: 89-93.

Kulkarni, S., S. Surange, S.C. Nautiyal, 2000. Crossing the limits of *Rhizobium* existence in extreme conditions, Curr Microbiol., 41: 402-409.

Nogales, J., R. Campos, H. Ben Abdelkhalek, J. Olivares, C. Lluch, J. Sanjuan, 2002. Rhizobium tropici genes involved in free-living salt tolerance are required for the establishment of efficient nitrogen-fixing symbiosis with *Phaseolus vulgaris,* Mol Plant–Microbe Interact., 15: 225-232.

Priefer, U.B., J. Aurag, B. Boesten, I. Bouhmouch, R. Defez, A. Filali-Maltouf, M. Miklis, H. Moawad, B. Mouhsine, J. Prell, A. Schluter, B. Senatore, Characterization 2001. of Phaseolus symbionts isolated from Mediterranean soils and analysis of genetic factors related to pH tolerance, J Biotechnol, 91: 223-236.

Rehman, A., C.S. Nautiyal, 2002. Effect of drought on the growth and survival of the stress-tolerant bacterium *Rhizobium sp*. NBRI2505 sesbania and its drought-sensitive transposon Tn5 mutant, Springer-Verlag New York LLC., 45(5): 368-377.

Sadiki, M., K. Rabih, 2001. Selection of chickpea (*Cicer arietinum* L.) for yield and symbiotic nitrogen fixation ability under salt stress, J Agron., 21: 659-666.

Shamseldin, A., 2005. Improvement of common bean (*Phaseolus vulgaris*) nodulation by selected rhizobial strains from Egyptian soils through genotypic characterization, symbiotic effectiveness and competitiveness under salt stress conditions, Thesis for degree of ph.D.

Shamseldin, A., D. Werner, 2004. Selection of competitive strains of *Rhizobium* nodulating *Phaseolus vulgaris* and adapted to environmental conditions in Egypt, using the gus-reporter gene technique, World J Microbiol Biotechnol, 20: 377-382.

Surange, S., A.G. Wollum, N. Kumar, C.S. Natutiyal, 1997. Characterisation of *Rhizobium* from root noudules of leguminous trees growing in alkaline soils, Can J Microbiol., 43: 891-894.

Velagaleti, R.R., S. Marsh, 1989. Influence of host cultivars and *Bradyrhizobium* strains on the growth and symbiotic performance of soybean under salt stress, Plant Soil. 119: 133-38.

Vincent, J.M., A manual, 1970. for the practical study of the root-nodule bacteria, IBP Handbook no. 15 Oxford: Blackwell Scientific Publications.

Yap, S.F., S.T. Lim, 1983. Response of *Rhizobium* sp. UMKL 20 to sodium chloride stress, Arch Microbiol., 135: 224-228.

Zahran H.H., HH, Rhizobium–legume symbiosis and nitrogen fixation under severe conditions and in an arid climate, Microbiol Molecul Biol Rev., 63: 968-989.

Zhang, X.P., M. Karsisto, R. Harper, K. Lindstrom, 1991. Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees, Int J Syst Bacteriol., 41: 104-113.