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Osmoadaptation and performance of Indigenous *Sinorhizobium meliloti* isolates from Oman in comparison with exogenous strains tested for efficiency on alfalfa (*Medicago sativa*).

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Laboratory studies on both exotic & indigenous strains of *Sinorhizobium meliloti* revealed that the native rhizobia from Oman exhibited normal growth similar to the exotic rhizobia tested. All the strains growth is inhibited at higher concentrations of NaCl. The sequence of tolerance at 0.5M NaCl concentration was Canada > Buraimi (Dahira) > Barka (Batinah) > Australia > Sharqia. The Sharqia strain did not exhibit any growth at this level of concentration in solid agar medium. This work was expanded to determine the capability of the native rhizobium strains to establish a symbiotic relationship under salt stress conditions. The strains interacted differently with the three tested local alfalfa cultivars; Sharqia, Batini & Interior. The addition of the introduced Australian rhizobium showed a trend of increase in nodules number and nitrogen percentage. This was evident with the high nitrogen percentage obtained. These results although showed no direct relation of the strains tolerance in the laboratory when applied to host plants under growth chamber conditions, are of immense importance for the productivity of alfalfa in Oman by using salt tolerant` strains of *Sinorhizobium meliloti*.

Key words: Indigenous Exotic *Sinorhizobium* Osmoadaptation SAR EC.

If rhizobial strains capable of tolerating high concentrations of salt (osmo adaptation) are found, they will be of high significance to agriculture in Oman. Water salinity is continuously increasing due to intrusion of seawater into ground water aquifers as a result of over pumping for use in irrigation of agricultural crops. This in turn is causing the soils salinity to rise in proportion to the water salinity due to secondary salinization. Alfalfa resembles the major fodder crop in the country. The estimated area was 25,000 fed. (MAF, 1995). However, many obstacles hinder its cultivation in the predominating sandy soils of Oman, of which the nitrogen nutrition merits more consideration. Therefore either fertilization with chemical fertilizers should be practiced, and/or the technology of biofertilization should be adopted through the search of osmo adapted, highly nitrogen fixing *Sinorhizobium meliloti* strains. Some strains were found to tolerate up to 500 mM NaCl (Anonymous, 1996; Rafig, 1999).

This work is therefore initiated to test the indigenous *Sinorhizobium meliloti* strains isolated from the most potential areas of crop production in Oman representing different climatic & salt stress conditions. Both osmoadaptation and efficiency in fixing atmospheric nitrogen upon interaction with different native alfalfa cultivars will be evaluated. The practical application of such research will be of enormous significance to the farmer in Oman who is currently using imported alfalfa inocula, the identity and the efficiency of which are questionable under

such stress conditions prevailing in the country.

MATERIALS AND METHODS

Laboratory Investigations

The *Sinorhizobium* strains used in this study were isolated in the soil Microbiology laboratory at ARC at Rumais from fresh root nodules collected from alfalfa plants growing in the respective regions using the classical isolation methods described by Vincent (1972). The native isolates were from Batinah Coast (Batinah or Barka strain), Sharquia region (Sharquia strain), and from Dahira region (Buraimi strain) obtained from the Faculty of Science, SQU. Two alien strains were used in the study; one from Canada obtained from the Research Station at Beverlodge, Agriculture Canada (Canada strain), and the other strain was isolated from alfalfa inoculum imported from Australia for use by Omani farmers. YEM broth was used as the medium for growing the selected strains. Different concentrations of NaCl were added to give concentrations of 0.1M, 0.25M, & 0.5M. Erlenmeyer flasks containing 150 mls of the sterile proper medium containing the calculated amount of NaCl were inoculated with the designated strain under aseptic conditions using a Laminar flow Hood. Three replications were used for each strain. The whole set was put in an orbital shaker adjusted at 160 rpm under laboratory conditions. An uninoculated control was included. Bacterial growth was monitored using a spectrophotometer Model 6102 adjusted at 525 nm wavelength for turbidity measurement by following changes in transmittance. Monitoring the growth continued for 14 days.

A related experiment was conducted to test the strains tolerance when the soil extract growth medium was adjusted to differing conditions of SAR (sodium adsorption ratio) and different EC (electrical conductivity) using different CaCl2 and NaCl concentrations to give a range of combinations of SAR (0-30) and EC (1 and 5).

A supporting experiment was conducted simultaneously by streaking the five *Sinorhizobium* strains in solid agar medium containing the same concentration of NaCl; 0.1M, 0.25M, and 0.5M. The whole set was kept in an incubator at 30◦ C. Daily observations were taken through inspection of the plates starting the 4th day of inoculation. The growth of the different strains was rated as 4: maximum growth, 3 & 2: medium growth, 1 & 0: minimum growth. The growth curves were constructed using the computer facilities at the ARC at Rumais.

The tolerance of the Rhizobium strains to high temperatures was tested using an incubator with the temperature adjusted at 30 and 40°C. Growth was monitored using a spectrophotometer Model 6102 at 525 nm wavelength. The growth curves were constructed using MS Excel.

Growth chamber experiment

Two Omani local alfalfa cultivars; Batinah and Dakhylia obtained from the local farmers in the respective regions in Oman were used in this trial. The irrigation water used was 0.8 DS/m). The soils were chosen with low EC=0.1. Pots with 4 kg- capacity were used in this study. The pots were irrigated with 1 liter of sweet water before sowing (2 days before planting).

Twenty alfalfa seeds from each of the two cultivars were treated with ethanol (95%) for surface sterilization and five healthy seedlings were sown 2-cm below the soil surface. Immediate irrigation with one liter of sweet water followed. The growth continued for 2-month from sowing. Upon harvest, the pots were irrigated and left for 2 hrs. The roots were freed from the attached soil. The nodules were counted and the tissues were dried at 65 for 48 hours for dry weight determinations. Statistical analysis was conducted using MSTATC.

RESULTS AND DISCUSSION

Laboratory Investigations

The data are presented in table 1 and figures 1, 2, 3, 4, 5. The figures summarize the mean transmittance percentage during the whole growth period plotted for the strains tested at different salt concentrations i.e. 0.1M, 0.25M, and 0.5M.

All the treated strains consistently showed maximum growth at lower levels of salt concentrations (figure 1). However, at 0.5M salt concentration, the sequence of salt tolerance was in the order Canada > Buraimi > Barka > Australia > Sharqia.

This result was further confirmed by the strain growth in solid agar media containing the same salt concentration. The rest of the strains, however, grew in the solid medium at

Table 1: Days taken by *Rhizobium* strains to attain maximum growth at different NaCl concentrations.

Rhizobium Strain	*Days taken at the specified level of NaCl concentration*		
	0.1M	**0.25M**	**0.5M**
1. Canada	11	2	7
2. Australia	10	2	4
3. Buraimi	7	3	5
4. Barka	5	2	4
5. Sharquia	13	2	7

Table 2: Growth of the Rhizobium strains in solid agar medium with different NaCl concentrations.

NaCl concentration	*Growth of the Rhizobium* strains(days)*				
	Canada	**Buraimi**	**Barka**	**Austr.**	**Shar.**
Control	4	4	4	4	4
0.1M	4	4	4	4	4
0.25M	4	4	4	4	4
0.5M	2	4	3	3	1

* Growth rate values (visual rating) 4: maximum growth, 3 & 2: medium growth 1: minimum growth

Table 3: the response of local alfalfa cultivars to inoculation with indigenous strains of Sinor*hizobium meliloti* tested for salinity tolerance

Alfalfa Cultivar Growth Traits	**Sharquia Nodule No.**	**Tissue Dry. Wt (g)**	**Batinah Nodule No.**	**Tissue Dry.Wt (g).**	**Dakhylia Nodule No.**	**Tissue Dry.Wt (g).**
Sinorhizobium Strains						
1)Australia	90a*	0.552bcd	69ab	0.478bcd	24bc	0.207d
2)Batinah	18bc	0.331cd	34bc	0.268cd	68ab	0.677bcd
3)Canada	18bc	0.210d	50abc	0.574bcd	53abc	0.911ab
4)Sharquia	52abc	0.691bcd	72ab	0.741bcd	46abc	0.674bcd
5)Nitrogen Control	0.0c	0.626bcd	0.0c	0.663bcd	0.0c	1.419a
6)Uninoc. Control	0.0c	0.794bc	0.0c	0.426bcd	0.0c	0.211d

*Numbers followed by the same letters within and between every two adjacent columns are not significantly different at the 0.05 level of probability by the Duncan Multiple Range Test

the 0.5M level of concentration (table 2). The growth curves of the tested strains reflect normal bacterial growth curves (Figs. 1, 2, 3, 4, & 5).The *Sinorhizobium* strains response to the salt concentration was variable (table1). All the tested strains attained maximum growth at the 0.5M level of concentration between 4-7 days, with Barka and Sharquia strains showing the slowest growth (7days). At the 0.1M level of concentration. However, the strains growth was slower for all the tested strains ranging from 5-13 days. The Sharquia strain was the slowest as it attained maximum growth in 13 days. Apparently, the level and the growth stage of the added inoculum masked the adverse effect of the salt concentration in the medium. While refrigerated slants were used in inoculating the 0.1M flasks, inocula from recently streaked plates were used for inoculating the 0.5M concentration flasks. On the other hand, the 0.25M concentration flasks were inoculated from cultures adjusted to give the same transmission reading i.e. in the logarithmic phase of growth. This explains the discrepancies observed of which the high level of growth is observed after 2-3days for all the tested strains at the 0.25M concentration. Alternatively, other media ingredients could be responsible for such variations in the growth. Botsford, (1981) found that different media ingredients influenced the level of tolerance to NaCl of the *Sinorhizobium meliloti* strains tested Further investigations are therefore needed to look for the effect of the media ingredients and their interaction with the level of NaCl added. The current study clearly reveals that indigenous alfalfa rhizobia in Oman resemble other *Sinorhizobium* strains in growth behavior. Their tolerance to salt concentration, however, is erratic.

Figure 1: Growth curves of Braka strain at different NaCl concentrations

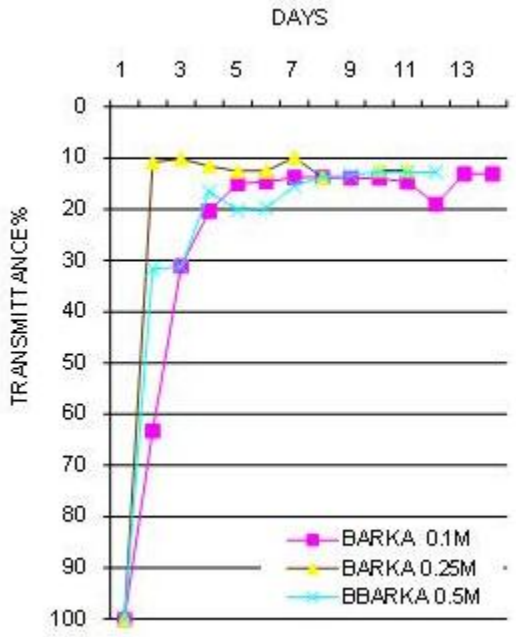
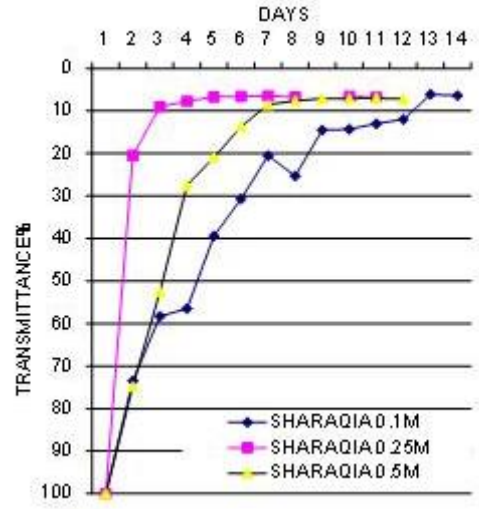


Figure 2: Growth curves of Sharq strain at different NaCl concentrations.



Nevertheless, the sequence of tolerance is Buraimi>Barka>Sharquia, which clearly indicates that the level of tolerance is not related to the region of isolation, or to the level of salinity prevailing in these regions, be it water salinity or soil salinity. Further testing of a large number of isolates from these regions may confirm these results. Such basic information is essential for future selection of suitable *Sinorhizobium* inocula for alfalfa in Oman. The need for further investigations should be stressed to avail the capability of the native *Sinorhizobium* strains in fixing atmospheric dinitrogen under the prevailing salt stress conditions in Oman, especially in Al-Batinah region.

Figure 3: Growth curves of Canada strain at different NaCl concentrations.

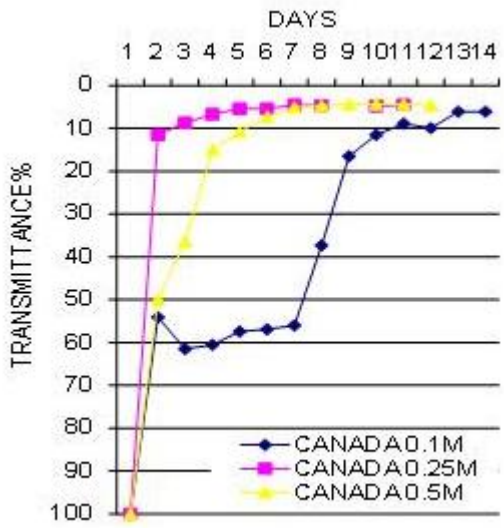
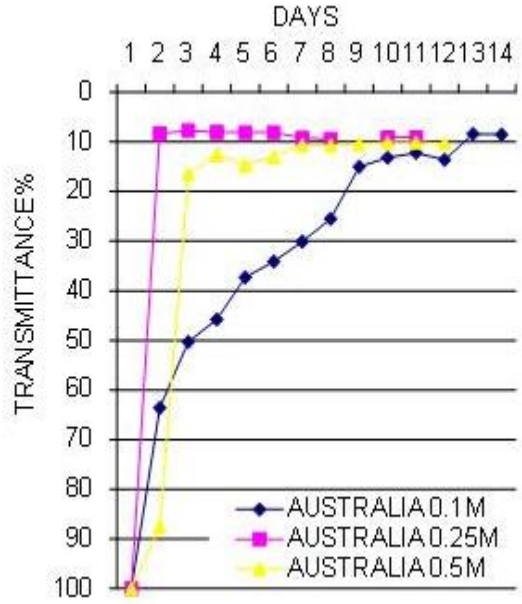


Figure 4: Growth curves of Australia strain at different NaCl concentrations.

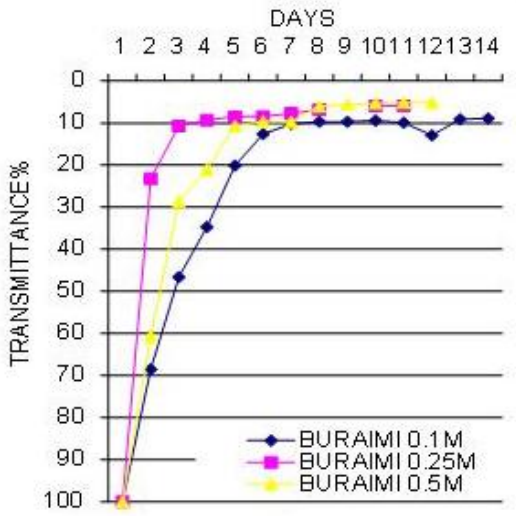


Growth chamber experiment

High nodulation was obtained with the Batinai cultivar over the Dakhlia cultivar irrespective of the strain, inoculum carrier, or the level of salinity used (table 2).This might imply a significant genetic character associated with the Batini cultivar being well adapted to the environmental conditions prevailing at the experimental site. Hadad & Al-Hashmi, (2001) found similar results where variance in nodulation with water salinity was rather

erratic.

Figure 5: Growth curves of Buraimi strain at different NaCl concentrations.



Inconsistent results were obtained with either cultivar at the two salinity levels tested. This might be due to the fact that both cultivars were selected naturally for salinity tolerance since both are grown in the Batinah region, where creeping water salinity from the sea is threatening the whole area (Hadad, et al. 1998) The performance of the *Sinorhizobium* strains tested consistently showed superiority of the Australian strain together with the mixture of the local strains over the strains from Batinah and Sharquia (added as single strains) on the Batinah cultivar. However, with the cultivar from Dakhylia, the *Sinorhizobium* strain performance could be rated as Batinah > Sharquia >Australia > Mixture.

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