

SALINITY AND ALKALINITY IN RELATION TO LEGUME— *RHIZOBIUM* SYMBIOSIS

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Effect of chlorides of sodium, potassium and magnesium and carbonate and bicarbonate of sodium, on bacterial (*R. meliloti*) and host (lucerne) components of a symbiotic system was studied. While *R. meliloti* could tolerate NaCl levels upto 3 per cent, seeds of lucerne could not germinate beyond 1.5 per cent. Beyond 0.7 per cent nodulation of lucerne seedlings with *R. meliloti* was inhibited under aseptic conditions. Almost similar results were obtained with KCl and MgCl₂.

The threshold of tolerance for carbonate and bicarbonate was much lower than in the case of chlorides. Nodulation was inhibited at 0.1 to 0.2 per cent levels, though rhizobia could tolerate up to 0.5 per cent and lucerne seedlings up to 0.5–0.75 per cent of carbonate/bicarbonate.

Thus, the degrees of salinity/alkalinity conducive for good nodulation were found to be definitely different from the limits of tolerance of *Rhizobium* and the host to the respective salts.

INTRODUCTION

Saline alkaline soils are wide spread in India especially in the Indo-Gangetic basin in the north. These soils are generally not suitable for raising food and fodder legumes. While salinity in these soils is due to the accumulation of soluble salts of calcium, magnesium and sodium, mostly chlorides and sulphates, alkali soils are developed as a result of the predominance of carbonates and bicarbonates. Potassium salts, though present, are only in smaller quantities.

There are three interrelated factors involved in studying the effects of these salts on legume-*Rhizobium* symbiosis.

- (i) The effect of these salts on the growth of *Rhizobium*
- (ii) The limits of salt tolerance of the legume, and
- (iii) The limits of salt tolerance of the host as well as the bacterium for successful nodulation and symbiosis.

The present paper summarizes our observations covering all the three aspects outlined above on nodulation of lucerne (*Medicago sativa*) under the influence of different salts.

Earlier studies in this laboratory (Pillai and Sen 1966) were concerned with the first aspect of the problem. Yadav and Vyas (1971) have also studied in detail the response of root nodule bacteria to saline, alkaline and acid conditions. Ayers (1948), Brown and Hayward (1956), Ayers and Eberhard (1960), and Abel and Mackenzie (1964) have studied the salt tolerance of birdsfoot trefoil, alfalfa, broad bean and soybean. Bernstein and Ogata (1966) have briefly reported on the effects of salinity on nodulation, nitrogen fixation and growth of soybeans and alfalfa.

MATERIALS AND METHODS

Aseptic culturing (Jensen 1942; Subba Rao *et al.*, 1971; Singh *et al.*, 1972) of plants was followed for studies on nodulation. The influence of salts on the growth of rhizobia was measured in Hilger absorptiometer.

RESULTS AND DISCUSSION

Results are summarized in Table I to IV. Variations occurred not only within the genus (Table I) but also within the species (Table II) as regards tolerance of various salt concentrations by the *Rhizobium*. The levels of salts at which seeds of lucerne could germinate also varied. Total inhibition of germination occurred with NaCl and KCl at 2 per cent, $MgCl_2$ at 3 per cent and with bicarbonate and carbonate at 0.75 per cent. More interesting, however, were the observations on the effect of the various salts on legume-*Rhizobium* symbiosis as evidenced in nodulation and development of other plant characters (Tables III and IV). Certain facts stand out from these investigations.

- (i) The lower symbiont (*Rhizobium*) can withstand higher levels of all the salts than the higher symbiont (lucerne) in experiments conducted under controlled conditions.

TABLE I

Salt concentration (%) at which rhizobial growth was reduced by more than 50%

Rhizobial strains	$MgCl_2$	KCl	Na_2CO_3	$NaHCO_3$
<i>R. meliloti</i> 2001	3.0	3.0	0.4	0.1
<i>R. trifolii</i> RO ₁	1.5	1.0	0.1	0.3
<i>R. lupini</i> 3001	1.5	1.0	0.1	0.4
<i>R. leguminosarum</i> 1007	1.5	1.0	0.1	0.2
<i>R. japonicum</i> SB 16	1.5	1.0	0.5	0.3
			(above)	

TABLE II

Sodium chloride concentration (%) at which growth of R. meliloti was reduced by more than 50%

Strains	NaCl (%)
SU 10	1.00
SU 47	2.25
SU 216	2.50
SU 2001	2.50
SU 101	2.75
D 11	3.00

TABLE III

Effect of chlorides of sodium, magnesium and potassium on nodulation and other plant characters in lucerne (Medicago sativa L.; Based on 3 different experiments).

Salt concn. %	Age of initial nodulation	At the end of 40 days			
		Nodules	Leaves	Number of Lateral roots	Dry wt. in mg†
NaCl	0.0	1.7	1.9	5.7	2.36
	0.3	1.4	2.0**	7.6	3.54
	0.4	0.6**	0.7**	5.5	2.91
	0.5	0.6**	0.2**	2.4**	2.73
	0.6	0.3**	0.1**	4.3	2.73
	0.7	—	0.1**	2.5**	1.83
MgCl ₂	0.0	2.8	3.9	6.7	4.75
	0.1	2.5	3.3	6.5	4.75
	0.2	1.6*	3.7	4.9	4.00
	0.3	1.3*	2.9*	4.9	3.61
	0.4	1.8*	5.1*	5.3	3.05
	0.5	0.8*	2.1*	3.7*	3.05
	0.75	0.5*	1.6*	3.4*	2.89
	1.00	0.2*	1.3*	3.3*	2.89
KCl	0.0	4.0	3.5	5.3	9.58
	0.1	4.0	5.2	6.4	8.09
	0.2	3.4	3.9	6.6	5.42
	0.3	1.8*	3.0*	6.1	4.58
	0.4	1.3*	3.8	6.4	4.58
	0.5	0.1*	3.9	2.5*	3.19
	0.75	—	1.5*	2.7*	2.27

*Significant at 1% level **Significant at 5% level

†Samples were pooled, hence no statistical analysis.

(ii) Alkaline salts had a much more drastic effect on germination and plant growth than those which induce salinity. Those effects, persisted upto 40 days, when experiments were terminated.

(iii) Growth inhibitory effects were manifest firstly in the delay in initial nodulation and later in the reduction in the number of nodules, laterals and leaves and reduction in dry weight, the latter being an index of symbiotic efficiency.

The above study clearly demonstrates that successful symbiosis between the *Rhizobium* and the legume can take place only at salt levels that are much below those

TABLE IV

Effect of carbonate and bicarbonate on nodulation of lucerne (Medicago sativa, mean of 12 plants)

Salt concn. %	Age of initial nodulation	Number of		Dry wt. mg**
		Nodules	Lateral roots	
Carbonate				
0.0	14.16	3.92	9.75	3.63
0.1	12.83*	1.16*	5.16*	3.63
0.2	20.00*	0.08*	3.91*	2.73
0.3	—	—	—	—
Bicarbonate				
0.1	15.83*	0.26*	5.83*	2.73
0.2	0.0	0.0	0.0	0.0

*Significant at 1% level

**Replicate samples were pooled together hence no statistical analysis.

tolerated by *Rhizobium*. Thus emphasis should be more on creating conditions conducive to optimum symbiosis rather than on evolving salt tolerant strains/varieties of either the bacterium or the leguminous host.

REFERENCES

- Abel, G. H., and Mackenzie, A. J. (1964). Salt tolerance of soybean varieties (*Glycine max* L. Merrill) during germination and later growth. *Crop. Sci.*, **4**, 157-161.
- Ayers, A. D. (1948). Salt tolerance of birdsfoot trefoil. *Agron. J.*, **40**, 331-334.
- Ayers, A. D., and Eberhard, D. L. (1960). Response of edible broadbean to several levels of salinity. *Agron. J.*, **52**, 110-111.
- Bernstein, L., and Ogata, G. (1966). Effects of salinity on nodulation, nitrogen fixation and growth of soybeans and alfalfa. *Agron. J.*, **58**, 201-203.
- Brown, J. W., and Hayward, H. E. (1956). Salt tolerance of alfalfa varieties. *Agron. J.*, **48**, 18-20.
- Jensen, H. L. (1942). Nitrogen fixation in leguminous plants II. Is symbiotic nitrogen fixation influenced by *Azotobacter*? *Proc. Linn. Soc. N.S.W.*, **57**, 205-212.
- Pillai, R. N., and Sen, A. (1966). Salt tolerance of *Rhizobium trifolii*. *Indian J. agric. Sci.*, **36**, 80-84.
- Singh, C. S., Lakshmi Kumari, M., Amita Biswas, and Subba Rao, N. S. (1972). Nodulation of lucerne (*Medicago sativa* L.) under the influence of chlorides of magnesium and potassium. *Proc. Indian Acad. Sci.*, B **76**, 90-96.
- Subba Rao, N. S., Lakshmi Kumari, M., Singh, C. S., and Magu, S. P. (1972). Nodulation of lucerne (*Medicago sativa* L.) under the influence of sodium chloride. *Indian J. agric. Res.*, **42**, 386-388.
- Yadav, N. K., and Vyas, S. R. (1971). Response of root nodule rhizobia to saline, alkaline and acid conditions. *Indian J. agric. Sci.*, **41**, 875-881.