J. Phytol. Res. 18(2) : 223-226, 2005

ROLE OF ARBUSCULAR MYCORRHIZA ON TOLERANCE TO SALINITY OF THE TREE LEGUME *DALBERGIA SISSOO* ROXB. INOCULATED BY *RHIZOBIUM*

V.M. RAO, ANJU KESHAWAT, GAURAV BHUSHAN, SANTOSH KUMAR SHARMA, and H.C. JAIN
Department of Botany, University of Rajasthan, Jaipur-302004, Rajasthan, India.

The effect of different levels of salinity (concentrations of NaCl) on growth, nodulation and N_2-fixation of single (*Rhizobium* or AM alone) and dual inoculated (*Rhizobium* + AM) tree legumes, has been studied in a pot culture experiment using sterilized soil. The parameters growth, nodulation, nitrogen fixation and % AM colonization of roots were considerably influenced with the increase in salt concentration from 0.5% to 4% NaCl. It was observed that the mycorrhizal tree legume (inoculated with the most preferred AM *Glomus fasciculatum*) performed better in the increasing levels of salinity in comparison to non-mycorrhizal ones. These observations suggested a protective role played by AM in providing resistance to the tree legume against injurious effects of salinity. Inoculation of efficient strain of AM fungi (*Glomus fasciculatum*) during the course of the study, prevented the injurious effects of salinity in the test plants due to enhanced water and nutrient uptake thereby promoting growth, nodulation and nitrogen fixation of the tree legume under investigation.

Keywords : Arbuscular mycorrhiza; *Dalbergia sissoo; Glomus fasciculatum; Rhizobium*; Salinity.

Introduction

Mycorrhiza are mutualistic symbiosis between certain groups of soil fungi and plant root system. Among this Arbuscular Mycorrhizae (AM) form obligate symbiotic association with plant roots and increase phosphorus (P) availability by mobilizing the P with the help of their extramatrical hyphae, particularly in soil with less of available P. Available reports indicate that plants infected with mycorrhizal fungi grow better under stress conditions[1]. The requirement of P is high in legumes[2] and therefore leguminous plants respond more to mycorrhizal infection than cereals, which indirectly enhances the biological nitrogen fixation through increased P availability specially in soils with low P content[1,3]. Rosendahl and Rosendahl[4] eported that the vesicular arbuscular mycorrhizal fungi have the ability to protect plants from salt stress. Mycorrhizal association helps the plants to withstand stress at maximum NaCl regime[5]. No reports are available on the levels of mycorrhization on growth and nutrient uptake of tree legume grown in Rajasthan under salinity stress condition. In the present study, the role of AM fungi (the most preferred AM *Glomus fasciculatum*) and the percentage of AM colonization on growth and nutrient uptake of tree legume *Dalbergia sissoo* under salinity are discussed.

Materials and Methods

Role of most preferred AM on tolerance to different salinity (NaCl) levels of test plants inoculated with most efficient *Rhizobium* was studied. A pot culture experiment was conducted for 6 months with sterilized soil using plastic pots to prevent leaching of NaCl from the bottom. The surface sterilized seeds pelleted with rhizobial isolates were sown in pots on AM inoculum pad containing 250 spores /50 gm soil. After 15 days of seedling establishment the pots were saturated with different concentrations of NaCl viz., 0.5%, 1%, 2%, 3% and 4%. Pots with no addition of salt (NaCl) served as control. Three replicates of pots, each containing 5 seedlings were maintained for each treatment and were periodically irrigated with the sterilized tap water. The various treatments included were T_0-0%, T_1-0.5%, T_2-1.0%, T_3-2.0%, T_4-3.0% and T_5-4.0% NaCl. Each of these treatments consisted of uninoculated control, *Rhizobium* alone, AM alone and *Rhizobium* + AM inoculated sets.

The parameters selected for the study were shoot-root length, shoot and root dry weight, total plant protein[6], total chlorophyll[7], total nitrogen[8] and phosphorus content[9], nodule number, nodule dry weight and maximum nodule size[10], nitrogenase activity[11] of root nodules and AM colonization by roots (%).

Results and Discussion

The effect of Arbuscular mycorrhizal inoculation on plant growth (shoot and root length), plant dry matter production (shoot and root dry weight) and nutrient uptake level under different levels of salinity stress condition are given in Table 1.

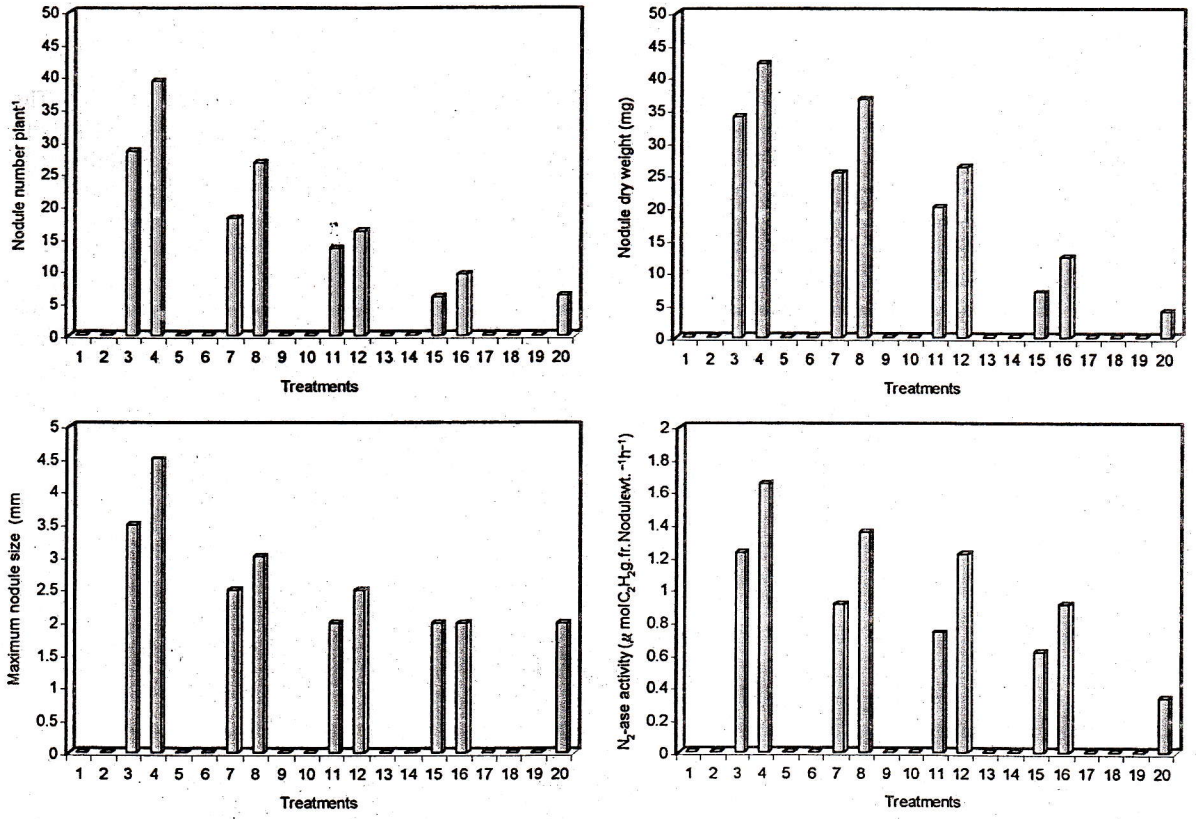
The highest value of growth in terms of shoot length (44.3 cm), shoot dry weight (0.97g), root length (62.4 cm) and root dry weight (0.96g) was observed in *Rhizobium*

isolate DS Rhz-9 + *Glomus fasciculatum* inoculated plants at the first level of salinity (T_0-0% NaCl) which also served as control. The same parameters in other dual inoculated plants decreased at the subsequent levels (T_1-0.5%, T_2-1.0%, T_3-2.0%, T_4-3.0% and T_5-4.0% NaCl) of salinity. Among *Rhizobium* (DS Rhz-9) or AM (*Glomus fasciculatum*) alone inoculated seedlings, highest values of shoot length (32.5 cm), shoot dry weight (0.78g), root length (54.3 cm) and root dry weight (0.84g) was recorded in DS Rhz-9 inoculated plants at T_0 (0% NaCl). All the above parameters decreased with the increasing salinity levels. The uninoculated sets recorded the least values of all the above parameters.

The dual inoculated seedlings recorded maximum values of total plant protein, total chlorophyll, total N and total P at the first level (T_0) of salinity. The values decreased at the subsequent levels. Among single inoculated plants, the values in *Rhizobium* (DS Rhz-9) inoculated plants were highest at first level as compared to other levels of salinity. The uninoculated seedlings recorded the lowest values (Table 1).

The nodulation (nodule number, nodule dry weight, maximum nodule size) and nitrogen fixation in terms of nitrogenase activity by nodules, were recorded highest in seedlings inoculated with dual combination of *Rhizobium* (DS Rhz-9) + *Glomus fasciculatum* (Fig. 1). The maximum nitrogenase activity of nodulated roots was recorded 1.65μ mol C_2H_4 g^{-1} fresh nodule h^{-1} in seedling inoculated with dual combination of *Rhizobium* (DS Rhz-9) + *Glomus fasciculatum* in comparison to the nitrogenase activity of 1.23 μ mol C_2H_4 g^{-1} fresh nodule h^{-1} in the seedlings inoculated with *Rhizobium* (DS Rhz-9) alone, at

Fig. 1 Effect of salinity (salt concentration) on growth, nodulation, N_2-fixation and AM root colonization of *Dalbergia sissoo* Roxb. inoculated with *Rhizobium* (DS Rhz-9) and AM (*Glomus fasciculatum*) (Values are Mean ± Standard deviation of 15 replicates)



Treatment : T_0 (0% NaCl)- 1. Uninoculated control, **2.** *Golmus fasciculatum*, **3.** DS Rhz-9, **4.** DS Rhz-9+*Glomus fasciculatum*, **T_1 (0.5% NaCl) - 5.** Uninoculated control, **6.** *Golmus fasciculatum*, **7.** DS Rhz-9, **8.** DS Rhz-9+*Glomus fasciculatum*, **T_2 (1.0% NaCl) - 9.** Uninoculated control, **10.** *Golmus fasciculatum*, **11.** DS Rhz-9, **12.** DS Rhz-9+*Glomus fasciculatum*, **T_3 (2.0% NaCl)- 13.** Uninoculated control, **14.** *Golmus fasciculatum*, **15.** DS Rhz-9, **16.** DS Rhz-9+*Glomus fasciculatum*, **T_4 (3.0% NaCl) - 17.** Uninoculated control, **18.** *Golmus fasciculatum*, **19.** DS Rhz-9, **20.** DS Rhz-9+*Glomus fasciculatum*.

Table 1. Effect of salinity (salt concentration) on growth, nodulation, N_2-fixation and AM root colonization of *Dalbergia sissoo* Roxb. inoculated with *Rhizobium* (DS Rhz-9) and AM (*Glomus fasciculatum*). (Values are Mean ± Standard deviation of 15 replicates)

Salinity levels	Treatments	Shoot length (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Total plant protein (mg/g)	Total plant chlorophyll (mg/l)	N-content (%) (Dry weight)	P-content (%) (Dry weight	AM colonization (%)
T_0 (0% NaCl)	Uninoculated Control	26.5±3.2	0.64±0.04	51.3±8.7	0.73±0.05	87.66±1.02	0.83±0.00	0.99±0.00	0.11±0.00	Zero
	Glomus fasciculatum	31.7±3.6	0.77±0.08	60.3±6.3	0.78±0.03	116.50±2.31	1.17±0.01	1.02±0.01	0.14±0.00	34.8±4.3
	DS Rhz-9	32.5±3.5	0.78±0.06	54.3±7.4	0.84±0.04	119.50±1.17	1.40±0.01	1.07±0.01	0.12±0.00	Zero
	DS Rhz-9+*Glomus fasciculatum*	44.3±4.2	0.97±0.07	62.4±5.5	0.96±0.05	196.56±2.50	1.95±0.02	1.26±0.02	0.29±0.00	38.4±5.7
T_1 (0.5% NaCl)	Uninoculated Control	24.3±3.1	0.63±0.05	50.5±5.5	0.71±0.06	86.17±1.56	0.83±0.02	0.98±0.01	0.08±0.00	Zero
	Glomus fasciculatum	30.3±2.7	0.74±0.06	58.7±5.5	0.77±0.05	112.68±2.36	1.17±0.02	0.99±0.01	0.14±0.00	24.0±3.5
	DS Rhz-9	31.8±2.8	0.77±0.06	54.3±8.1	0.78±0.05	118.64±2.30	1.37±0.01	1.04±0.01	0.12±0.00	Zero
	DS Rhz-9+*Glomus fasciculatum*	42.1±3.7	0.96±0.08	60.5±7.3	0.84±0.06	162.76±2.53	1.81±0.02	1.24±0.01	0.23±0.00	28.0±4.6
T_2 (1.0% NaCl)	Uninoculated Control	21.3±2.1	0.57±0.04	40.7±5.3	0.61±0.03	83.45±1.13	0.76±0.00	0.86±0.01	0.08±0.00	Zero
	Glomus fasciculatum	26.3±2.1	0.72±0.08	52.5±5.7	0.74±0.04	103.67±1.58	1.01±0.00	0.96±0.01	0.12±0.00	20.4±3.5
	DS Rhz-9	28.7±2.1	0.73±0.05	46.3±5.3	0.74±0.05	105.57±1.97	1.29±0.01	0.99±0.01	0.09±0.00	Zero
	DS Rhz-9+*Glomus fasciculatum*	40.5±2.7	0.95±0.07	53.8±5.5	082±0.06	111.37±1.93	1.48±0.02	1.11±0.01	0.14±0.00	24.7±3.8
T_3 (2.0% NaCl)	Uninoculated Control	18.1±2.1	0.57±0.03	32.9±4.5	0.41±0.03	56.35±1.93	0.67±0.01	0.75±0.01	0.07±0.00	Zero
	Glomus fasciculatum	20.2±2.1	0.61±0.04	41.8±3.6	0.71±0.04	98.66±1.53	1.00±0.01	0.80±0.00	0.09±0.00	18.6±4.4
	DS Rhz-9	22.3±2.2	0.62±0.05	40.3±3.7	0.71±0.05	100.45±1.50	1.13±0.02	1.87±0.00	0.08±0.00	Zero
	DS Rhz-9+*Glomus fasciculatum*	32.7±2.7	0.72±0.05	45.7±4.4	0.74±0.05	103.67±1.50	1.32±0.00	0.96±0.01	0.12±0.00	20.5±4.2
T_4 (3.0% NaCl)	Uninoculated Control	10.1±1.1	0.43±0.03	19.3±2.1	0.22±0.03	48.12±0.01	0.64±0.00	0.57±0.01	0.06±0.00	Zero
	Glomus fasciculatum	13.7±1.1	0.46±0.03	24.7±2.3	0.36±0.02	83.67±1.05	0.88±0.01	0.67±0.00	0.08±0.00	17.6±4.6
	DS Rhz-9	15.2±1.1	0.49±0.3	23.9±2.3	0.37±0.04	87.35±1.07	0.98±0.00	0.76±0.01	0.07±0.00	Zero
	DS Rhz-9+*Glomus fasciculatum*	21.5±1.8	0.51±0.03	32.5±3.1	0.44±0.03	96.17±1.17	1.19±0.01	0.81±0.01	0.09±0.00	18.6±4.4
T_5 (4.0% NaCl)	Uninoculated Control									
	Glomus fasciculatum									
	DS Rhz-9	Plants survived for 10-15 days of the treatment								
	DS Rhz-9+*Glomus fasciculatum*									

the first level (T_0) of salinity. The nodulation and nitrogen fixation were very poor and decrease drastically at the subsequent levels of salinity (Fig. 1).

Maximum percentage AM root colonization (Table1) was recorded to be 38.4% in seedlings inoculated with dual combination of *Rhizobium* (DS Rhz-9) + *Glomus fasciculatum* and in only AM (*Glomus fasciculatum*) inoculated plants, percentage AM root colonization was recorded 34.8% at the first level of salinity (T_0). The value decreased with the increase in salinity levels. The salinity levels of 4% did not support the growth of the test individuals. At 4% NaCl treatment individuals survived for 10-15 days only.

The result from the experiment clearly indicated that *Rhizobium* isolate DS Rhz-9 and *Glomus fasciculatum* formed an effective dual combination resulting in highest values of all the parameters studied at the first level of salinity (T_0). The percentage AM root colonization enhanced further when AM was associated with *Rhizobium*. These results were also supported by other workers[12-15].

Thus under salinity stress condition, it was found that the growth rate of plants decreased according to the increase in the levels of salinity. Even though the plants had low growth compared to the uninoculated control plants, the treated plants (treated with AM) showed more growth than their non-mycorrhizal counter parts. The data presented suggested that inoculation of efficient strain of AM fungi (*Glomus fasciculatum*) during the course of the study, prevented the injurious effects of salinity in the test plants due to enhanced water and nutrient uptake thereby promoting growth, nodulation and nitrogen fixation of the tree legume under investigation. It was noted that the percentage of mycorrhization was directly proportional to the growth rate, nodule and nitrogenase activity under salinity stress condition. The above observations have been supported by Rosendahl and Rosendahl[4] and Hirrel and Gerdemann[5].

Acknowledgement

The authors wish to express their thanks to the Head, Department of Botany, University of Rajasthan, Jaipur, for providing necessary facilities and encouragement.

References

1. Sieverding E 1983, Influence of soil water regimes on VA mycorrhiza. iii. Effect of soil temperature and water content on growth, nutrient uptake and water utilization of *Eupatorium odoratum L. J. Agronomy and Crop Sci.* **152** 56-57.
2. Bagyaraj DJ 1991, Ecology of vesicular arbuscular mycorrhizae. Dilip K. Arora, Bharat Rai, K.G. Mukerji and Guy R. Knudsen (eds.), *Hand Book of Applied Mycology,* Vol. **1**, pp. 3-34.
3. Anilkumar KK and Muraleedhara Kurup G 2003, Effect of Vesicular Arbuscular Mycorrhizal fungi inoculation on growth and nutrient uptake of leguminous plants under stress conditions. *J. Mycol. Pl. Pathol.* **33(1)** 33-36.
4. Rosendahl CN and Rosendahl S 1991, Influence of VAM fungi (*Glomus*) on the response of cucumber (*Cucumis sativus*) to salt stress. *Environ. Exp. Bot.* **31** 313-318.
5. Hirrel MC and Gerdemann JW 1979, Effects of salinity on the growth and phosphorus nutrition of vesicular arbuscular mycorrhizal bell pepper and on the germination of *Gigaspora margarita. (Abst.) Fourth North American Conf. on Mycorrhiza.* Fort Collins, Colarado; 1970.
6. Lowry OH, Farr Rosebrough AL and Randall RJ 1951, Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.* **193** 265-275.
7. Arnon DJ 1949, Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris. Plant Physiol.* **24** 1-15.
8. Bremner JM 1960, Determination of nitrogen in soil by kjeldahl method. *J. Agric. Sci.* **55** 11-33.
9. Chapman HD and Pratt P E 1961, Methods of analysis for soils, plants and waters. Univ. of California. pp. 169-170.
10. Hartee EF 1957, Haematin compounds. In : *Modern Methods of Plant Analysis* R. Peach and M.V. Tracey (eds.). 4. Springer Verlag, pp. 197-245.
11. Hardy RWF, Holsten RD, Jackson EK and Burns RC 1968, The acetylene-ethylene assay for N_2-fixation : Laboratory and field evaluation. *Plant Physiol.* **43** 1185-1207.
12. Bagyaraj DJ, Manjunath A and Patil RB 1979, Interaction between VA mycorrhizae and *Rhizobium* and their effects on soybean in field. *New Phytol.* **82** 141-145.
13. Manjunath A and Bagyaraj DJ 1984, Response of pigeon pea to cowpea to phosphate and dual inoculation with VAM and *Rhizobium. Trop. Agric.* **61** 48-52.
14. Subba Rao NS, Tilak KVBR and Singh CS 1986, Dual inoculation in chickpea (*Cicer arietinum L.). Plant and Soil* **95** 351-359.
15. Singh CS 1990, Vesicular arbuscular (VA) mycorrhiza in presence of *Rhizobium* sp. enhances nodulation, N_2 fixation, utilization of pigeon pea (*Cajanus cajan*) as assessed with a ^{15}N technique. In : B.L. Jalali and H. Chand (eds.) *Curr. Trends in Mycorrhizal Res.* pp. 152.