*J. Phytol. Res.* 18(2) : 203-206, 2005

# GROWTH, NODULATION AND $N_2$ FIXATION IN *DALBERGIA SISSOO* INOCULATED BY *RHIZOBIUM* (COWPEA MISCELLANY) AND *GLOMUS FASCICULATUM* UNDER DIFFERENT MOISTURE LEVELS

**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 various levels of moisture on growth, nodulation and $N_2$-fixation of single (*Rhizobium* or AM alone) and dual inoculated (*Rhizobium* + AM) *Dalbergia sissoo,* 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 moisture stress from –0.3 MPa to –1.8 MPa. At –0.3 MPa level, the growth of plants, total N content, percentage of AM colonization, nodulation and nitrogenase activity were found to be maximum in plants raised after dual inoculation with *Rhizobium* and *Glomus fasciculatum*. In other watering regimes, though the values of all the above parameters decreased with increase in moisture stress, the plants with dual inoculation performed better than single inoculation. These observations suggested a protective role played by AM in providing resistance to *Dalbergia sissoo* Roxb. against injurious effects of moisture stress.

**Keywords :** *Dalbergia sissoo; Glomus fasciculatum;* Moisture stress; Nodulation; *Rhizobium.*

## Introduction

Mycorrhizal fungi occur in nearly all soils throughout the world and form symbiotic relationship with the roots of most terrestrial plants. They exist in the soil as spores or as vegetative propagates in root fragments unlike other soil borne and most root-inhabiting fungi. Vesicular Arbuscular Mycorrhizal fungi tolerate a wide range of soil water regimes and are found in habitats as diverse as arid deserts and aquatic environments[1]. Moisture stress can have profound metabolic influence on plant, resulting not only in impaired gas exchange but also in considerable alteration of physiological processes[2,3]. VAM association in plants contributes towards the development of resistance to water stress[4,5]. Available reports indicate that plants infected with mycorrhizal fungi grow better even under stress conditions[6]. The requirement of P is high in legumes[1] 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[6]. In this paper, the effect of inoculation of *Rhizobium* alone and in combination with *Glomus fasciculatum* on growth and nitrogen fixation in *Dalbergia sissoo,* a tree legume, under different moisture stress levels has been discussed.

## Materials and Methods

Role of most preferred AM *Glomus fasciculatum* on resistance and recovery from moisture stress of test plants inoculated with most efficient *Rhizobium* was studied by conducting a pot culture experiment using sterilized soil for six months. The surface sterilized seeds pelleted with rhizobial isolates were sown in pots on AM inoculum pad containing 250 spores/50 g soil. Three seedlings were maintained in each of the five replicates for each treatment. Each of these treatments consisted of uninoculated control, *Rhizobium* alone, AM alone and *Rhizobium* + AM inoculated sets. These were separately maintained at four different soil moisture levels. Soil moisture content was determined in terms of MPa (matric potential) level (1MPa = 10 bars) by using thermocouple psychrometer[7]. The four watering regimes were (i) watering once to the field capacity in 24h (-0.3 MPa), (ii) 48h (-0.6 MPa), (iii) 72h (-1.4 MPa) and (iv) 96h (-1.8 MPa). The plants were subjected to above moisture regimes after two month of their establishment. The above mentioned matric potential were approximately equal to soil moisture levels.

Following parameters were selected for the study: shoot-root length, shoot and root dry weight, total plant protein[8], total chlorophyll[9], total nitrogen[10] and phosphorus content[11], nodule number, nodule dry weight and maximum nodule size[12], nitrogenase activity[13] of root nodules and percentage AM colonization by roots.

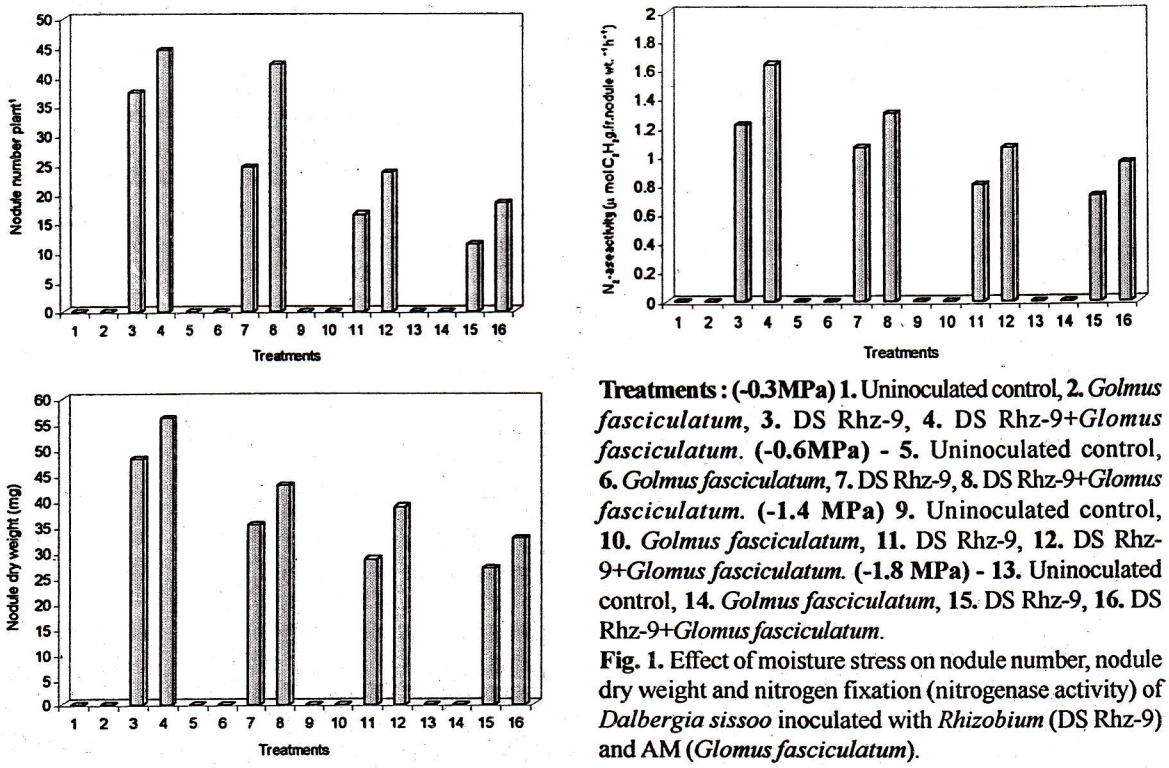
## Results and Discussion

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 moisture stress condition are given in Table-1. The highest value of growth in terms of shoot length, shoot dry weight, root length and root dry weight was observed in *Rhizobium* isolate DS Rhz-9 + *Glomus fasciculatum* inoculated plants at the first level of moisture

**Table 1.** Effect of moisture stress on growth 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)

| Interval of watering | MPa 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 root colonization (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Once in 24 h | (-0.3 MPa) | Uninoculated Control | 12.83±0.93 | 0.61±0.06 | 20.87±2.50 | 0.68±0.04 | 87.50±1.13 | 0.83±0.08 | 0.99±0.04 | 0.11±0.04 | Zero |
| | | *Glomus fasciculatum* | 28.50±2.24 | 1.23±0.04 | 32.45±4.73 | 0.93±0.05 | 117.33±1.35 | 1.16±0.10 | 1.05±0.07 | 0.1±0.05 | 28.30±3.43 |
| | | DS Rhz-9 | 30.81±2.87 | 1.56±0.08 | 36.08±4.55 | 1.73±0.08 | 119.33±1.57 | 1.39±0.12 | 1.02±0.07 | 0.12±0.04 | Zero |
| | | DS Rhz-9+*Glomus fasciculatum* | 34.75±2.51. | 1.67±0.09 | 41.13±4.75 | 1.89±0.09 | 196.67±1.85 | 1.96±0.16 | 1.25±0.08 | 0.20±0.06 | 32.97±5.73 |
| Once in 48 h | (-0.6 MPa) | Uninoculated Control | 9.38±0.86 | 0.56±0.04 | 18.23±2.34 | 0.61±0.05 | 62.67±8.22 | 0.78±0.08 | 0.91±0.04 | 0.09±0.02 | Zero |
| | | *Glomus fasciculatum* | 22.50±2.50 | 0.99±0.05 | 31.51±2.57 | 0.86±0.04 | 103.15±1.14 | 1.02±0.10 | 1.01±0.06 | 0.14±0.04 | 32.63±3.79 |
| | | DS Rhz-9 | 24.67±2.55 | 1.17±0.05 | 34.47±4.32 | 1.63±0.03 | 103.33±1.48 | 1.18±0.11 | 1.01±0.07 | 0.11±0.02 | Zero |
| | | DS Rhz-9+*Glomus fasciculatum* | 28.55±2.45 | 1.24±0.08 | 36.15±4.55 | 1.78±0.06 | 189.56±1.67 | 1.69±0.14 | 1.16±0.08 | 0.21±0.06 | 34.57±6.13 |
| Once in 72 h | (-1.4 MPa) | Uninoculated Control | 7.89±1.73 | 0.45±0.02 | 14.79±2.46 | 0.51±0.03 | 51.34±1.63 | 0.73±0.09 | 0.81±0.03 | 0.08±0.01 | Zero |
| | | *Glomus fasciculatum* | 14.25±2.32 | 0.68±0.03 | 29.15±3.16 | 0.78±0.04 | 96.17±0.85 | 0.89±0.09 | 0.97±0.05 | 0.15±0.05 | 32.35±4.22 |
| | | DS Rhz-9 | 17.76±2.46 | 1.08±0.03 | 32.78±4.25 | 1.34±0.06 | 100.56±0.97 | 0.98±0.10 | 0.98±0.06 | 0.09±0.02 | Zero |
| | | DS Rhz-9+*Glomus fasciculatum* | 18.95±2.53 | 1.18±0.03 | 34.34±4.58 | 1.46±0.08 | 181.78±1.25 | 1.46±0.14 | 1.11±0.07 | 0.23±0.06 | 37.45±6.08 |
| Once in 96 h | (-1.8 MPa) | Uninoculated Control | 6.63±0.64 | 0.28±0.02 | 13.85±2.53 | 0.41±0.04 | 48.12±0.58 | 0.71±0.08 | 0.67±0.03 | 0.06±0.00 | Zero |
| | | *Glomus fasciculatum* | 12.74±1.87 | 0.51±0.01 | 27.53±3.51 | 0.61±0.04 | 82.89±0.75 | 0.78±0.08 | 0.91±0.05 | 0.15±0.06 | 38.15±4.54 |
| | | DS Rhz-9 | 14.77±1.53 | 0.73±0.03 | 31.36±4.13 | 1.08±0.05 | 86.17±0.78 | 0.91±0.09 | 0.92±0.06 | 0.08±0.00 | Zero |
| | | DS Rhz-9+*Glomus fasciculatum* | 18.35±2.35 | 0.78±0.02 | 34.10±4.52 | 1.22±0.05 | 131.27±1.01 | 1.36±0.12 | 0.96±0.06 | 0.24±0.07 | 43.68±5.93 |




**Treatments : (-0.3MPa) 1.** Uninoculated control, **2.** *Golmus fasciculatum*, **3.** DS Rhz-9, **4.** DS Rhz-9+*Glomus fasciculatum*. **(-0.6MPa) - 5.** Uninoculated control, **6.** *Golmus fasciculatum*, **7.** DS Rhz-9, **8.** DS Rhz-9+*Glomus fasciculatum*. **(-1.4 MPa) 9.** Uninoculated control, **10.** *Golmus fasciculatum*, **11.** DS Rhz-9, **12.** DS Rhz-9+*Glomus fasciculatum*. **(-1.8 MPa) - 13.** Uninoculated control, **14.** *Golmus fasciculatum*, **15.** DS Rhz-9, **16.** DS Rhz-9+*Glomus fasciculatum*.

**Fig. 1.** Effect of moisture stress on nodule number, nodule dry weight and nitrogen fixation (nitrogenase activity) of *Dalbergia sissoo* inoculated with *Rhizobium* (DS Rhz-9) and AM (*Glomus fasciculatum*).

(-0.3 MPa) in comparison to single inoculated (DS Rhz-9 alone or *Glomus fasciculatum* alone) plants. The uninoculated plants recorded the lowest growth. In other watering regimes, though, the values decreased with increase in moisture stress, the plants with dual inoculation performed better than single inoculation (Table 1).

The dual inoculated plants recorded maximum values of total protein, total chlorophyll and total N at the first level of moisture. The values decreased at the subsequent levels. Total P content per plant increased only in dual and AM alone inoculated plants with the increase in moisture stress. Therefore maximum total P content was observed in dual inoculated plants at fourth level of moisture (- 1.8 MPa) (Table 1).

The nodulation (nodule number, nodule dry weight, maximum nodule size) and nitrogen fixation in terms of nitrogenase activity by nodules, were recorded maximum in plants inoculated with dual combination of *Rhizobium* (DS Rhz-9) + *Glomus fasciculatum* (Fig. 1). The maximum nitrogenase activity of nodulated roots was recorded 1.64 μmol $C_2H_4$ $g^{-1}$ fresh nodule $h^{-1}$ in dual inoculated plants in comparison to the plant inoculated with *Rhizobium* (DS Rhz–9) alone, at the first level of moisture. The nodulation and nitrogen fixation were very poor and decrease drastically at the subsequent levels of moisture stress (Fig. 1).

The colonization percentage (Table 1) increased with higher levels of moisture stress. Maximum value of percentage AM root colonization was recorded at fourth level of moisture (-1.8 MPa) in dual inoculated plants as compared to only AM inoculated plants.

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. The percentage AM root colonization enhanced further when AM was associated with *Rhizobium*. These results were also supported by other workers[14-17]. In green house studies mycorrhizae have been shown to increase the drought resistance of cultivated crops such as wheat[18,19], soybean[20,21], onion[22], pepper[23] and red clover[24] as well as several native plant species[25-30]. The observations presented in this paper are in agreement with the above findings.

Under moisture stress condition it was found that the growth of plants decreased according to the increase in moisture stress. Even though the plants had low growth compared to the non-stressed mycorrhizal plants, the treated plants showed more growth than their non-mycorrhizal counter parts[31]. The data presented suggested that inoculation of efficient AM fungi (*Glomus fasciculatum*) during the course of the study, prevented the injurious effects of moisture stress in the test plants

due to enhanced water and nutrient uptake thereby promoting growth, nodulation and nitrogen fixation of the *Dalbergia sissoo* under investigation.

**Acknowledgement**

We thank Head, Department of Botany, University of Rajasthan, Jaipur for providing necessary facilities and encouragement.

**References**

1. Bagyaraj D J 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.
2. Hsiao T C 1973, Plant response to water stress. *Ann. Rec. Pl. Physiol.* **24** 519-570.
3. Ramakrishnan B, Johri B N and Gupta R K 1990, The response of mycorrhizal maize plants to variations in water potentials. In: *Current Trends in Mycorrhizal Research.* B.L. Jalali and H. Chand (eds.), pp. 61-62.
4. Sunderesan P, Raja Ubalthoose, Gunasekaran P and Lakshmanan M 1987, Mycorrhizal association in cowpea (*Vigna unguiculata*) increases phosphate uptake under water stress conditions. In: *Mycorrhiza Round Table comp.*, pp. 461-470.
5. Rao V M and Shukla R 2002, Effect of moisture on Growth and $N_2$ Fixation in *Digitaria adscendens* inoculated with *Azospirillum brasilense* and *Glomus mossae. Indian J. Microbiol.* **42** 299-301.
6. 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. Agron. and Crop Sci.* **152** 56-57.
7. Millar B D 1982, Accuracy and usefulness of psychrometer and pressure chamber for evaluating water potential of *Pinus radiata* needles. *Aust. J. Pl. Physiol.* **9** 499-507.
8. Lowry O H, Farr Rosebrough A L and Randall R J 1951, Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* **193** 265-275.
9. Arnon D J 1949, Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris. Plant Physiol.* **24** 1-15.
10. Bremner J M 1960, Determination of nitrogen in soil by kjeldahl method. *J. Agric. Sci.* **55** 11-33.
11. Chapman H D and Pratt P E 1961, *Methods of analysis for soils, plants and waters.* Univ. of California. pp. 169-170.
12. Hartee E F 1957, Haematin compounds. In: *Modern Methods of plant Analysis*, R. Peach and M.V. Tracey (eds.). **4**. Springer Verlag, pp. 197-245.
13. Hardy R W F, Holsten R D, Jackson E K and Burns R C 1968, The acetylene-ethylene assay for $N_2$-fixation: Laboratory and field evaluation. *Plant Physiol.* **43** 1185-1207.
14. Bagyaraj D J, Manjunath A and Patil R B 1979, Interaction between VA mycorrhizae and *Rhizobium* and their effects on soybean in field. *New Phytol.* **82** 141-145.
15. Manjunath A and Bagyaraj D J 1984, Response of pigeon pea to cowpea to phosphate and dual inoculation with VAM and *Rhizobium. Trop. Agric.* **61** 48-52.
16. Subba Rao N S, Tilak K V B R and Singh C S 1986, Dual inoculation in chickpea (*Cicer arietinum* L.), *Plant and Soil* **95** 351-359.
17. Singh C S 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 : *Current Trends in Mycorrhizal Research.* B.L. Jalali and H. Chand (eds.), pp. 152.
18. Allen M F and Boosalis M G 1983, Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytol.* **93** 67-76.
19. Ellis J R, Larsen H J and Boosalis M G 1985, Drought resistance of wheat plants inoculated with vesicular arbuscular mycorrhizae. *Plant Soil* **86** 369-379.
20. Safir G R, Boyer J S and Gerdemann J W 1972, Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiol.* **49** 700-703.
21. Brusse M D and Ellis J R 1985, Vesicular Arbuscular Mycorrhizal (*Glomus fasciculatum*) influence on soybean drought tolerance in high phosphorus soil. *Can. J. Bot.* **63** 2290-2294.
22. Nelsen C E and Safir G R 1982, The water relations of well-watered, mycorrhizal and non-mycorrhizal onion plants. *J. Am. Soc. Hortic. Sci.* **107** 271.
23. Waterer D R and Coltman R R 1989, Response of bell peppers to inoculation timing, phosphorus and water stress. *Hortic. Sci.* **24** 688-690.
24. Fitter A H 1988, Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal colonization of phosphorus supply before and during drought. *J. Exp. Bot.* **39** 595-603.
25. Stahl P D and Smith W K 1984, Effects of different geographic isolates of *Glomus* on the water relations of *Agropyron smithiri. Mycologia* **76** 261-267.
26. Allen E B and Allen M F 1986, Water relations of xeric grasses in the field. Interactions of mycorrhizas and competions. *New Phytol.* **104** 559-571.
27. Hetrick B A D, Kitt D G and Wilson G T 1987, Effects of drought stress on growth response in corn, sudan grass and big blue stem to *Glomus etunicatum. New Phytol.* **105** 403-410.
28. Wallance L L 1987, Mycorrhizas in grass lands: Interactions of ungulates, fungi and drought. *New Phytol.* **105** 619-632.
29. Zajcek J M, Hetrick B A D and Albrecht M L 1987, Influence of drought stress and mycorrhizae on growth of two native forbs. *J. Am. Soc. Hortic. Sci.* **112** 454-459.
30. Fitter A H 1986, Effect of Benomyl on leaf phosphorus concentration in alpine grass lands: A test of mycorrhizal benefit. *New Phytol.* **103** 767-776.
31. Anilkumar K K 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.