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EFFECT OF MOISTURE LEVELS ON THE GROWTH, NODULATION AND NITROGEN FIXATION IN *Dalbergia sissoo* BY *Azospirillum brasilense* AND *Acaulospora laevis*

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Nodulation

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ABSTRACT

In present study, influence of single and combined inoculation of *Azospirillum brasilense* and *Acaulospora laevis* on the growth, nodulation and N_2 fixation in *Dalbergia sissoo* was tested at various moisture levels under pot culture conditions. Combined inoculation of *A.brasilense* and *A.laevis* was found best in raising maximum growth, nodulation, nitrogen fixation and percentage of AM colonization in roots. These characters were considerably influenced with the increasing moisture stress from -0.3MPa to -1.5MPa. Furthermore, the values of all the above mentioned parameters show decrement with the increasing of moisture stress. Plants with dual inoculation performed better than single inoculated plants. Observation of the present study counted a protective role played by AM in providing resistance to *D.sissoo* against injurious effects of moisture stress.

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1 Introduction

Like most root inhibiting and soil born fungi, Mycorrhizal fungi also existing in the form of spores in soil or in the vegetative propagate part, throughout the world and form symbiotic relationship with the most terrestrial plant's roots. These fungi are also habitants of arid deserts and aquatic environment (Bagyaraj, 1991). Arbuscular mycorrhizal fungi have a wide range of soil water regimes tolerant. Negative impact of drought stress on nodule (Sprent, 1971), photosynthesis and disturbance in delicate mechanism of oxygen control in nodules is a constant agricultural constraint in the semi-arid tropics. Symbiosis of AMF with nodule forming plants can protect host plants against detrimental effects caused by drought stress (Ssnchez-Diaz & Honrubia, 1994; Ruiz-Lozano et al., 1999) and can also reduce the impact of environmental stresses such as salinity (Ruiz-Lozano & Azcon, 1996).

Metabolic influence of moisture stress can show extreme result not only on impaired gas exchange but also in considerable alteration of physiological process (Hsiao, 1973; Ramakishnan et al., 1990). Available reports indicate that plants infected with mycorrhizal fungi grow better even under stress condition (Sunderesan et al., 1987) and AM association in plants plays vital role towards the development of resistance to water stress (Rao & Shukla, 2002; Sieveding, 1983). Leguminous plants are responding more to mycorrhizal association due to higher requirements of Phosphorus than cereals; this association indirectly enhances the biological nitrogen fixation, especially in soil with low P content by increased P availability. Present study was proposed to discusses the effects of individual and combined inoculation of *A. brasilense* alone and *A. laevis* on growth and nitrogen fixation in *D. sissoo* a tree legume, under different moisture stress.

2 Materials and Methods

The role of the most preferred AM fungi (*A.laevis*) on resistance recovery from moisture stress of test plants inoculated with most efficient *A.brasilense* was studied by conducting a pot experiment using sterilized soil for six months. Surface sterilized seeds pelleted with *A.brasilense* isolates, were sown in pots on AM inoculum pad containing 250 spores/50 g soils. Four seedlings were maintained in each of the five replicates for their individual treatment. Each of these treatments consists of uninoculated control, *A.brasilense* alone, *A. laevis* alone and *A. brasilense* + *A. laevis* inoculated sets. These were separately maintained at five different soil moisture levels. Soil moisture content was determined in terms of MPa (matric potential) level (1MPa=10bars) by using thermocouple psychometer (Millar, 1982).

The tested five water regimes were watered once to the field capacity in 24h (-0.3MPa), 36h (-0.6MPa), 48h (-0.9MPa), 72h (-1.2MPa) and 96h (-1.5MPa). The plants were subjected to above moisture regimes after two months of their establishment. The above maintained matric potential were approximately equal to soil moisture levels. Following parameters were selected and they tested by standard methodology, shoot-root length, shoot and root dry weight, total plant protein (Lowry et al., 1951), total chlorophyll (Arnon, 1949), total nitrogen (Bremner, 1960), phosphorus content (Chapman & Pratt, 1962), nodule number, nodule dry weight, maximum nodule size (Hartree, 1955), nitrogenous activity (Hardy et al., 1968) of root nodules and percentage of AM colonization by roots.

Results and Discussion

The effects of *Arbuscular* mycorrhizal inoculation on plants growth (shoot and root length), plant dry matter production (shoots and root dry weight) and nutrient uptake level under different levels of moisture stress condition was given in Table-1. The highest value of growth in terms of shoot length, shoot dry weight, root length and root dry weight was reported in the combination of *A. brasilense* isolate + *A. laevis* at the first level of moisture (-0.3MPa). The lowest growth was reported from the uninoculated plants. In other water regimes, though, the values decreases with increase in moisture stress, the plants with dual inoculation performed better than single inoculated plants (Table-1).

The dual inoculated plants recorded maximum values of total protein, total chlorophyll and total N at the first level of moisture. The value decreased at the subsequent increasing the levels of moisture. In case of total Phosphorus level, results are different than the other parameters and level of P content per plant increase only in dual and in individual AM inoculated plants with increase in moisture stress. Therefore maximum total P content was observed in dual inoculated plants at fifth level of moisture (-1.5MPa) (Table-1).

The effect of various treatments on nodulation (nodule number, nodule dry weight, maximum nodule size) and nitrogen fixation in terms of nitrogenous activity by nodules were represented in Figure 1. Among various tested treatment, maximum nodulation was reported in plants inoculated with dual combination of *A. brasilense* + AM (Figure 1). The maximum nitrogen activity of nodulated roots was recorded at the first level of moisture in 2.0 µmol C_2H_2 g^{-1} fresh nodule h-1 in dual inoculated plants as compared to plant inoculated with *A. brasilense* alone. The nodulation and nitrogen fixation were very poor and decrease drastically at the subsequent increasing the levels of moisture stress (Figure 1).

The colonization percentage (Table-1) increased with levels of moisture stress. Maximum value of percentage AM root colonization was recorded at fifth level of moisture (-1.5MPa) in dual inoculated plants as compared to only AM inoculated plants.

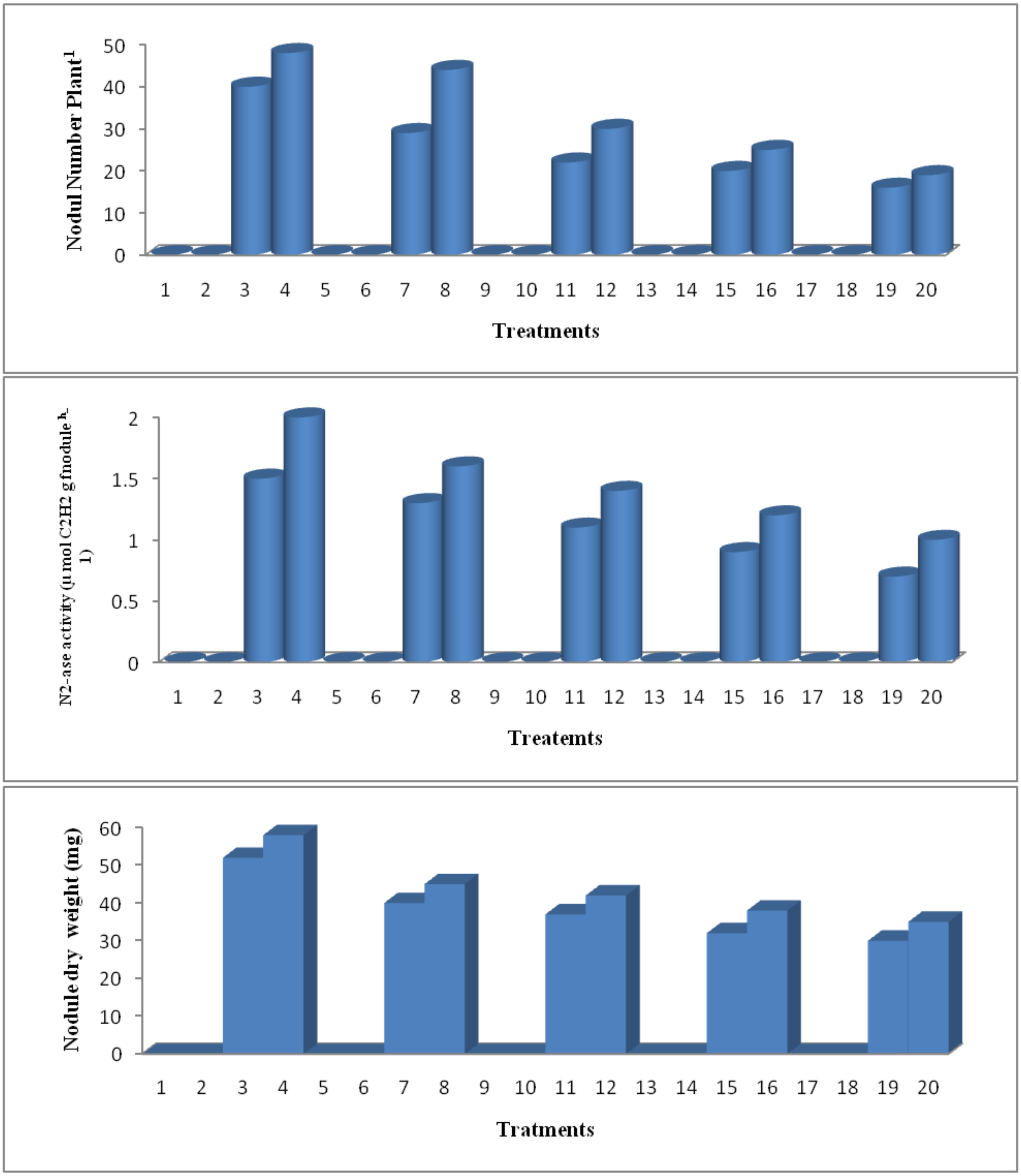

Figure 1 Treatments: (-0.3MPa)1. Uninoculated control, 2. *Acaulospora laevis* 3. *Azospirillum brasilense* 4. *Acaulospora laevis + Azospirillum brasilense.* (-0.6MPa) 5. Uninoculated control, 6. *Acaulospora laevis* 7. *Azospirillum brasilense* 8. *Acaulospora laevis + Azospirillum brasilense.* (-0.9MPa) 9. Uninoculated control, 10. *Acaulospora laevis* 11. *Azospirillum brasilense* 12. *Acaulospora laevis + Azospirillum brasilense.* (-1.2MPa) 13. Uninoculated control, 14. *Acaulospora laevis* 15. *Azospirillum brasilense* 16. *Acaulospora laevis + Azospirillum brasilense.* (-1.5MPa) 17. Uninoculated control, 18. *Acaulospora laevis* 19. *Azospirillum brasilense* 20. *Acaulospora laevis + Azospirillum brasilense.*

Table1. Effect of moisture stress on growth, nodulation and AM root colonization of *Dalbergia sissoo* inoculated with *A.brasilense* and AM (*A. laevis*).

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 colonization (%)
Once in 24h (-0.3MPa)	Uninoculated Control	17.85 ± 1.03	1.11 ± 0.06	25.72 ± 0.03	0.94 ± 1.05	92.80 ± 1.02	1.18 ± 0.04	1.04 ± 0.09	0.15 ± 0.18	Zero
	A. laevis	23.34 ± 1.21^{aa}	1.72 ± 0.14^{aa}	37.14 ± 0.01^{aa}	1.25 ± 0.07^{aa}	122.92 ± 1.18^{aa}	1.46 ± 0.07^{aa}	1.09 ± 0.08^{aa}	0.11 ± 0.13^{aa}	35.80 ± 0.43
	A brasilense	35.92 ± 0.98^{aa}	2.05 ± 0.03^{aa}	41.24 ± 0.08^{aa}	2.04 ± 0.11^{aa}	124.87 ± 1.09^{aa}	1.70 ± 0.01^{aa}	1.07 ± 0.06^{aa}	0.17 ± 0.17^{aa}	Zero
	A brasilense + *A. laevis*	39.54 ± 1.25^{aa}	2.14 ± 0.01^{aa}	46.92 ± 0.12aa	2.26 ± 0.18^{aa}	201.32 ± 1.11^{aa}	2.22 ± 0.06^{aa}	1.34 ± 0.02^{aa}	0.25 ± 0.19^{aa}	41.28 ± 0.73
Once in 36h (-0.6MPa)	Uninoculated Control	14.35 ± 0.88	1.06 ± 0.04	23.23 ± 0. 18	0.89 ± 0.05	71.32 ± 1.36	1.05 ± 0.03	0.96 ± 0.09	0.14 ± 0.11	Zero
	A. laevis	27.32 ± 0.54^{aa}	1.48 ± 0.11^{a}	36.92 ± 0. 21^{a}	1.17 ± 0.02^{aa}	107.18 ± 1.00^{aa}	1.39 ± 0.04^{aa}	1.06 ± 0.11^{aa}	0.19 ± 0.10^{aa}	38.93 ± 0.07
	A brasilense	29.71 ± 1.01^{aa}	1.68 ± 0.07^{a}	39.24 ± 0.06^{a}	1.84 ± 0.22^{aa}	106.93 ± 1.24^{aa}	1.65 ± 0.09^{aa}	1.07 ± 0.08^{aa}	0.16 ± 0.12^{aa}	Zero
	A brasilense + *A. laevis*	23.52 ± 0.11^{aa}	1.75 ± 0.02^{a}	41.12 ± 0.17^{a}	2.04 ± 0.09^{aa}	194.32 ± 1.55^{aa}	2.10 ± 0.08^{aa}	1.29 ± 0.01^{aa}	0.27 ± 0.16^{aa}	43.87 ± 1.75
Once in 48h (-0.9MPa)	Uninoculated Control	12.81 ± 1.75	0.94 ± .009	19.96 ± 0.52	0.85 ± 0.32	64.79 ± 1.06	0.86 ± 0.03	0.85 ± 0.13	0.12 ± 0.11	Zero
	A. laevis	19.05 ± 1.08^{aa}	1.15 ± 0.18^{aa}	35.21 ± 0.05^{aa}	1.09± 0.01^{aa}	98.25 ± 1.44^{aa}	1.19 ± 0.06aa	1.00 ± 0.03^{aa}	0.20 ± 0.17^{aa}	38.56 ± 0.09
	A brasilense	23.41 ± 0.92^{aa}	1.54 ± 0.16^{aa}	37.49 ± 0.19^{aa}	1.76 ± 0.15^{aa}	101.39 ± 1.21^{aa}	1.41 ± 0.01^{aa}	1.01 ± 0.07^{aa}	0.14 ± 0.19^{aa}	Zero
	A brasilense + *A. laevis*	24.85 ± 0.69^{aa}	1.68 ± 0.03^{aa}	39.02 ± 0.12^{aa}	1.94 ± 0.06^{aa}	189.34 ± 1.72^{aa}	1.95 ± 0.07^{aa}	1.20 ± 0.08^{aa}	0.28 ± 0.21^{aa}	45.93 ± 1.21
Once in 72h (-1.2MPa)	Uninoculated Control	11.92 ± 0.71^{aa}	0.77 ± 0.08	18.32 ± 0.11^{aa}	0.70 ± 0.11	57.04 ± 0.06	0.80 ± 0.02	0.81 ± 0.01	0.11 ± 0.14	Zero
	A. laevis	17.44 ± 0.13^{aa}	1.01 ± 0.04^{aa}	32.82 ± 0.04^{aa}	0.96 ± 0.03^{aa}	89.25 ± 0.21^{aa}	0.92 ± 0.09^{aa}	0.95 ± 0.16^{aa}	0.21 ± 0.17^{aa}	39.63± 0.05
	A brasilense	19.25 ± 0.16^{aa}	1.21 ± 0.01^{aa}	36.56 ± 0.09^{aa}	1.54 ± 0.28^{aa}	91.62 ±0.34^{aa}	1.09 ± 0.07^{aa}	0.99 ± 0.04^{aa}	0.14 ± 0.05^{aa}	Zero
	A brasilense + *A. laevis*	23.11 ± 0.37^{aa}	1.29 ± 0.21^{aa}	38.04 ± 0.01^{aa}	1.89 ± 0.71^{aa}	154.29 ± 0.07^{aa}	1.71 ± 0.04^{aa}	1.12 ± 0.07^{aa}	0.30 ± 0.13^{aa}	47.84 ± 0.15
Once in 96h (-1.5MPa)	Uninoculated Control	9.24 ± 1.53	0.48 ± 0.05	16.72 ± 0.13	0.64 ± 0.80	49.24 ± 1.11	0.74 ± 0.02	0.67 ± 0.17	0.08 ± 0.09	Zero
	A. laevis	15.72 ± 0.79^{aa}	0.91 ± 0.04^{a}	30.59 ± 0.21^{aa}	0.82 ± 0.03^{aa}	81.92 ± 1.39^{aa}	0.86 ± 0.01^{aa}	0.82 ± 0.09^{aa}	0.16 ± 0.02^{aa}	41.08 ± 0.32
	A brasilense	19.26 ± 0.25^{aa}	1.03 ± 0.08^{a}	34.72 ± 0.91^{aa}	1.22 ± 0.01^{aa}	86.22 ± 1.14^{aa}	0.92 ± 0.08^{aa}	0.92 ± 0.21^{aa}	0.11 ± 0.14^{aa}	Zero
	A brasilense + *A. laevis*	23.24 ± 1.21^{aa}	1.12 ± 0.11^{a}	37.61 ± 0.22^{aa}	1.30 ± 0.09^{aa}	138.04 ± 1.55^{aa}	1.42 ± 0.03^{aa}	0.99 ± 0.05^{aa}	0.18 ± 0.19^{aa}	51.88 ± 0.16

The results from the experiments clearly indicated that *A. brasilense* and *A. laevis* formed an active dual combination resulting in significant improvement in all the parameter studied. The percentage of AM root colonization enhanced further when AM was associated with *A. brasilense*. The results were also supported by other workers (Bagyaraj et al., 1979; Manjunath and Bagyaraj, 1984; Subba et al., 1986; Singh, 1990). In green house studies mycorrahizae have been shown to increase the drought resistance of cultivated crops such as wheat (Allen & Boosalis, 1983; Ellis et al., 1985) , Soya been (Safir et al., 1972; Busse & Ellis, 1985), onion (Nelson & Safir, 1982) , pepper (Waterer & Coltman, 1989) and red clover (Fitter, 1988) as well as several native plant species (Stahl & Smith, 1984; Allen & Allen, 1986; Hetrick et al., 1987; Wallace, 1987; Zajeek et al., 1987; Fitter, 1986).

The observations presented in this paper are in agreement with the above findings. The probable mechanism reported earlier behind the above results was the osmotic adjustment, which was found to maintain cell turgidity in many plant species as the water potential decreases, enabling water uptake and the maintenance of plant metabolic activity and therefore growth and productivity (Zlatev & Lidon, 2012). However, in the earlier studies it was suggested that AM colonization improves the osmotic adjustment originating from NSC, K^+, Ca^{2+} and Mg^{2+}, resulting in the enhancement of drought tolerance (Wu & Xia, 2006). The root nodulation was also found to be highly influenced by the water potential. The earlier data indicates that the water stress directly affects the interaction between the bacteria and the host plant by altering the nodule structure and enzymatic activity. It was also suggested that the water stress limits many processes of nodule activity. Further, it was reported that the water stress results decline in the enzyme activity in metabolic paths concerning the nitrogen fixation, NADH-dependent glutamate synthase (NADH-GOGAT), glutamine synthetase (GS), phosphoenolpyruvate carboxylase (PEPEC) and increase in glutamate dehydrogenase (GDH). Further, it was clarified that NADH-GOGAT was the most sensitive enzyme under water stress and its activity in the nodule decreases rapidly with an increase in the water stress (Figueiredo et al., 2001). Therefore, nodulation and nodule activity are reported inversely proportional to the water stress.

Under moisture stress condition it was found that the growth of plants decreases with the increase in moisture stress. This finding is in agreement with the previous report revealing that the water stress substantially alters plant metabolism, decreasing plant growth and photosynthesis (Tezara et al., 1999). Even though the plants showed less growth compared to the non-stressed mycorrhizal plants, the treated plants showed more growth than their non mycorrhizal counter parts (Anilkumar & Muraleedhara, 2003). The data presented, suggested that inoculation of efficient AM fungi (*A. laevis*) 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 *D. sissoo* under investigation.

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