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Effect of arbuscular mycorrhizal fungi, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* on the growth and nutrient status of *Dalbergia sissoo* Roxb.

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Abstract: *Dalbergia sissoo,* an important tree of tropical regions, is grown as monoculture or in agroforesty systems because of the quality of its wood and its effects on soil fertility through N_2 fixation. Low soil fertility in tropical regions results in poor plant growth. This is significant in the case of forest trees, since they are generally transplanted without considering the fertility status of soil. The present study was conducted to evaluate the effects of arbuscular mycorrhizal fungi (AMF) (a plant-growth promoting fungus through higher nutrient uptake), *Rhizobium leguminosarum* strain DSP_2 (a N_2 fixer) and *Pseudomonas fluorescens* strain GRPr (a plant-growth promoting bacterium), on the growth and nutrient acquisition of *Dalbergia sissoo* Roxb. Mollisol and Entisol were used to compare the effects of different soils. A tetrapartite interaction of AMF, *P. fluorescens* strain GRPr, *R. leguminosarum* strain DSP_2 and *D. sissoo* showed improved plant growth response in the Entisol compared to uninoculated plants. The interaction of AMF with DSP_2 was found; however, AMF did not show the same growth responses in combination with either GRPr or DSP_2 and GRPr regardless of soil type. AMF and GRPr showed decreased plant growth, suggesting that enhanced plant growth was dependent on the bacteria - AMF combination used. We suggest that, in the case of *D. sissoo*, choice and testing of the combination of beneficial organisms is necessary to get desired plant growth promotion.

Resumen: *Dalbergia sissoo*, un árbol importante de regiones tropicales, se cultiva en monocultivo o sistemas agroforestales debido a la calidad de su madera y sus efectos en la fertilidad por la fijación de N_2. La baja fertilidad del suelo en regiones tropicales provoca un crecimiento vegetal pobre. Esto es significativo en el caso de árboles del bosque, puesto que por lo general éstos son transplantados sin considerar el estatus de la fertilidad del suelo. El presente estudio se llevó a cabo con el fin de evaluar los efectos de hongos micorrícicos arbusculares (HMA) (promotores del crecimiento vegetal por medio de una mayor captación de nutrientes), de *Rhizobium leguminosarum* cepa DSP_2 (un fijador de N_2) y de *Pseudomonas fluorescens* cepa GRPr (una bacteria promotora del crecimiento vegetal), sobre el crecimiento y la adquisición de nutrientes de *Dalbergia sissoo* Roxb. Se usaron un Molisol y un Entisol para comparar los efectos de suelos diferentes. La interacción tetrapartita del HMA, *P. fluorescens* cepa GRPr, *R. leguminosarum* cepa DSP_2 y *D. sissoo* mostró una respuesta mejorada en el crecimiento vegetal en el Entisol, en comparación con las plantas uninoculadas. Se encontró una interacción del HMA con DSP_2; sin embargo, el HMA no produjo las mismas respuestas en el crecimiento en combinación ya sea con GRPr o DSP_2 y GRPr, independientemente del tipo de suelo. El HMA y GRPr produjeron una disminución en el crecimiento vegetal, lo que sugiere que el aumento en el crecimiento dependió de la combinación usada bacteria-HMA. Sugerimos que en el caso de *D. sissoo*, hace falta seleccionar y probar la combinación de organismos benéficos para promover el crecimiento vegetal deseado.

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Resumo: A *Dalbergia sissoo*, uma importante árvore das regiões tropicais, é cultivada em regime nonocultural ou em sistemas agroflorestais por causa da qualidade da sua madeira e dos seus efeitos na fertilidade do solo através da fixação do N_2. A baixa fertilidade do solo nas regiões tropicais tem como resultado um fraco crescimento das plantas. Isto é significativo no caso das espécies arbóreas florestais, porque elas são transplantadas sem ser tomada em atenção a fertilidade do solo. O presente estudo foi efectuado para avaliar os efeitos do fungo micorrízico arbuscular (AMF) (um promotor de crescimento vegetal através de uma absorção de nutrientes), a linha DSP_2 de *Rhizobium leguminosarum* (um fixador de N_2) e a linha GRPr de *Pseudomonas fluorescens* (uma bactéria promotora de crescimento de plantas), no crescimento e absorção de nutrientes da *Dalbergia sissoo* Roxb. Os solos *Mollisol* e *Entisol* foram usadas para comparar os efeitos em solos diferentes. Uma interacção quadripartida entre a AMF, a linha GRPr de *P. fluorescens*, a linha DSP_2 de *R. leguminosarum* e a *D. sissoo* mostraram uma resposta melhorada nos Entisol em comparação com as plantas não inoculadas. Encontrou-se uma interacção da AMF com a DSP_2; contudo, a AMF não mostrou a mesma resposta ao crescimento em combinação quer com a GRPr ou DSP_2 e GRPr, independentemente do tipo de solo. A AMF e GRPr mostraram um decréscimo no crescimento das plantas, sugerindo que um aumento de crescimento das mesmas estava dependente da combinação bactéria-AMF utilizada. Sugere-se que, no caso da *D. sissoo*, a escolha e testagem da combinação dos organismos benéficos são necessárias para conseguir-se a promoção desejável do crescimento das plantas.

Key words: Arbuscular mycorrhizal fungi, *Dalbergia sissoo*, entisol, mollisol, *Pseudomonas fluorescens, Rhizobium leguminosarum*.

Introduction

Microorganisms play a major role in biogeochemical process, such as solubilization of nutrients, N_2 fixation, and the mobilization, immobilization and mineralization of various plant nutrients. Plant-growth promoting effects of bacterial inoculation have been demonstrated in specialized N_2-fixing structures in non-legume symbiotic relationships, including *Parasponia*-rhizobia (Gresshoff *et al.* 1984), *Alnus-Azolla-Anabaena* (Peters & Meeks 1989), *Gunnera-Nostoc* (Bergman *et al.* 1992) and cycads-cyanobacteria (Korzhenevskaya *et al.* 1999). Arbuscular mycorrhizal fungi (AMF) facilitate higher water absorption and nutrient uptake in plants, which in turn helps to combat various diseases and enhances plant growth (Sharma *et al.* 1992). Phosphorus, nitrogen, zinc, and copper are the most commonly reported elements whose uptake is enhanced by AMF in plants; however, acquisition of other mineral nutrients required for plant growth may also be enhanced (Singh & Adholeya 2004). AMF are also known for increasing phosphorus uptake and plant growth promotion at low P levels in the soil solution (Sharma *et al.* 1999). By contrast, some AMF depress growth in host plants when grown on soils containing high levels of P (Graham & Abbott 2000). Significant efforts have been made to elucidate the role of soil microbiota in relation to mycorrhizal association and their effects on development of the host plant and its productivity. Beneficial effects of AMF, such as growth promotion, increased root branching in lengths of lateral roots, specific root length, root diameter (Koide 1993), transplant performance, protection against pathogens (Sharma *et al.* 1992) and tolerance to abiotic stresses (Göhre & Paszkowski 2006), could be due to positive interactions between mycorrhizae and associated microorganisms such as *Pseudomonas, Arthrobacter* and *Burkholderia* in a particular environment (Mansfeld-Giese *et al.* 2002).

Plant-growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which, when grown in association with a host plant, result in the stimulation of its growth. The mycorrhizal fungi influence the composition of the rhizospheric bacterial community (Linderman 1988). Bacteria can interact with mycorrhizal fungi in a wide variety of ways, as they do with plant root itself (Andrade *et al.* 1997). Total viable counts of bacteria in the rhizosphere of guinea grass were reduced by *Acaulospora leavis* and

increased by *Glomus fasciculatum* irrespective of an increased *Pseudomonas fluorescens* population reported in the rhizosphere of AM plants (Meyer & Linderman 1986). Successful bacterial response in the rhizosphere depends on its ability to colonize the rhizosphere (Bais *et al.* 2006). However, bacterial inoculation induces a carbon sink on the root surface, which in turn affects photosynthesis and the carbon balance of the plant (Schulze & Pöschel 2004). Several bacteria described as good root colonizers are also capable of attaching to hyphal surfaces (Artursson *et al.* 2005).

Excessive industrial exploitation, clearing of land for agricultural purposes and procurement of firewood in India have led to deforestation during recent decades (Bandyopadhyay & Shyamsundar 2004). Planting of trees along roadsides, on degraded lands and as part of agroforestry projects is being practiced for environmental protection and economic gain. Among the different tree species planted, *Dalbergia sissoo* is one of the most important because of its economic importance, and could be used successfully on degraded lands to prevent wind and rain erosion, and to control the erosion of sand dunes. *D. sissoo* is also a source of wood and provides fodder for livestock. Being a leguminous tree species, *Dalbergia sissoo* is expected to have an association with *Rhizobium* sp. as well as AMF. Although a great deal is known about crop plants with respect to AMF (Sharma & Johri 2002), relatively little information exists for trees, leaving ample scope for improving seedling quality in forest tree nurseries using AMF**.** Bacterial growth-promoting effects have been shown with different host plants, including herbaceous (Alten *et al.* 1993; Paula *et al.* 1992; Requena *et al.* 1997) and tree species (Dunstan *et al.* 1998). In addition, studies have been conducted to assess the role of rhizosphere bacteria on AMF, which is not always synergistic (Johansen & Jensen 1996; Walley & Germida 1997).

Efficiency of microorganisms residing in the rhizosphere depends on the soil type, which provides nutrients and habitat (Cardoso & Kuyper 2006). Amongst the abiotic factors, soil plays a major role in the interaction of soil microorganisms. The present study was conducted to evaluate the interaction of AMF and *Pseudomonas fluorescens* strain GRPr and *Rhizobium leguminosarum* strain DSP_2, on the growth and nutrient content (P, N, Cu, Mn, Zn and S) of *Dalbergia sissoo* Roxb. under glasshouse conditions in two different soil types.

Materials and methods

AMF inoculum production and identification

Soil samples were collected for isolation of AMF at the depth of 0-20 cm from an undisturbed site in GBPUAT, Pantnagar, US. Nagar, Uttarakhand, situated at 29º N latitude, 79º 3' E longitude and at an altitude of 243 m above MSL in tarai belt in Shivalik range of Kumaun Himalayas (Garg 2002). These sites were dominated by *Lathyrus aphaca* L., *Ageratum conyzoides* L., *Cyperus rotundus* L., *Cynodon dactylon* L., *Erigron bonariensis* L., *Cyperus deformis* L., *Elucine coracana* L., *Cannabis sativa* L. Soils were sieved, air dried and processed by wet sieving and decanting method (Gerdeman & Nicolson 1963) for collection of AMF spores. These were picked and identified after sucrose gradient density centrifugation (Tommerup & Kidby 1979). The inoculum was produced in steam sterilized soil:sand (1:1) mixture placed in 2 kg pots. Four holes were made in the soil and 50 spores were placed in each hole. One maize seed (*Zea mays* L., variety "Naveen") was sown in each hole and grown for two cycles each of 60 days of plant growth to ensure sufficient production of fresh spores. The plants were watered as required. After two cycles of AMF multiplication, the most probable number (MPN) of infectious AMF propagules was assessed using a dilution technique (Porter 1979). Spores were identified on the basis of number of spore walls, size, colour, hyphal attachment and ornamentation using the manual of Schenck & Perez (1990). After two cycles of AMF culturing, the soil-based inoculum had approximately 25 AM infectious propagules g^{-1} of soil, which was used for carrying out the experiments. The dominant AMF genera were *Gigaspora albida, Glomus intraradices* and *Acaulospora scrobiculata*.

Bacterial isolates

The bacterial isolate used in the investigation was *Pseudomonas fluorescens*-GRPr (G), isolated from the rhizoplane of *Glycine max* L. capable of siderophore production and P solubilization (Bisht 2000). The *Rhizobium leguminosarum* strain DSP_2 (R) was procured from the Department of Microbiology, College of Basic Sciences & Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar. *P. fluorescens* and rhizobial strains were cultured in King's B and

YEM broth, respectively, at 30ºC on a rotary shaker at 200 rpm.

Soil types

Soils (Mollisol from Research Station of G.B. Pant University of Agriculture & Technology, Pantnagar and Entisol from Ujhani Research Station of Sardar Ballabh Bhai Patel University of Agriculture & Technology, Meerut) were passed through a 2-mm-mesh sieve. The Mollisol (mollic epipedon, high base saturation, dark soils with argillic horizon, more cation exchange capacity) has a higher nutrient regime than the Entisol (weakly developed mineral soils without natural genetic subsurface horizons, little profile development, ochric epipedon common) (Table 1). Methods employed to determine soil physico chemical properties were: soil organic carbon (g kg^{-1}) by Walkley & Black (1934), alkaline $KMnO_4$ hydrolyzed nitrogen (kg ha^{-1}) by Kjeldahl method (Subbiah & Asija 1956), neutral normal ammonium acetate extractable potassium (kg ha^{-1}) using flame photometer (Jackson 1958), Olsen's Phosphorus (mg kg^{-1}) by Olsen *et al.* (1954) and micronutrients were extracted using DTPA and estimated by Atomic Absorption Spectometer. Soils were steam-sterilized three times on alternate days.

Table 1. Physico-chemical properties of soils.

Property	Mollisol	Entisol
Texture	Clay loam	Sandy loam
pH	7.8	7.3
Organic carbon (g kg^{-1})	18	5
Alkaline $KMnO_4$ hydrolyzed Nitrogen (kg ha^{-1})	12992	1792
Neutral normal Ammonium acetate extractable Potassium (kg ha^{-1})	380.8	313.6
Olsen's Phosphorus (mg kg^{-1})	14.9	6.3
DTPA extractable Zinc (mg kg^{-1})	5.6	2.39
DTPA extractable Copper (mg kg^{-1})	4.8	0.082
DTPA extractable Manganese (mg kg^{-1})	30.94	21.28
DTPA extractable Iron (mg kg^{-1})	29.72	19.12

Tetrapartite interaction in different soil types

Steam-sterilized soil was air-dried and placed in 11 kg earthen pots. Forty-five-day-old seedlings of *D. sissoo* (PSC 99-1) were transplanted and provided with 50 g of mycorrhizal inoculum (containing 25 AM infectious propagules g^{-1}) and were inoculated with GRPr and/or rhizobial culture broth (final density 10^{-10} cfu) in the planting hole depending on the treatment. Eight different treatments, each replicated twelve times, were used: (1) Uninoculated (C); (2) AMF consortium (M); (3) GRPr (G); (4) DSP_2 (R); (5) G+M; (6) M+R; (7) G+R; and (8) G+R+M. In each of the eight treatments, twenty-one days old seedlings of *D. sissoo* (grown on sterilized sand after disinfecting the seeds with 0.1% $HgCl_2$ for two minutes followed by three rinses with sterilized water) were transplanted (2 seedlings pot^{-1}) (Bisht 2000). After three months of establishment, plants were harvested and analyzed for biomass production in terms of root & shoot dry weight (dried in oven at 68 ºC for 48 h). Mycorrhizal colonization was assessed using the method of Biermann & Lindermann (1981) after staining the roots with acid fuschin and toludine blue O. The roots were cut in 1-cm segments and boiled at 90^{0}C in 10% KOH for 1 h, followed by acidification in 1N HCl for 10 min (Sharma *et al.* 1988). To determine P, Fe, Zn, Cu and Mn content, dried plant samples were digested in a tri-acid mixture (HNO_3:H_2SO_4:$HClO_4$, 10:1:4). After digestion the volume was made with 1N HCl to 25 ml. Phosphorus and sulphur were determined using the vandomolybdate method (Bhargava & Raghupathi 1984) and the method of Chesnin & Yien (1950), respectively. Fe, Zn, Cu and Mn were estimated using an atomic absorption spectrophotometer (GBC, Model No. 902, Australia). The nitrogen content was estimated using the Kjeldahl method (Cliffes 1958). Photosynthetic and transpiration rates were measured using an infrared gas analyzer (CID, USA). Data were analyzed using two-way ANOVA using SPSS software (Version 7.5, SPSS Inc., Illinois, USA). Interaction was evaluated by calculating percent increase over uninoculated by the treatment. Synergistic and antagonistic effect was calculated on the basis of percent increase over control. In addition, if the sum of the individual treatment was lower than the combination of all three microorganisms, it was

considered synergistic; if not, it was considered antagonistic.

Results

Biomass production

Significantly higher root & shoot dry weights were recorded in all seven treatments compared to control. The plants inoculated with the combination of *Rhizobium* sp. and AMF had the highest biomass, followed by mycorrhizal inoculation alone. Amongst all the treatments, lowest shoot and root dry weight were observed in plants inoculated with combination of GRPr and AMF in both the soils types used in investigation. The Mollisol supported higher root & shoot dry weight than Entisol in all the treatments (Table 2).

Table 2. Effect of soil type and different microbial inoculation treatments on root & shoot dry weight of *D. sissoo*. LSD value represents the value of interaction of treatments and soil types. The same letter in superscript in each parameter of plant in a column are not significantly different. M= Arbuscular mycorrhiza, G = *Pseudomonas fluorescens* strain GRPr, and R = *Rhizobium leguminosarum* strain DSP_2.

Treatments	Root dry weight (g)		Shoot dry weight (g)	
	Mollisol	Entisol	Mollisol	Entisol
AM (M)	1.01^{f}	0.91^{ef}	5.63^{de}	4.47^{cd}
GRPr (G)	0.75^{de}	0.65^{cd}	3.95^{c}	3.10^{bc}
DSP_2 (R)	0.70^{d}	0.60^{cd}	3.85^{c}	2.95^{d}
G+R	0.82^{de}	0.62^{cd}	4.11^{c}	3.82^{c}
G+M	0.65^{cd}	0.56^{c}	2.95^{b}	2.10^{ab}
R+M	1.20^{g}	0.98^{ef}	6.03^{e}	5.20^{d}
G+R+M	0.78^{de}	0.85^{e}	4.50^{cd}	3.50^{bc}
Uninoculated	0.35^{b}	0.20^{a}	2.18^{ab}	1.85^{a}
LSD (P=0.05)	0.13		0.75	

Mycorrhizal colonization

In the case of the Entisol, significantly higher mycorrhizal colonization was found in plants inoculated with AMF, followed by the combined inoculation of all three microorganisms, with the lowest colonization observed in plants inoculated with GRPr and AMF. This was not the case for the Mollisol. In general, Entisols supported higher mycorrhizal infection than the Mollisols (Fig. 1). In the Mollisol, the lowest percent mycorrhizal colonization was recorded for the combination of GRPr & AMF, and the highest percent mycorrhizal infection was recorded for plants inoculated with *Rhizobium* sp. & AMF.

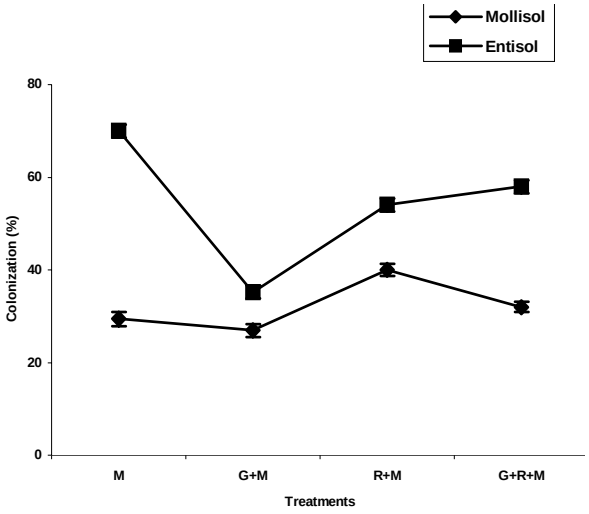

Fig. 1. Percent mycorrhizal colonization as influenced by AM, *P. fluorescens* and *Rhizobium leguminosarum* in two soils. Bars represent one standard deviation The data are shown where AMF was added. No AMF infection was observed in the treatments not provided with AMF. M= Arbuscular mycorrhiza, G = *Pseudomonas fluorescens* strain GRPr, and R = *Rhizobium leguminosarum* strain DSP_2. LSD (P=0.05) for the Mollisol = 2.52; for the Entisol = 2.60.

Nutrient contents

Phosphorus content in both root and shoot tissue was significantly higher in plants inoculated with a combination of *Rhizobium* sp. & AMF, followed by mycorrhizal inoculation alone in both soil types (Table 3). However, a significantly lower value (P=0.05) was obtained with triple inoculation of GRPr, DSP_2 and AMF when compared to the inoculation with AMF alone. In general, plants grown in Entisols had a lower phosphorus content. The nitrogen content of root and shoot tissue was significantly higher for plants treated with a combination of DSP_2 & AMF, followed by DSP_2 alone. A significantly lower N content was obtained for uninoculated plants, below the nitrogen value for plants inoculated with a combination of GRPr & AMF. Plants grown in Mollisols had a higher nitrogen content in plant tissues in all the treatments (Table 3).

Table 3. Effect of soil type and different microbial inoculation treatments on phosphorus and nitrogen content of dry root & shoot of *D. sissoo*. LSD value represents the value of interaction of treatments and soil types. The same letters in superscript in each nutrient presented in column are not significantly different. M= Arbuscular mycorrhiza, G = *Pseudomonas fluorescens* strain GRPr, and R = *Rhizobium leguminosarum* strain DSP_2.

Treatments	Root				Shoot			
	Phosphorus (%)		Nitrogen (%)		Phosphorus (%)		Nitrogen (%)	
	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol
AM (M)	2.15^{i}	1.70^{f}	7.85^{j}	6.95^{f}	2.35^{h}	1.31^{c}	7.05^{e}	6.55^{d}
GRP_r (G)	1.82^{g}	1.13^{bc}	6.85^{f_f}	5.85^{c}	2.00^{g}	0.81^{a}	6.55^{d}	4.60^{b}
DSP_2 (R)	1.76^{fg}	1.23^{d}	8.33^{l}	7.65^{i}	1.95^{fg}	0.99^{b}	7.25^{e}	6.55^{d}
G+R	2.00^{h}	1.30^{e}	8.23^{kl}	7.45^{h}	2.04^{g}	1.04^{b}	6.95^{e}	6.22^{d}
G+M	1.15^{c}	1.04^{ab}	6.53^{e}	5.70^{b}	1.73^{e}	0.87^{a}	5.55^{c}	4.32^{b}
R+M	2.82^{j}	2.00^{h}	8.64^{m}	8.15^{k}	2.95^{i}	1.55^{d}	7.30^{e}	7.47^{e}
G+R+M	1.86^{g}	1.25^{de}	7.15^{g}	6.15^{d}	1.86^{f}	0.86^{a}	5.98^{cd}	4.22^{ab}
Uninoculated	1.01^{a}	1.08^{b}	5.85^{c}	4.66^{a}	1.54^{d}	0.79^{a}	4.83^{b}	3.66^{a}
LSD (P=0.05)	0.06		0.14		0.09		0.65	

The sulphur content of root & shoot tissues was significantly higher in AM inoculated plants. However, in the shoots, significantly lower S content was found in plants inoculated with DSP_2 alone. In the roots, a lower S content was obtained with inoculation of all the three organisms. Combined inoculation of DSP_2 & AMF showed higher Zn content, followed by AMF for root and shoot tissues. The combined effect of GRPr with either AMF or DSP_2 showed significantly lower (P=0.05) Zn content in shoot tissues (Table 4). Mn and Cu levels were higher (P=0.05) in root and shoot tissue of plant inoculated with DSP_2 and AMF. The rest of the treatments, i.e. C; M; G; R; G+M; G+R; and G+R+M, showed significant differences in nutrient content from each other; however, combined inoculation of GRPr and AMF showed lower nutrient content in root and shoot tissues for both soils (Table 4).

Photosynthetic and transpiration rates

Significantly higher photosynthetic and transpiration rates were observed in plants dually inoculated with a combination of DSP_2 and AMF, followed by AM fungal inoculation alone, although these treatments were not significantly different from each other. Lower rates of photosynthesis and respiration were found in uninoculated plants and those inoculated with both GRPr and AMF (Table 5). The presence of DSP_2 in combination with GRPr and AMF nullified the effect of AMF and GRPr to some extent; hence, the combined effect of these three organisms on rate of photosynthesis and transpiration was significantly better than the dual effect of AMF and GRPr. Mollisols supported higher photosynthetic rates, whereas Entisols supported higher transpiration rates.

Nature of interaction

Interaction of AMF, DSP_2 and GRPr was lower than their individual effect for all the parameters measured, hence the combined effect of all these three organisms was considered to be antagonistic. This antagonistic effect was noticed in both soil types. (Tables 6 & 7).

Discussion

The results of this study clearly showed that (1) mycorrhizal symbiosis enhanced growth of *D. sissoo*, and (2) in general, the combination of DSP_2 with mycorrhiza stimulated plant growth and nutrient uptake. It is well known that many N_2-fixing trees and shrubs depend on mycorrhiza to absorb mineral nutrients required for plant growth and efficient N_2 fixation (Cornet *et al.* 1982; Duponnois *et al.* 2001). Enhanced growth of *Acacia holosericea* was recorded when the plants were inoculated with *Glomus intraradices* (Duponnois & Plenchette 2003), *G. fasciculatum* (Senghor 1998) and *G. aggregatum* (Duponnois *et al.* 2001). The present study is in accordance with the above findings that association of consortial inocula of AMF with *D. sissoo* stimulates plant growth under glasshouse conditions, which could be of importance for its survival and growth in natural conditions.

Table 4. Effect of soil type and different microbial inoculation treatments on micronutrient content of dry root & shoot of *D. sissoo*. LSD (P=0.05) value represents the value of interaction of treatments and soil types. The data with same letters in superscript in each nutrient presented in column are not significantly different. M= Arbuscular mycorrhiza, G = *Pseudomonas fluorescens* strain GRPr, and R = *Rhizobium leguminosarum* strain DSP_2.

Treatments	Root								Shoot							
	Sulphur (%)		Zinc (mg kg^{-1})		Manganese (mg kg^{-1})		Copper (mg kg^{-1})		Sulphur (%)		Zinc (mg kg^{-1})		Manganese (mg kg^{-1})		Copper (mg kg^{-1})	
	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol	Mollisol	Entisol
AM (M)	3.35^{h}	1.85^{d}	143.00^{m}	121.00^{i}	17.50^{e}	14.50^{d}	54.00^{e}	44.00^{e}	3.05^{g}	2.60^{f}	104.50^{i}	95.50^{g}	25.95^{h}	23.00^{g}	37.00^{i}	29.00^{g}
GRPr (G)	2.15^{e}	1.15^{b}	91.00^{e}	83.00^{c}	13.00^{cd}	11.10^{bc}	51.00^{d}	39.10^{d}	2.15^{d}	1.80^{bc}	87.00^{e}	82.50^{d}	19.00^{f}	10.70^{cd}	28.00^{f}	25.00^{e}
DSP2 (R)	2.55^{f}	1.25^{c}	91.00^{e}	84.80^{d}	11.10^{bc}	10.00^{b}	46.50^{bc}	36.50^{bc}	1.93^{c}	1.70^{b}	99.00^{h}	92.00^{f}	18.00^{ef}	12.00^{d}	30.75^{h}	26.00^{ef}
G+R	2.65^{f}	1.10^{b}	93.10^{f}	86.10^{d}	12.15^{c}	9.70^{b}	48.00^{c}	38.20^{c}	2.15^{d}	1.76^{bc}	85.95^{e}	81.70^{d}	17.00^{e}	10.06^{c}	27.00^{f}	26.00^{ef}
G+M	2.75^{g}	1.25^{c}	125.10^{j}	101.80^{h}	10.15^{b}	7.75^{a}	46.00^{b}	35.20^{b}	2.28^{e}	1.99^{cd}	80.10^{c}	73.00^{b}	12.00^{d}	8.10^{b}	18.75^{c}	16.75^{b}
R+M	3.25^{g}	1.75^{d}	151.10^{n}	136.20^{l}	19.06^{f}	17.03^{e}	54.10^{e}	44.40^{e}	2.97^{g}	2.10^{d}	109.40^{j}	99.00^{h}	26.70^{h}	18.30^{ef}	37.50^{i}	30.75^{h}
G+R+M	2.15^{e}	1.11^{bc}	126.20^{k}	98.82^{g}	14.00^{d}	10.15^{b}	49.00^{c}	36.20^{c}	2.10^{d}	1.86^{c}	99.10^{n}	92.10^{f}	20.20^{f}	12.06^{d}	29.10^{g}	22.00^{d}
Uninoculated	1.75^{d}	0.87^{a}	78.70^{b}	71.40^{a}	8.50^{ab}	8.00^{a}	25.10^{a}	17.20^{a}	1.86^{c}	1.55^{a}	74.50^{b}	65.50^{a}	10.00^{c}	5.10^{a}	16.00^{b}	13.10^{a}
LSD (P= 0.05)	0.14		1.50		1.50		1.50		0.13		1.50		1.44		1.50	

Table 5. Effect of soil type and different microbial inoculation treatments on photosynthetic rate (μ mole m^{-2} sec^{-1}) and transpiration rate (m mole m^{-2} sec^{-2}) of *D. sissoo* in two soil types. LSD value represents the value of interaction of treatments and soil types. The data containing same letter in superscript in each parameter in column is not significantly different. M= Arbuscular mycorrhiza, G = *Pseudomonas fluorescens* strain GRPr, and R = *Rhizobium leguminosarum* DSP_2.

Treatments	Photosynthetic rate (μ mole m^{-2} sec^{-1})		Transpiration rate (m mole m^{-2} sec^{-2})	
	Mollisol	Entisol	Mollisol	Entisol
AM (M)	4.60^{f}	3.40^{e}	2.80^{d}	1.90^{c}
GRPr (G)	2.75^{d}	2.40^{cd}	1.60^{c}	1.10^{b}
DSP_2 (R)	2.35^{cd}	2.35^{cd}	1.90^{c}	0.91^{ab}
G+R	2.25^{cd}	2.05^{c}	1.40^{bc}	1.03^{ab}
G+M	1.55^{bc}	1.15^{b}	1.20^{bc}	0.72^{a}
R+M	5.05^{f}	3.62^{e}	3.00^{e}	2.50^{d}
G+R+M	3.88^{e}	2.93^{de}	2.12^{d}	1.65^{c}
Uninoculated	0.75^{ab}	0.45^{a}	0.91^{ab}	0.60^{a}
LSD (P=0.05)	0.56		0.45	

In the presence of mycorrhiza-helping bacteria (MHBs), AMF root colonization is enhanced, which promotes plant growth and nutrient uptake (Boer *et al.* 2005). According to Boer *et al.* (2005) the frequency of mycorrhizal colonization achieved with *G. intraradices* alone was about 20%, while co-inoculation with *P. fluorescens*, or *Agrobacterium rhizogenes* enhanced fungal colonization up to 40%, 50% and 60%, respectively. Our results showed a higher degree of root colonization and proliferation of AM hyphae in the plants grown in Entisols relative to those grown in Mollisols when inoculated with AMF alone. Higher root colonization by AMF may be because of lower phosphorus levels in the Entisol in comparison to Mollisol. Inhibition of AMF infection due to higher phosphorus level has been reported by Abbott & Robson (1984). The mycorrhizal infection was lower when the bacterial inoculation GRPr was given in combination with AMF. This shows an antagonistic effect of the rhizobacteria on AMF used in the present study. Ravanskov *et al.* (1999) reported the negative effect of *G. intraradices* on the population size of *P. fluorescens* DF57 both in rhizosphere and hyphosphere soil.

Table 6. Nature of interaction between Mycorrhiza, *Pseudomonas fluorescens* strain GRPr and *Rhizobium leguminosarum* strain DSP_2 in *D. sissoo* under Mollisol.

Parameters	Mycorrhiza (M)	GRPr (G)	*Rhizobium* (R)	G +R+M
Root dry weight (g)	188.57	114.29	100.00	128.57*
Shoot dry weight (g)	158.26	81.19	76.61	106.42*
P in shoot (mg kg^{-1})	52.59	29.87	26.62	20.78*
P in root (mg kg^{-1})	112.87	80.20	74.26	84.16*
N in shoot (mg kg^{-1})	51.14	14.91	45.96	23.81*
N in root (mg kg^{-1})	34.19	17.09	40.68	22.22*
S in shoot (%)	63.98	15.59	3.76	12.90*
S in root (%)	91.43	22.86	45.71	22.86*
Zn in shoot (mg kg^{-1})	40.27	16.78	32.89	33.02*
Zn in root (mg kg^{-1})	81.70	15.63	15.63	66.53*
Mn in shoot (mg kg^{-1})	159.50	90.00	80.00	102.00*
Mn in root (mg kg^{-1})	105.88	52.94	30.59	64.71*
Cu in shoot (mg kg^{-1})	134.38	75.00	92.19	81.88*
Cu in root (mg kg^{-1})	115.14	103.19	85.26	95.22*
Photosynthetic rate (μ mole m^{-2} sec^{-1})	513.33	266.67	213.33	417.33*
Transpiration rate (m mole m^{-2} sec^{-1})	216.67	83.33	51.67	175.00*

* Values showed in interaction of all three microbes are not more than the sum of individual treatment and hence mentioned as the nature of interaction antagonistic.

Table 7. Nature of interaction between Mycorrhiza, *Pseudomonas fluorescens* strain GRPr and *Rhizobium leguminosarum* strain DSP_2 in *D. sissoo* under Entisol.

Parameters	Mycorrhiza (M)	GRPr (G)	*Rhizobium* (R)	G+R+M
Root dry weight (g)	355.00	225.00	200.00	325*
Shoot dry weight (g)	141.62	67.57	59.46	89.19*
P in shoot (mg kg^{-1})	65.82	2.53	25.32	8.86*
P in root (mg kg^{-1})	57.41	4.63	13.89	15.74*
N in shoot (mg kg^{-1})	104.10	18.03	69.65	15.30*
N in root (mg kg^{-1})	49.14	25.54	64.16	74.89*
S in shoot (%)	67.74	16.13	9.68	20.00*
S in root (%)	112.64	32.18	43.68	27.59*
Zn in shoot (mg kg^{-1})	45.80	25.95	40.46	40.61*
Zn in root (mg kg^{-1})	69.47	16.25	18.77	38.38*
Mn in shoot (mg kg^{-1})	350.98	109.80	135.29	136.47*
Mn in root (mg kg^{-1})	81.25	38.75	25.00	26.88*
Cu in shoot (mg kg^{-1})	134.73	90.84	98.47	67.94*
Cu in root (mg kg^{-1})	69.23	53.85	46.15	43.08*
Photosynthetic rate (μ mole m^{-2} sec^{-1})	655.56	433.33	422.22	551.11*
Transpiration rate (m mole m^{-2} sec^{-1})	208.79	75.82	31.87	132.97*

* Value showed in interaction of all three microbes are not more than the sum of individual treatment and hence mentioned as the nature of interaction antagonistic.

Plant response to any microbial treatment is the result of complex interactions with rest of the soil microbial inhabitants. Of these, arbuscular mycorrhizal association is a ubiquitous and normal condition with obvious benefits for most plant species (Bisht 2000). Plant growth responses to pseudomonad inoculation were highly variable and depended on the growth conditions imposed. Schroth & Weinhold (1986) also reported inconsistent growth response by beneficial bacteria. The interaction between pseudomonads and the arbuscular mycorrhizal fungus, *Glomus clarum* NT4 on spring wheat grown under gnotobiotic condition was investigated by Walley & Germida (1997). Although plant growth responses varied, a positive response to pseudomonad inoculants was obtained. Shoot dry weight enhancement ranged from 16 to 48%, whereas enhancement ranged from 82 to 137% for roots. Typically, dual inoculation positively influenced the magnitude of response associated with any organism applied alone (Walley & Germida 1997). However, our results do not support these findings, as the shoot and root dry weights were lower in comparison to the individual inoculation with either of the organisms used. Higher root and shoot weight recorded in the Mollisol relative to the Entisol may be because of textural and nutrient differences between these soils. Higher levels of different nutrients, *viz.* P, N, S, Zn, Mn and Cu, were associated with AMF inoculation, and were further increased by the combination of AMF and DSP_2. This showed a synergistic effects of these two microorganisms. These results are in agreement with the findings of others (Clark & Zeto 2002; Jakobsen 1995; Sharma *et al.* 1994).

Maximum photosynthetic rates in the present investigation were observed in mycorrhizal plants, an effect corroborated by increased root biomass. Since mycorrhizal infection often results in increased allocation of C to the root system, it implies increased root biomass, increased root respiration and mycelial biomass which could explore a larger soil volume for nutrient, consequently resulting in higher uptake rates (Jakobsen 1995). The transpiration rates for plants grown in a Mollisol were higher, which could also explain higher nutrient content in the shoots of plants grown in these soils. According to our results, lower photosynthetic and transpiration rates were observed in combination of GRPr and AMF, with maximum rates observed in plants

dually inoculated with DSP_2 and AMF followed by AM fungal inoculation alone. Changes in transpiration could cause a change in the rate of photosynthesis changing the supply of carbohydrate to the fungus. Alternatively, higher nutrient uptake due to higher transpiration rates could be due to mass flow of nutrients towards the root (Sharma *et al.* 1991, Tables 1-5).

The results of the present study suggest that the bacterial strain used in the investigation i.e. GRPr, though a good biocontrol agent and P solubilizers, has an antagonistic effect on plant growth with AMF. In general, in both the soils, interaction of GRPr and DSP_2 was less effective than their individual inoculation and interaction of GRPr, AM and DSP_2 was found to be antagonistic suggesting that the microorganisms might compete for nutrient, producing nutrient starvation in plants (Johansen & Jensen 1996). Clearly, application of bacterial inoculants would be self-defeating if plant productivity is compromised by antagonistic interactions between the inoculants and potentially beneficial soil microorganisms, such as AMF. Results from this study demonstrate the potential for such negative interactions. Although the mechanisms by which antagonistic interactions occur are not known, it is possible that spore germination is inhibited by pseudomonads. Walley & Germida (1997) observed that plant growth responses to inoculation with AMF and pseudomonads typically were not additive in co-inoculated plants. Moreover, a nonvolatile, diffusible substance or substances produced by pseudomonad strains R85 and R104 was inhibitory to *G. clarum* NT4 spore germination. These observations suggest that substances produced by some pseudomonad inoculants, implicated in the biocontrol of pathogenic fungi, similarly may antagonize AMF. Shastry *et al.* (2000) also documented inhibitory effects of coinoculation of AMF and PGPR, suggesting that microbial components with stimulatory effects when inoculated independently may not always have a positive, synergistic effect on plant growth when coinoculated. Negative effects of *Glomus intraradices* on populations of PGPR *P. fluorescens* DF57 were shown by Ravnskov *et al.* (1999), who suggested that competition for inorganic nutrients might explain the effect, since the mechanism did not require cell-to-cell contact. Marschner *et al.* (1997) suggested that similar negative effects of *Glomus intraradices* on *P. fluorescens* 2-79RL might be due to mycorrhizal-induced decreases in root exudation, affecting the composition of the rhizosphere soil solution.

Conclusions

The present study clearly demonstrated the benefits of arbuscular mycorrhizal fungi and *Rhizobium* for enhancing the growth of *D. sissoo*. However, the combination of both of these bioagents i.e. AMF and DSP_2 with *Pseudomonas fluorescens*, GRPr did not result in synergistic enhancement of the growth of *Dalbergia sissoo.* This might be because of the siderophore production property of *Pseudomonas fluorescens,* GRPr.

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