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Potential Biofertilizer Application for the Enhancement of Tree Legumes

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Abstract: ***Nitrogen is one of the major elements available to plants through biological nitrogen fixation, which has received much attention in recent years. The present study aims at improving tree legumes viz., Albizzia lebbeck, Pithecolobium dulce, Sesbania grandiflora, Albizzia amara, Enterolobium saman, Erythrina indica, Leucaena leucocephala, Acacia mellifera, Pongamia glabra and Acacia auriculiformis through dual inoculation with arbuscular mycorrhizal fungi and suitable rhizobial isolates. Rhizobial isolates viz., AcM05 (from Acacia mellifera), AlL01 (from Albizzia lebbeck), EnS08 (from (Enterolobium saman), LeL02 (from Leucaena leucocephala) and ErI06 (from Erythirina indica) were characterized. Based on the biochemical characterization of rhizobial isolates and dry matter yield of test tree legume species, strain AcM05 and Glomus fasciculatum were scored as the most efficient strain and these two were used to inoculate Acacia mellifera. Dual inoculation with Rhizobium and Glomus fasciculatum increased the nodule nitrogenase activity (36-213%), dry matter yield (156-279%), total nitrogen content (12-159%) and total chlorophyll content of leaves of Acacia mellifera (125-395%) compared to the uninoculated control or single inoculation with either Rhizobium or Glomus fasciculatum alone. The impact of dual inoculation on soil enzymes viz., amylase, chitinase, protease and phosphatase was also studied. Initially the soil had high potassium content (115mg/kg soil) and low nitrogen (14mg/kg soil) and phosphorous (1.1mg/kg soil) content. In contrast, dual inoculation significantly enhanced the nitrogen (60.0mg/Kg soil) and phosphorous (24.3mg/Kg soil) contents of soil, but to a lesser extent with respect to potassium content (155mg/Kg soil). The per cent increase of the activity of soil enzymes amylase, protease, phosphatase and chitinase was more upon dual inoculation. Higher percent mycorrhizal root colonization was found in Acacia mellifera with dual inoculation than compared with plants inoculated with either Rhizobium or Glomus fasciculatum alone. In conclusion, it may be stated that tripartite association not only enhances plant growth but also improves the nutrient status and activities of soil enzymes.***

Keywords: *Glomus fasciculatum, Rhizobium, Acacia mellifera,* tree legumes, soil enzymes

1. Introduction

The symbiotic association between certain plants and microorganisms plays an important role in soil fertilization, and improves their growth and mineral nutrition. Microorganisms implicated in this symbiotic interaction are from two groups: bacteria and fungi. The bacteria group is implicated on nitrogen fixation (Pawlowski and Bisseling, 1996; Platt and Morton, 2012) while the fungal group is involved in the uptake of nutrients with low mobility.

Nitrogen fixing tree legumes provide timber, wood fuel, pulp fodder and even feed of human food. For example, pods of *Prosopis juliflora* are highly nutritive (Dulton, 1992) and leaves, flowers and pods of *Albizzia lebbeck* are sources of carbohydrate and nitrogen (Lowry, 1989).

BNF in legume – *Rhizobium* association demands a steady supply of ATP, it is need to synthesize. Interestingly, majority of the root systems of the legumes are naturally infected with arbuscular mycorrhizal (AM) fungi, which augments efficient uptake of phosphorus from the soil. In legume plants the importance of AMF – symbiosis has been attributed to high phosphorus requirements and enhanced phosphorus uptake. That AM fungi mediated 'P' mobilization has been shown to improve soil fertility in response of P – availability in the soils of the tropics (Dadd, 2000). Mycorrhizal fungi also improve the absorption of nitrogen from ammonical (NH_4^+ - N) mineral fertilizers, transporting it to the host plant (Berendsen *et al.*, 2012). Phosphorus absorption by plants increases plant biomass production in soils with limiting levels of potassium, calcium and magnesium (Liu *et. al.*, 2002).

Soil microorganisms produce quite a number of extra cellular enzymes to decompose the complex organic matter before it is absorbed as a source of energy. Seasonal variations in enzymes activities in forest soils are seen to bear correlation with the counts of fungi and bacteria. Though much has been explored on the interactions of rhizobia for grain legumes and for some tree legumes, little is known as the response of tree legumes.

2. Materials and Methods

The activity of the oxidative enzyme, catalase was determined by the method of Graham and Parker (1964). The salt tolerance was determined with 0.5,2,5 and 10 per cent of sodium chloride. Phosphate solubilization was estimated by the method of Sundara Rao and Sinha (1963) One gram of soil based inoculum containing 180-200 spores and sporocarps of *Glomus fasciculatum* was spread over the lower layer of sterile soil (1.5kg). Then 1kg of sterile soil was layered over the inoculum. Seeds of *Acacia mellifera* obtained from the Oddukkum Seed Centre, Nallampatti, Tamilnadu were surface sterilized with 0.1% $HgCl_2$ and sown in earthen pots containing garden soil and sand (2:1 ratio w/w). Plant growth conditions and *Rhizobium* (Cowpea miscellany isolated from *Acacia mellifera*) inoculation were as described by Rajagopalan and Raju (1972). The plants were watered with sterile tap water and harvested at 45 DAI (Day After Inoculation).

The per cent colonization was measured by the gridline intersect method (Giovannetti & Mosse, 1980). Dry matter yield (plant materials dried to constant weight), total nitrogen content by microkjeldahl method (Umbriet *et al.*, 1972), total chlorophyll content (Arnon, 1949) and nodule

nitrogenase activity by acetylene reduction technique (Stewart *et al*., 1968) were determined.

Total bacterial and fungal population by dilution technique, isolation of AM spores by wet sieving and decanting method (Gerdemann and Nicolson, 1963), determination of nitrogen, phosphorus and potassium content (Jackson, 1973), assay of amylase, chitinase (Skujins, 1976) phosphatase (Tabatabai & Bremner, 1969), protease (Nannipieri *et.al*., 1980) in the soil were carried out. The data was subjected to statistical analysis by using Costat package for one-way ANOVA and Student Newman Kauls test.

3. Result and Discussion

All the isolates were positive to catalase and nitrate reductase activity (Table 1). They were negative to gelatinase and hydrogen sulphide production. Further, all of them grew well at pH 8.0. However, differential growth response was found at pH9.0. Two isolates (LeL02 and EnS08) failed to grow at this pH. Expect LeL02, all the isolates grew well at 5% salt concentration. None of them showed alkali production. All the isolates exhibited phosphate-solubilizing property. Among the phosphate solubilizers, maximum (952.38µg pi/mg protein), phosphate solubilizing property was observed in AcM05 and minimum (212 µg pi/mg protein) inEnS08. Except ErI06, all the isolates showed siderophore producing property. None of the isolates produced catechol type of siderophores. Among the hydraxamate type of siderophore producing isolates, maximum amount of hydraxamate production was observed in AcM05 (547 µg hydraxamate / mg protein) and minimum (286µg hydraxamate / mg protein) in LeL02 (Table 2). The per cent mycorrhizal infection was significantly high in roots of dual inoculated *Acacia mellifera* (80%), as compared with the ones inoculated with *Glomus fasciculatum* alone (64%) (Table 2). These results are in agreement with earlier reports of Manjunath *et al*., (1984). As has been shown (Gupta and Rahangale, 1999), dry matter yield, nodule nitrogenase activity and total nitrogen content of *Acacia mellifera* were in higher order in plants inoculated with *Glomus fasciculatum* or *Rhizobium* or both as compared to the uninoculated control plants. Also, AM fungi in association with nitrogen fixing bacteria increased the plant biomass, nodulation and nitrogen fixation (Pringle *et al*., 2009). Mobilization of P by AM fungi to the host plant could be the reason for better nodulation and nitrogen fixation in dual inoculated plants. Total chlorophyll content in leaves of inoculated plants was higher than the uninoculated control plants (Table 4). This increase in chlorophyll content in inoculated plants is to meet the C requirements of the microsymbionts, since both *Rhizobium* and AM fungi depend on the host for their C requirements (Bakker *et al*., 2013).

Microbial inoculation induced significant changes in soil characteristics (Table 3). Dual inoculation significantly enhanced the N (60.0mg/Kg soil) and P (24.3mg/Kg soil) content of the soil, but to a lesser extent in the case of K (155mg/Kg soil). The results of present study are in conformity with the findings of other workers (Dwivedi *et al*., 2003). Native barren soil had low microbial population. However, the total bacterial (4.7 X 10^7 cfu/ g soil) and fungal (8 X 10^5 cfu/g soil) population and AM spores (256 spores / g soil) in soil increased substantially with dual inoculation. The activities of amylase, protease, chitinase and phosphatase in the soil also increased significantly following microbial inoculation (Table 4). For amylase and protease, the increase was much pronounced with dual inoculation, followed by single inoculation with *Rhizobium* or *Glomus fasiculatum*. For phosphatase and chitinase, the increase was less pronounced with *Rhizobium* inoculation as compared to *Glomus fasciculatum* inoculation. The increased phophatase activity in soil inoculated with *G. fasciculatum* could be the result of hydrolytic cleavage of organic P by the fungus, since a positive correlation has been established between soil enzymes and soil microbial biomass (Tabatabai, 1994).

In conclusion, inoculation of tree legumes species with *Rhizobium* and AM fungus enhanced plant growth by providing a balanced nutrient supply due to their beneficial association with root system of the host plant.

Table 1: Biochemical characteristics of the rhizobial isolates

Biochemical characteristics	Strains				
Acid production on YMA medium	+	+	-	+	+
Catalase activity	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-
Nitrate reductase activity	+	+	+	+	+
Growth at 44^0C	+	-	-	+	+
Utilization of asparagine as nitrogen	+	+	-	+	+
Oxidase test	+	+	+	+	+
α Amylase activity	+	+	+	+	+
Growth at pH	+	-	+	+	+
5	+	+	-	+	+
8	+	+	+	+	+
9	+	-	-	+	+
10	-	-	-	-	-
Salt (NaCl) 0.5%	+	+	+	+	+
2%	+	+	+	+	+
5%	+	-	+	+	+
10%	-	-	-	-	-
Solubilization of Ca_3 Po_4 (µg pi/mg	+ (291.61)	+	+ (212)	+ (385.95)	+ (952.38)
H_2S production	-	-	-	-	-

+ Positive reaction
- negative reaction

Table 2: Siderophore production by Rhizobial isolates

Rhizobial isolates	*$FeCl_3$ Test*	*Absorption maximum in nm (Neilands assay)*	*Arnow's assay for catechol*	*Tetrazolium test for hydroxamate*	*µg hydroxamate/ mg protein*
AlL01	+	445	-	+	428.13
EnS08	+	430	-	+	345
ErI06	+	-	-	-	-
LeL02	+	435	-	+	286
AcM05	+	435	-	+	547

+ Positive reaction - negative reaction

Table 3: Dual inoculation on per cent colonization, nitrogenase activity, dry matter yield, total nitrogen content and total chlorophyll content in leaves of *Acacia mellifera*

Treatments	**Control**	***Rhizobium***	***Glomus fasciculatum***	***R+Glomus fasciculatum***	**LSD P<0.05**
% Colonization	-	-	$65b^{b}$±11	80.3^{c} ± 9	2.717***
Dry weight (g/plant)	0.139^{a}±0.005	0.449^{b}±0.13(223)	0.356^{b}±0.056(156)	0.527^{b} ±0.089 (279)	0.159**
Total nitrogen content (mg N/g dry wt)	20.06^{a}±2.25	40.3^{b}±4.20(101)	22.4^{a}±3.27(12)	51.8^{c}± 3.95 (159)	6.593***
Nitrogenase Activity (n moles C_2H_4 formed /h / g fresh nodules)	3.70^{a}±0.71	9.55^{c}±2.82(157)	5.07^{b}±1.64(36)	11.62^{d}±3.35(213)	0.0266***
Total Chlorophyll content mg Chl/g fresh leaves	1.86^{a}±0.58	5.6^{b}±1.5(201)	4.2^{b}±0.79(125)	9.2^{c}±1.1 (394)	1.982***

± = Standard deviation; values in parenthesis indicate per cent increase over control Values suffixed with different letter on same row indicate significant differences *, **, *** Extent of Significance

Table 4: Impact of dual inoculation on NPK content and enzymes in the rhizosphere soil of *Acacia mellifera* at 45 DAI

	Treatments			
Parameters	*Control*	*Rhizobium*	*Glomus fasciculatum*	*R+Glomus fasciculatum*
Soil N (mg/kg soil)	14 ± 2.51	56 ± 2	52 ± 1.73	60 ± 3.05
Soil P (mg/kg soil)	1.1 ± 0.25	11.3 ± 0.87	8.8 ± 0.47	24.3 ± 1.17
Soil K (mg/kg soil)	115 ± 1.82	145 ± 2.64	140 ± 2.52	155 ± 1.71
Heterotrophic Bacteria (cfu/g soil)	1.1×10^7	2.6×10^7	1.8×10^7	4.7×10^7
Fungi (cfu / g soil)	2×10^5	5×10^5	3×10^5	8×10^5
AM spores Number / g soil	18	28	228	256
Soil amylase (µg starch degraded / h / g soil)	2187.0±123.3	7173.0±219.5(227)	5260.0±428.3(140)	11062.5±929.0(405)
Soil phosphatase (µg PNP formed / h/ g soil)	3348.1±103.4	5649.5±163.3(68)	6250.9±111.1(86)	8507.8±212.4(154)
Soil chitinase (µg glucose liberated/ h / g soil)	391.7±29.3	1315.6±217.5(235)	1372.1±35.03(250)	1555.2±236.6(297)
Soil protease (µg amino acid released / h / g soil)	104.06±6.93	384.3±9.43(269)	296.12±10.73(185)	479.3±16.9(360)

± - Standard deviation
Values in parenthesis indicate per cent increase over control

Reference

[1] Arnon D I **1949.** Plant physiol 24:1-15.
[2] Bakker P., Berendsen RL., Doornbos R F. **2013**. *Front. Plant Sci.*
[3] Berendsen R L., Pieterse C M J., Bakker P A H M. 2012.. *Trends Plant Sci.* 4:1-19.
[4] Dulton, RW. **1992.** *Prosopis* species aspects of their value research and development. CORD,University of Durham, U.K.
[5] Dwivedi, O P. Vyas, D. and Vyas. K M. **2003**. Indian phytopath. 56 (4): 467-469.
[6] Gerdemann, JW. and Nicolson, TH. **1963**. *Trans Brit mycol. Soc.* 46: 235-244.
[7] Graham, PH. and Parker, CA. **1964**. Plant and Soil. 20:383-396.
[8] Gupta, N. and Rahangdale, R. **1999**. Indian J. Expt. Biol. 37: 1005-1011.
[9] Jackson, M L. **1973**. Soil chemical analysis. Prentice Hall of India (Pvt.) Ltd., New Delhi.

[10] Liu, A., Hamel,C., Elmi, A., Costa, C., Ma, B., Smith, DL. **2002**. Can.J.Soil. Sci.82 (2): 271-278.
[11] Nannipieri, P. Ceccanti, B. Cerevelli, S. and Matarease, E. **1980**. Soil Sci. Soc. Am. J. 44: 1011 – 1016.
[12] Platt TG., Morton E R. **2012.** *Annu. Rev. Microbiol.* 66 265–283.
[13] Pringle A., Bever J D., Gardes M., Parrent J L., Rillig M C., Klironomos J N. **2009**. *Annu. Rev. Ecol. Evol. Syst.* 40 699–715
[14] Pawlowski and K.,Bisseling,T.**1996**. Plant Cell. 8: 281-315.
[15] Rajagopalan, N. and Raju, P. N**. 1972.** Phytopath. Z 73: 285-3-9.
[16] Skujins, J. **1976.** Critical Rev. Microb. **4**:383 – 421.
[17] Stewart, D W D P. Fitzgerald, G P. and Burris, R H. **1968.** Arch. Microbiol. 62: 336-348.
[18] Tabatabai, M A. and Bremner, J M. **1969**. Soil. Biol. Biochem. 1: 301 – 307.
[19] Tabatabai, MA. **1994.** *Methods of soil Analysis Part 2*. Microbiological and Biochemical properties. ed : Weaver, RW., Angle, S., Bezdicek, D., Smith, S., Tabatabai, M A. and Wollum, A. Soil Science society of America Book Series, No.5. pp. 775-834.
[20] Umbriet, W. W. Burris, R. H. and Stauffer, J. F. **1972**. Manometric and Bio-chemical techniques 5th Ed: Burgess publishing company. Minnesota, pp. 259-260.