Isolation, characterization and evaluation of tree legume rhizobia

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ABSTRACT: Tree legumes contribute much to global nitrogen fixation. 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 isolation and characterization of tree legume rhizobia and evaluation of tree legume rhizobia on the basis of dry matter yield, nodule dry weight, total nitrogen content and total chlorophyll content of the leaves. Rhizobial isolates viz., AlL01 (from Albizzia lebbeck), PiD07 (from Pithecolobium dulce), SeG01 (from Sesbania grandiflora), AlA02 (from Albizzia amara), EnS08 (from Enterolobium saman), ErI06 (from Erythrina indica), LeL0 (from Leucaena leucocephala,), AcM05 (from Acacia mellifera), PoG01 (from Pongamia glabra) and AcA04 (from Acacia auriculiformis) and were characterized. AcM05 (from Acacia mellifera) was the most efficient strain.
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Key words: Rhizobia, tree legume, nitrogen fixation, bio chemical characterization, dry matter yield.

INTRODUCTION

It has been established in agriculture that nitrogen is the most important and major plant nutrient that decides the success in crop productivity. Crop fails to produce enhanced yield, if nitrogen becomes a limiting factor. In general, farmers apply a substantial quantity of nitrogenous chemical fertilizers to increase the productivity of crop plants. Unfortunately, the cost of production and distribution of nitrogenous fertilizers are increasing day by day, since it involves substantial quantities of fossil fuel (hydrocarbon) as energy source. Poor and marginal farmers are ill afforded to purchase fertilizers for crops. Therefore, scientists and policy makers have turned their attention to alternate sources of this important plant nutrient. This would hopefully reduce the cost of application of chemical fertilizers and hence the cost of the crop. Biological nitrogen fixation (BNF) appears surely an alternate technology to circumvent the fertilizers crisis. For India and other developing countries, biological nitrogen fixation offers great scope in the agricultural sector (Rao, 2002).

It is surprising to find that nearly 75% of the total fixed nitrogen comes from biological fixation. It is still more surprising to realize that leguminous plants contribute much to biological nitrogen fixation. Legumes are naturally endowed with the ability of trapping elemental nitrogen from the atmosphere. Each root nodule of legumes functions as a mini nitrogen-fixing factory. Legume-*Rhizobium* symbiosis is responsible for an estimated 180 X 10^6 tonnes of biological nitrogen fixation per year worldwide (Postgate, 1998).

Nitrogen fixing tree legumes occupy a pivotal place in afforestation programmes since these trees require a little nitrogenous fertilizer as they obtain their nitrogen requirements through biological nitrogen fixation. In addition to enhancing soil fertility through nitrogen fixation, 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).

The symbiotic bacteria producing nodules in the root system of legumes are classified into six genera *viz.*, *Rhizobium, Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium,* and *Sinorhizobium*. The success of a tree legume harvesting nitrogen from the atmosphere largely depends on the selection and evaluation of the rhizobial strain that matches with its host. Screening of the microsymbiont (bacteria) is often done based on the host compatibility, nitrogen fixing efficiency, ability to

synthesize phytohormones and siderophores or other substances influencing plant growth (Giller and Wilson, 1991). The present study is aimed to isolation, characterization and evaluation of tree legume rhizobia.

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% of sodium chloride such plates was inoculated with the rhizobial isolates. Phosphate solubilization was estimated by (Sundara Rao and Sinha, 1963), bacteriocin production by (Schillinger and Lucke, 1989; Venema *et al.*, 1993), siderophore production by (Neilands, 1981), and antibiotic sensitivity test was determined by the method of Bauer *et al.*, (1966).

POT CULTURE STUDIES

Seeds of *Albizzia lebbeck*, *Pithecolobium dulce*, *Sesbania grandiflora*, *Albizzia amara*, *Enterolobium saman*, *Erythrina indica*, *Leucaena leucocephala*, *Acacia mellifera*, *Pongamia glabra* and *Acacia auriculiformis* were 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 rhizobia (Cowpea miscellany) inoculation were as described by Rajagopalan and Raju (1972). The plants were watered with sterile tap water and harvested at 45 DAI.

Nitrogenase activity was assayed by using the acetylene reduction technique (Stewart *et. al.*, 1968). The chlorophyll content of leaf tissue was estimated following the method of Arnon (1949). Dry matter yield (plant materials dried to constant weight), total nitrogen content by microkjeldahl method (Umbriet *et al*., 1972).

RESULT AND DISCUSSION

Growth and phenotypic features can be used to distinguish fast-and slow-growing rhizobia. Growth at 44^{o}C indicated that only six out of ten rhizobial isolates were able to tolerate this temperature. Variation in temperature tolerance by *Rhizobium* strains has been reported earlier (Khokhar *et al.*, 2001). The results on phenotypic characters (Table 1) showed that all the isolates were positive to catalase, nitrate reductase, oxidase and α-amylase production. None of the isolates produced gelatinase and H_2S (Table 1). These results are in agreement with the report of Graham and Parker (1964) and indicate that the isolates are more related to fast – growing rhizobia. The results on pH and salt tolerance showed that most of the isolates showed similarities with fast – growers. It has been reported that among the rhizobia, tolerance to 2% NaCl is restricted to the fast – growing species of *Rhizobium meliloti* (Graham and Parker, 1964; Zerhari *et al*., 2000). Furthermore, most of the isolates produced acid and hence they are fast – growers. Acid production by fast growing rhizobia has been found by earlier investigators (Graham and Parker, 1964; Vincent, 1974). As has been shown by earlier reports (Halder, *et al*., 1990), phosphate soubilizing property of the isolates of this study correlated with acid production.

The results presented in Table 2 showed that six out of ten isolates were able to produce hydroxamate type of sidierophores. Siderophore producing isolates of *R. meliloti* have been found to control soil and seed – borne diseases caused by *Macrophomia phaseolina* (Arora *et al.*, 2001) (Table 2).

Antibiotic resistance in microbes has been successfully used to screen the competitive ability of elite strains with the indigenous strains (Brockwell *et al*., 1976) (Table 2). All the ten isolates of this study were of different identity, since each isolate had a unique innate antibiotic resistance pattern.

Five isolates (AlL01, EnS08, ErI06, LeL02, and AcM05) were able to produce nodules in the all the ten tree – legume species, in contrast to narrow host – range for the isolate AlA02 (Table 3). Legume – rhizobia interactions exhibit specificity where by rhizobial strains nodulate a limited range of legumes. For example, the African tree legume *Faridherbia albida* nodulated only with *Bradyrhizobium* (Drefus and Dommergues, 1981) However, *Rhizobium* strain NGR 234 is reported to form nodules on a wide range of host legume species including *Leucaena leucocephala* (Trinick, 1980). Roughley, (1987) showed that some species of Australian *Acacia* are very specific, nodulating with a few strains isolated from those species, while other nodulated freely with strains isolated from a wide range of *Acacia* spp. Rao, (2002) has classified nitrogen fixing tree legume species into three groups according to nodulation response with fast - and slow – growing tropical strains of rhizobia. They are (i) tree legume species that nodulate with fast growing strains (2) tree legume species that nodulate with slow-growing strains and (3) tree legume species that nodulate with fast-and slow-growing strains. According to Sprent (2000), nod factors produced by rhizobia determine the host - specificity. Thus, the differences in responses of tree legume species to different rhizobial isolates reported here could be the result of differences in nod factors of the isolates tested.

The results showed AcM05 induced higher nodule dry matter weight (Table 3), biomass accumulation (Table 4), total nitrogen content (table 5), and photosynthetic pigment concentration (Table 6) as compared to the uninoculated control or single inoculation with other rhizobial isolates. Bremner *et al.*, (1990) found the usefulness of plant biomass and nitrogen accumulation for determining the efficiency of *R. leguminosarum* strain. Thus it appears that AcM05 is the most efficient strain on the basis of above parameters. In conclusion, inoculation of tree legumes species with rhizobial isolates 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 cowpea rhizobial isolates

Characteristics	Cowpea rhizobial isolates									
	AlL01	**PiD07**	**SeG01**	**AlA02**	**EnS 08**	**ErI06**	**LeL02**	**AcM05**	**PoG01**	**AcA04**
Acid production on YM broth	+	+	+	-	-	+	+	+	-	+
Catalase activity	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-
Nitrate reductase activity	+	+	+	+	+	+	+	+	+	+
Growth at 44^0C	+	+	+	-	-	+	-	+	-	+
Utilization of asparagine as nitrogen source	+	+	+	-	-	+	+	+	-	+
Oxidase test	+	+	+	+	+	+	+	+	+	+
α amylase activity	+	+	+	+	+	+	+	+	+	+
Growth at different pH levels pH 4	+	+	+	-	+	+	+	+	+	+
pH 5	+	+	+	-	+	+	+	+	+	+
pH 8	+	+	+	+	+	+	+	+	+	+
pH 9	+	+	+	-	-	+	-	+	-	+
pH 10	-	-	-	-	-	-	-	-	-	-
Salt tolerance (NaCl) 0.5%	+	+	+	+	+	+	+	+	+	+
2%	+	+	+	+	+	+	+	+	+	+
5%	+	+	-	+	+	+	-	+	-	-
10%	-	-	+	-	-	-	-	-	-	-
Solubilization of Ca_3 Po_4 zone clearing(µg pi/mg protein)	+ (291.61)	Nil	+ (209.52)	Nil	+ (212)	+ (385.95)	+ (285)	+ (952.38)	Nil	+ (317.46)
H_2S production	-	-	-	-	-	-	-	-	-	-

+ Positive reaction
- Negative reaction

Table 2. siderophore production by cowpea rhizobial isolates and intrinsic antibiotic resistance

Antibiotics (100 µg/ml)	cowpea rhizobial isolates **Inhibition zone diameter (cm)**									
	AlL01	**PiD07**	**SeG01**	**AlA02**	**EnS08**	**ErI06**	**LeL02**	**AcM05**	**PoG01**	**AcA04**
Ampicillin	+	+	+	+	+	(1.2)	+	+	+	+
Amoxicillin	(1.0)	+	(1.1)	(0.2)	(1.8)	(0.6)	+	(0.6)	(1.5)	+
Chephaloxin	(0.5)	+	(0.6)	(0.6)	+	+	+	+	(1.8)	(1.0)
Tetracycline	+	+	(0.7)	(0.7)	(0.5)	(0.8)	(1.2)	(1.0)	(0.6)	(0.6)
Chloramphenicol	(0.5)	(0.6)	+	+	+	(0.4)	+	(0.5)	+	+
Gentamycin	(1.0)	+	(0.6)	(1.2)	+	(1.2)	+	(0.9)	(0.8)	(1.4)
Siderophore production $FeCl_3$ Test	+	-	-	+	+	-	+	+	-	+
Absorption maximum in nm (Neilands assay)	445	445	-	-	430	-	435	435	-	445
Arnow's assay for catechol	-	-	-	-	-	-	-	-	-	-
Tetrazolium test for hydroxamate	+	+	-	-	+	-	+	+	-	+
µg hydroxamate / mg protein	428.13	218.7	-	-	345	-	286	547	-	688

+ Indicates positive reaction – Indicates negative reaction
+ Resistant to antibiotics

Table 3. Effect of cowpea *Rhizobium* inoculation on nodule dry weight in tree – legume species at 45 DAI

Plant Species	g/ nodule dry weight											
	Control	**AlL01**	**PiD07**	**SeG01**	**AlA02**	**EnS08**	**ErI06**	**LeL02**	**AcM05**	**PoG01**	**AcA04**	**F Value**
Albizzia lebbeck	0 ^{a}	0.086 ^{c}	0 ^{a}	0.034 ^{ab}	0.04 ^{b}	0.037 ^{ab}	0.028 ^{ab}	0.046 ^{b}	0.11 ^{c}	0.033 ^{ab}	0.037 ^{ab}	14.36***
Pithecolobium dulce	0 ^{a}	0.055 ^{bcd}	0.062 ^{cd}	0.023 ^{abc}	0.017 ^{ab}	0.030 ^{abc}	0.029 ^{abc}	0.030 ^{abc}	0.071 ^{d}	0 ^{a}	0.038 ^{abcd}	6.42***
Sesbania grandiflora	0 ^{a}	0.063 ^{cd}	0.032 ^{bc}	0.034 ^{bc}	0 ^{a}	0.041 ^{bc}	0.038 ^{bc}	0.044 ^{bc}	0.077 ^{d}	0.026 ^{ab}	0.027 ^{ab}	9.21***
Albizzia amara	0 ^{a}	0.035 ^{e}	0.026 ^{d}	0 ^{a}	0.055 ^{f}	0.027 ^{d}	0.023 ^{cd}	0.030 ^{de}	0.063 ^{g}	0.011 ^{b}	0.018 ^{c}	84.86***
Enterolobium saman	0 ^{a}	0.043 ^{d}	0.03 ^{bcd}	0.037 ^{bcd}	0 ^{a}	0.106 ^{f}	0.03 ^{bcd}	0.039 ^{cd}	0.091 ^{e}	0.019 ^{b}	0.021 ^{bc}	57.43***
Erythrina indica	0 ^{a}	0.058 ^{bc}	0.05 ^{bc}	0.058 ^{bc}	0.053 ^{bc}	0.067 ^{bc}	0.081 ^{bc}	0.068 ^{bc}	0.085 ^{c}	0.055 ^{bc}	0.030 ^{ab}	4.94***
Leucaena leucocephala	0 ^{a}	0.036 ^{cd}	0.026 ^{bc}	0.033 ^{bc}	0.019 ^{b}	0.037 ^{cd}	0.038 ^{cd}	0.053 ^{d}	0.067 ^{e}	0 ^{a}	0.018 ^{b}	23.20***
Acacia mellifera	0 ^{a}	0.053 ^{c}	0.022 ^{b}	0.024 ^{b}	0.023 ^{b}	0.031 ^{bc}	0.035 ^{bc}	0.043 ^{bc}	0.101 ^{d}	0.032 ^{bc}	0.051 ^{c}	22.94***
Pongamia glabra	0 ^{a}	0.078 ^{cd}	0.044 ^{b}	0.102 ^{e}	0 ^{a}	0.075 ^{cd}	0.033 ^{b}	0.068 ^{c}	0.105 ^{e}	0.096 ^{de}	0 ^{a}	42.85***
Acacia auriculiformis	0 ^{a}	0.021 ^{b}	0 ^{a}	0 ^{a}	0 ^{a}	0.021 ^{b}	0.015 ^{b}	0.019 ^{b}	0.075 ^{c}	0 ^{a}	0.066 ^{c}	81.63***

Values suffixed with different letter on same row indicate significant difference *, **, *** = Extent of Significance LSD($P < 0.05$).

Table 4. Effect of cowpea *Rhizobium* inoculation on biomass accumulation in tree – legume species at 45 DAI

Plants Species	(g/plant)											
	Control	**AlL01**	**PiD07**	**SeG01**	**AlA02**	**EnS08**	**ErI06**	**LeL02**	**AcM05**	**PoG01**	**AcA04**	**F Value**
Albizzia lebbeck	0.157^{a}	0.218^{bc}	0.172^{ab}	0.161^{ab}	0.167^{ab}	0.183^{ab}	0.179^{ab}	0.194^{ab}	0.250^{c}	0.176^{ab}	0.170^{ab}	5.69***
Pithecolobium dulce	0.161^{a}	0.223^{c}	0.201^{bc}	0.192^{abc}	0.169^{ab}	0.204^{bc}	0.211^{c}	0.219^{c}	0.313^{d}	0.171^{abz}	0.176^{ab}	26.14***
Sesbania grantiflora	0.139^{a}	0.245^{b}	0.337^{c}	0.184^{ab}	0.194^{ab}	0.190^{ab}	0.202^{ab}	0.213^{ab}	0.185^{ab}	0.208^{ab}	0.205^{ab}	7.202 ***
Albizzia amara	0.146^{a}	0.279^{c}	0.211^{ab}	0.178^{ab}	0.238^{bc}	0.236^{bc}	0.192^{ab}	0.276^{c}	0.287^{c}	0.201^{ab}	0.152^{a}	9.85***
Enterolobium saman	0.128^{a}	0.265^{e}	0.176^{abc}	0.165^{ab}	0.228^{cde}	0.257^{de}	0.224^{cde}	0.242^{de}	0.271^{e}	0.154^{ab}	0.201^{bcd}	11.62***
Erythrina indica	0.163^{a}	0.303^{d}	0.171^{ab}	0.204^{abc}	0.212^{abc}	0.240^{c}	0.229^{b}	0.291^{d}	0.466^{e}	0.170^{ab}	0.218^{bc}	48.96***
Leucaena leucocephala	0.150^{a}	0.234^{bc}	0.207^{abc}	0.183^{ab}	0.205^{abc}	0.217^{abc}	0.212^{abc}	0.220^{abc}	0.269^{c}	0.186^{ab}	0.195^{abc}	3.64**
Acacia mellifera	0.116^{a}	0.311^{c}	0.255^{bc}	0.259^{bc}	0.277^{c}	0.294^{c}	0.286^{c}	0.303^{c}	0.373^{d}	0.224^{b}	0.263^{bc}	27.61***
Pongamia glabra	0.117^{a}	0.225^{b}	0.181^{b}	0.210^{b}	0.178^{b}	0.208^{b}	0.194^{b}	0.209^{b}	0.232^{b}	0.178^{b}	0.180^{b}	4.43**
Acacia auriculiformis	0.093^{a}	0.248^{d}	0.178^{bcd}	0.151^{abc}	0.124^{ab}	0.215^{cd}	0.217^{cd}	0.219^{cd}	0.254^{d}	0.209^{cd}	0.203^{cd}	9.32 ***

Values suffixed with different letter on same row indicate significant difference
*, **, *** = Extent of Significance LSD(P < 0.05).

Table 5. Effect of cowpea *Rhizobium* inoculation on total nitrogen content in tree – legume species at 45 DAI

Plant Species	mg N / g dry weight											
	Control	**AlL01**	**PiD07**	**SeG01**	**AlA02**	**EnS08**	**ErI06**	**LeL02**	**AcM05**	**PoG01**	**AcA04**	**F Value**
Albizzia lebbeck	8.33^{a}	41.13^{f}	15.2^{b}	22.93^{c}	23.53^{cd}	30.9^{de}	32.2^{de}	34^{e}	44.7^{f}	24.2^{c}	27.53^{cd}	63.46***
Pithecolobium dulce	8.2^{a}	25.86^{cd}	28.46^{d}	22.46^{c}	12.06^{ab}	27.2^{d}	29^{d}	12.6^{b}	30.3^{d}	10.06^{ab}	23^{c}	59.29***
Sesbania grandiflora	9.36^{a}	33.9^{e}	18.6^{b}	19.06^{b}	12.93^{a}	22.2^{bc}	25.06^{cd}	28.13^{d}	35.93^{e}	19.9^{bc}	20.8^{bc}	34.78***
Albizzia amara	13.36^{a}	39.7^{d}	21.73^{b}	23.8^{b}	22.4^{b}	28.26^{bc}	37.4^{d}	24.7^{b}	47.6^{e}	19.8^{b}	33.6^{cd}	25.91***

Enterolobium saman	6.5 [a]	22.3 [c]	21.2[c]	20.6 [c]	23.3 [c]	29.0[de]	25.06[cd]	11.46 [b]	31.0[e]	19.0[c]	21.86 [c]	21.64***
Erythrina indica	14.7 [a]	37.6[c]	24.0 [b]	25.6 [b]	25.3 [b]	30.40 [b]	45.7[d]	29.6[b]	47.8 [d]	22 [b]	27.4[b]	27.16***
Leucaena leucocephala	7.46[a]	24.6[e]	13.53[bc]	20.6[d]	16[c]	25.26[e]	29.4[f]	31.06[f]	34.26[g]	11.26[b]	22.53[de]	67.33***
Acacia mellifera	6.2 [a]	34.3[ef]	11.93 [b]	13.3 [b]	19.8 [c]	30.4 [e]	25.13 [d]	11.73[b]	35.26[f]	10.7 [b]	33.5[ef]	79.87***
Pongamia glabra	8.6 [a]	29.20[d]	22.06 [b]	23.0[bc]	12.8[a]	27.00 [cd]	30.86[de]	27.60 [cd]	33.86[e]	23.73[bc]	12.06 [a]	46.99***
Acacia auriculiformis	13.9 [a]	27.8 [d]	21.4 [ab]	20.86 [ab]	24.8 [ab]	27.46 [b]	41.6 [c]	40.63 [c]	55.06 [d]	23.53 [b]	27 [b]	39.19***

Values suffixed with different letter on same row indicate significant difference; *, **, *** = Extent of Significance LSD(P < 0.05).

Table 6. Effect of cowpea *Rhizobium* inoculation on chlorophyll content in leaves of tree – legume species at 45 DAI

Plants Species	**mg chl / g fresh leaves**											
	Control	**AlL01**	**PiD07**	**SeG01**	**AlA02**	**EnS08**	**ErI06**	**LeL02**	**AcM05**	**PoG01**	**AcA04**	**F Value**
Albizzia lebbeck	1.63[a]	5.5[b]	5.0[ab]	4.06[ab]	3.93[ab]	5.06[ab]	5.03[ab]	5.13 [ab]	6.3[b]	2.83[ab]	3.76 [ab]	3.25*
Pithecolobium dulce	2.02 [a]	5.9 [d]	4.03[abcd]	2.8 [ab]	3.2 [abc]	4.96 [bcd]	4.9 [bcd]	5.43[cd]	6.16 [d]	2.6 [ab]	2.53 [ab]	6.83***
Sesbania grandiflora	1.7 [a]	4.23 [bc]	4.53[bc]	4.56 [bc]	5.9 [bc]	6.43[c]	6.3[c]	3.8[b]	4.9[bc]	5.86[bc]	3.56[b]	7.77***
Albizzia amara	2.5 [a]	6.7 [c]	4.16 [abc]	4.36[abc]	4.53 [abc]	4.76 [ab]	4.6 [abc]	6.36 [bc]	6.80 [c]	3.7 [ab]	3.2 [a]	5.27***
Enterolobium saman	0.96 [a]	5.7 [b]	4.96 [b]	4.53 [ab]	4.36 [ab]	5.3[ab]	5.23 [ab]	5.46 [ab]	6.06 [b]	3.06 [ab]	4.1 [ab]	2.40*
Erythrina indica	2.1[a]	6.03 [bc]	4.23 [abc]	3.26 [ab]	3.5 [abc]	5.13[bc]	5.06 [bc]	5.76 [bc]	6.33 [c]	3.7 [abc]	3.70 [abc]	4.91***
Leucaena leucocephala	1.43 [a]	5.70[de]	3.73 [bc]	3.16[ab]	3.0[ab]	6.03[de]	4.5[bcd]	5.46[cde]	7.23[e]	2.83[ab]	2.86[ab]	12.82***
Acacia mellifera	0.93 [a]	6.26 [de]	2.93 [abc]	4.86 [bcde]	3.83 [abcd]	5.63 [bcde]	5.56 [bcde]	6.03 [cde]	7.63 [e]	2.7 [ab]	3.3 [abcd]	7.65***
Pongamia glabra	1.53[a]	6.1[e]	3.83[bcd]	2.8 [ab]	3.53 [abcd]	5.8[de]	5.7[de]	5.13 [cde]	6.53 [e]	3.9[bcd]	3.10 [abc]	9.57***
Acacia auriculiformis	1.1[a]	5.5 [bc]	5 [bc]	3.2[abc]	3.16 [abc]	5.06 [bc]	5.03 [bc]	5.13[bc]	6.3[c]	2.83 [ab]	3.76 [abc]	4.85***

Values suffixed with different letter on same row indicate significant difference
*, **, *** = Extent of Significance LSD(P < 0.05).

RESUMO

Tree legumes contribute much to global nitrogen fixation. 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 isolation and characterization of tree legume rhizobia and evaluation of tree legume rhizobia on the basis of dry matter yield, nodule dry weight, total nitrogen content and total chlorophyll content of the leaves. Rhizobial isolates viz., AlL01 (from Albizzia lebbeck), PiD07 (from Pithecolobium dulce), SeG01 (from Sesbania grandiflora), AlA02 (from Albizzia amara), EnS08 (from Enterolobium saman), ErI06 (from Erythrina indica), LeL0 (from Leucaena leucocephala,), AcM05 (from Acacia mellifera), PoG01 (from Pongamia glabra) and AcA04 (from Acacia auriculiformis) and were characterized. AcM05 (from Acacia mellifera) was the most efficient strain.

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