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Studies on association of arbuscular mycorrhizal fungi with *gluconacetobacter diazotrophicus* and its effect on improvement of *sorghum bicolor* (L.)

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ABSTRACT

Considerable attention has been paid on endophytic diazotrophs in recent times, because of its of ability to fix and transfer fixed nitrogen to the host plant. Arbuscular mycorrhizal (AM) fungi are ubiquitous and play a significant role in improving the growth of plants through better uptake of nutrients, especially phosphorus. Interaction between AM fungi and nitrogen fixing bacteria and its impact on the host plant has been studied in several instances. In the present study, an attempt has been made to know the combining ability of *G.diazotrophicus* with AM fungi on *S.bicolor.* Spores of ten species of AM fungi were isolated from the rhizosphere soils of S.bicolor from different localities of Madurai and Sivagangai districts of Tamil Nadu. *G.diazotrophicus* was isolated from stem tissues of sugarcane (Saccharrum officinarum L.) from Madurai districts. The AM fungi in association with *G.diazotrophicus* were evaluated on the basis of root colonization, fresh and drymatter yield, N, P, soluble sugars and photosynthetic pigments in leaves of *S.bicolor.* Fresh weight and dry weight was significantly higher in dual inoculated plants. The highest values were recorded with *Glomus fasciculatum* + *G.diazotrophicus* combination. AM fungal infection was significantly higher in dual inoculated plants. N concentration was significantly increased by *G.diazotrophicus* even more in association with the efficient fungal strains. Dual inoculated plants showed a significant increase in P, soluble sugars, photosynthetic pigments in leaves was observed in *G.diazotrophicus* + *Glomus fasciculatum* combination. Such morphological modification may enhance water and nutrient uptake. Our results confirm the importance of studying plant-microbial interrelationship to provide useful information for agricultural system management.

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1. Introduction

Sorghum bicolor (L.) Monech (Sweet sorghum) is used as forage or an intercalary cereal crop in rotation with winter cereals. In India and southern Tamil Nadu sorghum is cultivated on arid and marginal agricultural areas as substitute of maize. At present, the optimization of biomass yields is obtained by chemical fertilization [1]. To decrease production costs and pollution risks, the inoculation of plants with useful microorganisms is attempted.

Among rhizosphere microorganisms, Arbuscular mycorrhizal (AM) fungi are ubiquitous in distribution and occur on almost all vascular plants [2] .The increasing body of evidence for the sustainable beneficial effect of AM fungi on crop plants and the growing global awareness of sustainable food productivity encourages the use of these fungi as a biotechnological tool. The beneficial effect of inoculating crop plants with AM fungi for improving plant growth is well documented [3]. In general, diazotrophic bacteria play a major role in the nitrogen cycle. Most reports consider N fixing bacterial symbiosis with leguminous plants; several studies, however considered also the influence of diazotrophs, and the relationship between biological nitrogen fixation and sustainable agriculture [4].

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Gluconacetobacter diazotrophicus is an endophytic bacterium first isolated from the sugarcane growing regions of Brazil [5]. It was widely studied and used as a model system to assess the bacterial endophyte- plant interactions. After its first discovery, it was reported from variety of crops viz, coffee [6, 7, 8] and a latest report states *Gluconactobacter sp* as a natural colonizer of the wild rice (Porteresia coarctata Tateoka, formerly Oryza coarctata Roxb.) and a salt tolerant pokali rice variety [9] . These reports clearly indicated the wide occurrence of *G.diazotrophicus* in different plants than initially expected. *G.diazotrophicus* may fix atmospheric nitrogen in the presence of nitrate [10]. Such incomplete inhibition of nitrogen fixation is of ecological and agronomic relevance, since it may allow the complementation of biological nitrogen fixation in the presence of other N sources. This aspect may favour the re-cultivation of agronomic lands, following a prolonged period of chemical fertilization, alleviating immobilization of N by soil microorganisms.

In recent times, attention has been focused on dual inoculation involving AM fungi and nitrogen fixing bacteria on the growth of several crop plants [11]. Positive effects of dual colonization of non-leguminous plant roots by AM fungi and diazotrophic bacteria were investigated on sugarcane [12] and rice [13]. Dual inoculation could be especially advantageous in the case of *G.diazotrophicus,* since this bacterium has not been isolated from soil and the bacteria are mainly transmitted from plant to plant through vegetative propogation by stem pieces [5]. Investigations have shown that *G.diazotrophicus* may also be introduced in to sterile micropropgated sugarcane, sweet potato and sweet sorghum seedlings via arbuscular mycorrhizae [14, 15] . Inoculation of *G.diazotrophicus* with or inside AM spores allowed this bacterium to penetrate and to colonize the roots of plants and then passing to the aerial tissue. Inoculation of AM fungi seems therefore to be an essential condition for colonization of whole plants by nitrogen fixing bacteria.

Selection of AM fungal strains for the improvement of crop yields and diazotrophs efficiency should therefore consider inter symbiotic compatibility besides host-plant compatibility, in order to avoid unsuccessful field inoculations. In this paper an attempt is made to identify AM fungi compatible to *G.diazotrophicus* by an interaction study involving ten species of AM fungi and *G.diazotrophicus* on Sorghum bicolor, a sugar rich crop widely cultivated in India.

2. Materials and methods

2.1.Location of the sampling sites

Soil samples were collected from rhizosphere of Sorghum bicolor grown in different localities of south Tamilnadu viz Dindigul (Dindigul district), Kannivadi and Kovilpatti (Virudhunagar district), Paravai and Samanatham (Madurai district) for isolation of AM fungi and *Saccharum officinarum* from Madurai district for isolation of *G.diazotrophicus.*

2.2.Determination of soil characters

Soil pH was determined in the soil: water 1:1 ratio soon after bringing the soil samples to the laboratory. The total nitrogen (N), Electrical conductivity (EC) and total phosphorus (P), exchangeable potassium (K) were determined according to [16].

2.3.Media and cultural conditions

N-free semisolid LG1 medium supplemented with 0.5% sugarcane juice at pH 4.5 and cycloheximide (150 mgl-1) was used for enrichment culturing of N2-fixing *G.diazotrophicus.* For isolation and culturing, acetic acid LG1 agar plates supplemented with yeast extract (50mgl-1) and cyclohexamide (150mgl-1) and potato agar plates with 10% cane sugar were used [5].

2.4.Isolation of G.diazotrophicus

Stem of the selected plants were washed with tap water and the bud roots were exposed by removing the oldest leaves. The stems were washed with sterile distilled water and surface sterilized for 5 min with 5% calcium hypochlorite, and then washed five times with sterile distilled water. Thereafter, the samples were weighed and homogenized in a sterile sucrose solution using a sterile using pestle and mortar. Aliquots (500µl) were inoculated in to semisolid LG1 and incubated at 30°C for 4-6 days. Yellowish bacterial growth from the tubes was streaked on to LG1 plates [5] and incubated at 30°C for 5-6 days. The colony morphology was compared with *G.diazotrophicus* type strain PAL5.

2.5.Isolation of AM fungi

AM fungal spores were isolated from rhizosphere soils of S.bicolor by a modification of the wet sieving and decanting technique [17]. Aliquots (100g) of soil sample were dispersed in 1:1 water and the suspension left undisturbed for 15 min to allow soil particles to settle. The suspension was then decanted through 710-and 38µm sieves. The sievates were dispersed in water and filtered through gridded filter papers. Each filter paper was then spread on a petri dish and scanned under a dissection microscope at 40 X magnification and all intact spores (non-collapsed spores with cytoplasmic contents and free from parasitic attack) were counted. Sporocarps and spores clusters were considered as one unit. Intact AM fungal spores were transferred using a wet needle to polyvinyl alcohol-lactoglycerol with or without Melzers reagent on a glass slide for identification. Spores were identified from spore morphology and subcellular characters and compared to original descriptions [18]. Spore morphology was also compared to the culture database established by INVAM (http//invam.cag.wvu.edu).

2.6.Estimates of AM fungal colonization

The AM infected roots were cut into 1-cm fragments, cleared in 2.5% KOH [19] acidified with 5N HCL and stained with trypan blue (0.05% in lactophenol). Roots that remained dark after clearing were bleached in alkaline H2O2 prior to acidification. The stained roots were examined with a compound microscope (200-400X) for AM fungal structures and the percentage of root colonization was estimated according to a magnified intersection method [20].

2.7.Experimental design

Sweet sorghum (Sorghum bicolor) seeds obtained from var Co27 from Tamil Nadu seed testing centre, Madurai were sterilized with H2O2 for one hour, and germinated under aseptic conditions in steam-sterilized sand and soil mixture (twice at 120ºC for 30 min, with an interval of 24h between both autoclaving). Ten seeds after germination, were transplanted in 4:

1 pots (four seedlings/ per pot) in to sterilized soil and sand mixtures. Each seedling received 50g of mycorrhizal inoculum and 5ml of bacterial suspension. Twenty two treatments (three pots each) resulting from different combinations of G.diazotrophicus with ten species of AM fungi were established as follows: 1, Control (without inoculation)2, *Entrophospora infrequens* 3, *Gigaspora albida* 4, *Glomus albida* 5, *Glomus dimorphicum* 6, *Glomus tubaeformis* 7, *Glomus fasciculatum* 8, *Glomus mosseae* 9, *Glomus macrocarpum* 10, *Scutellospora heterogamma* 11, *Sclerocystis pachycaulis* 12, *G.diazotrophicus* 13, *G.diazotrophicus* + *Entrophospora infrequens* 14, *G.diazotrophicus + Gigaspora albida* 15, *G.diazotrophicus + Glomus albida* 16, *G.diazotrophicus + Glomus dimorphicum* 17,*G.diazotrophicus* + *Glomus tubaeformis* 18, *G.diazotrophicus* + *Glomus fasciculatum* 19, *G.diazotrophicus* + *Glomus mosseae* 20, *G.diazotrophicus + Glomus macrocarpum* 21, *G.diazotrophicus* + *Scutellospora heterogamma* 22, *G,diazotrophicus + Sclerocystis pachycaulis.*

Treatments were arranged in a completely randomized design. After inoculation, plants were grown for 35 days under controlled conditions. Control and AM inoculated plants received once a week Hoagland's nutrient solution [21] .Treatments inoculated with *G.diazotrophicus,* singularly or in combination with AM fungi, received once a week a modified Hoagland's solution (without NH4NO3) and with 1g 1-1CaCO3 added to stabilize the pH). Deionized water was added when needed.

2.8.Plant measurements and analyses

The plants were harvested at 35 days after inoculation with G.diazotrophicus and root, stem and leaves were cut in to bits for determination of dry matter yield, N, P, photosynthetic pigments, and soluble sugars. Dry matter yield was determined by the drying plant parts in an oven at 90°C for 3 days, N was measured by macro-kjeldahl mehod [22] , acid soluble P was estimated by the modified method of [23], total soluble sugars by anthrone method [24] and phtosyntetic pigments of fresh leaf tissue was estimated following the method of Arnon [25].

2.9.Statistical analyses

The data were subjected to statistical analysis by using IRRISTAT package for one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (P,<0.05) [26].

3.Results

3.1.Soil characteristics

Soil samples collected from the rhizosphere of S.bicolor grown in Paravai, Samanatham, Kovilpatti, Dindigul and Kannivadi were analysed for pH, electrical conductivity, available nitrogen, availablable phosphorus, and available potassium content (Table-1). The soil pH ranged from 5.4 to 6.2 and the electrical conductivity ranged from 0.07 to 0.41dsm-1. The phosphorus (10 to 28mg/ kg soil) and potassium (137 to 500 mg/kg soil) content of soil were high and the nitrogen content was low (49/to 71 mg/kg soil).

The FDA guidelines for tuna, mahi-mahi and related fish specified 5 mg/100 g as defect action level [13] and ≥50 mg/100 g as toxicity level [49,13]. The European Economic Community (ECC) has recently established regulation for species of fish belonging to the Scombridae and Clupedae families and fixed a three-class plan for maximum allowable levels of histamine in fresh fish (n=9; c=2; m = 100 ppm; M = 200 ppm) and enzymatically ripened fish products (n = 9; c = 2; m = 200 ppm; M = 400 ppm) where n is the number of units to be analyzed from each lot, m and M are the histamine tolerances, and c is the number of units allowed to contain a histamine level higher than m but lower than M [50].

Table 1. Source and Characteristics of soil

Sample Places	Physical Characteristics		Soil Nutrients (mg / kg soil)		
	pH	EC (d Sm-1)	N	P	K
Paravai	5.4	0.30	57	11.3	173
Samanatham	5.9	0.41	49	10	500
Kovilpatti	5.7	0.39	71	28	500
Dindigul	6.0	0.30	53	25.5	260
Kannivadi	6.2	0.07	59	17	485

EC=Electrical Conductivity
N=Available Nitrogen
P=Available Phosphorus
K=Available Potassium

Nitrogen
0 to 113 mg/kg soil - Low
113 to 181 mg/kg soil - Medium
181 above mg/kg soil - High

Phosphorus
0 to 4.5 mg/kg soil - Low
4.6 to 9.0 mg/kg soil - Medium
9.0 above mg/kg soil - High

Potassium
0 to 46 mg/kg soil - Low
47 to 113 mg/kg soil - Medium
113 above mg/kg soil - High

3.2.Identification of G.diazotrophicus and AM fungi

Effective strains of G.diazotrophicus were assessed in the sugarcane plant samples collected from Madurai districts, Tamil nadu, India. For preliminary screening, the cultures were isolated using LG1, a medium specifically used for G.diazotrophicus isolation [5]. The formation of orange colour colony on LG1 medium was taken as prime criterion to identify the G.diazotrophicus isolates. The isolates were maintained on LG1 agar slants.

Arbuscular Mycorrhizal fungal spores were isolated from rhizosphere of Sorghum bicolor grown in different localities of southern districts of Tamil Nadu using wet sieving and decanting technique and identified as *Entrophospora infrequens*, *Gigaspora albida*, *Glomus albida*, *Glomus dimorphicum*, *Glomus tubaeformis*, *Glomus fasciculatum*, *Glomus mosseae*, *Glomus macrocarpum*, *Scutellospora heterogamma* and *Sclerocystis pachycaulis*

3.3.Mycorrhizal infection and G.diazotrophicus effect on fungal colonization

Among the 10 species of AM fungi viz Entrophospora infrequens, Gigaspora albida, Glomus albida, Glomus dimorphicum, Glomus

tubaeformis, Glomus fasciculatum, Glomus mosseae, Glomus macrocarpum, Scutellospora heterogamma and Sclerocystis pachycaulis , Glomus fasciculatum was found to be the best root colonizer with 83% colonization in S.bicolor (Table-2). In contrast, Scutellospora heterogamma was found to be the poor root colonizer with 20% in *S.bicolor.* All the species of AM fungi produced vesicular infection but not produced arbuscular infection during the study period. AM infection was also significantly higher in dual inoculated with *G.diazotrophicus* + Glomus fasciculatum with 95% colonization in *S.bicolor.* In contrast, *G.diazotrophicus* + *Scutellospora heterogamma* was found to be the poor root colonizer with 24% in *S.bicolor*. (Table-2).

Table 2. Effect of AM fungi and Glucoanacetobacter diazotrophicus inoculation on per cent infection in roots of Sorghum bicolor grown in sterile- soil sand mixture at 35 DAI.

Treatments	Vesicular infection	Arbuscular infection	Hyphal infection	% infection*
Control	-	-	-	-
Entrophospora infrequens	+	-	+	30
Gigaspora albida	+	-	+	43
Glomus albida	+	-	+	40
Glomus dimorphicum	+	-	+	52
Glomus tubaeformis	+	-	+	64
Glomus fasciculatum	+	-	+	83
Glomus mosseae	+	-	+	70
Glomus macrocarpum	+	-	+	28
Scutellospora heterogamma	+	-	+	20
Sclerocystis pachycaulis	+	-	+	24
Gluconacetobacter diazotrophicus	-	-	-	-
+ Entrophospora infrequens	+	-	+	35
+ Gigaspora albida	+	-	+	45
+ Glomus albida	+	-	+	47
+ Glomus dimorphicum	+	-	+	54
+ Glomus tubaeformis	+	-	+	67
+ Glomus fasciculatum	+	-	+	95
+ Glomus mosseae	+	-	+	80
+ Glomus macrocarpum	+	-	+	30
+ Scutellospora heterogamma	+	-	+	24
+ Sclerocystis pachycaulis	+	-	+	25

* Average of 3 experiments.

3.4.Effect of AM fungi and G.diazotrophicus inoculation on S.bicolor

The results on fresh and dry matter yield of S.bicolor inoculated with AM fungi are presented in Table-3. The dual inoculation increased the fresh and dry matter yield of plants over single inoculation with either AM fungus or *G.diazotrophicus* and uninoculated control. This increase was more pronounced in the case of *G.fasciculatum* + *G.diazotrophicus* and *G.mosseae* + *G.diazotrophicus.*

Impact of AM fungi inoculation enhanced the nitrogen content in *S.bicolor,* as compared to the uninoculated control (Table-3). The nitrogen content increased significantly in mycorrhizal plants in the presence of G.diazotrophicus. Among the dual inoculated plants, the microbial effect was substantially higher in *G.fasciculatum* + *G.diazotrophicus* combination. In contrast, the microbial effect was least with *Scutellospora heterogamma* + *G.diazotrophicus* combination.

Dual inoculation with AM fungi and *G.diazotrophicus* enhanced the phosphorus content of *S.bicolor* over the other treatments viz uninoculated control, *G.diazotrophicus* treatment and AM fungi treatment (Table-3). Within the mycorrhizae alone inoculated plants, the inoculation effect was much significant in plants treated with *G.fasciculatum* and *G.mosseae.* Further, phosphorus accumulation was substantially higher in all the mycorrhizal plants in the presence of *G.diazotrophicus.*

AM fungi inoculation induced an increase in soluble sugars in *S.bicolor* as compared to *G.diazotrophicus* inoculation or uninoculated control (Table-4). There was little difference in soluble sugars between dual inoculated AM plants and singly inoculated AM plants. However, in the case of *G.diazotrophicus* + *G.fasciculatum* combination, the soluble sugar content was 12.5% higher than the plants inoculated with *G.fasciculatum* alone.

In general, dual inoculation significantly increased the chlorophyll content in leaves of *S.bicolor,* as compared to the control and single inoculation with either *G.diazotrophicus* or AM fungal species. Among the singly inoculated plants, the AM effect on chlorophyll content was higher with four species viz *Glomus fasciculatum, Glomus mosseae, Glomus dimorphicum* and *G. tubaeformis,* in contrast to lower effect with *Scutellospora heterogamma* and *Sclerocystis pachycaulis.* Within the dual inoculated plants, the diazotroph effect was much pronounced when it combined with *Glomus fasciculatum*. However, the diazotroph effect was poor in combination with *Scutellospora heterogamma .*

Table 3. Effect of AM fungi & Gluconacetobacter diazotrophicus inoculation on fresh wt, dry wt, N content of Sorghum bicolor grown in sterile soil-sand

Treatments	Fresh wt (g plant-1)	Dry wt (g plant-1)	N (mg g dry wt-1)
Control	2.562^{a} ± 0.046	0.431^{bc}±0.024	7.06^{a} ± 0.850
Entrophospora infrequens	4.046^{f} ± 0.047	0.626^{bc}±0.040	8.62^{cd} ± 0.7761
Gigaspora albida	4.495^{h} ± 0.016	0.919^{f} ± 0.036	2.30^{fg}±0.2081
Glomus albida	4.370^{g} ± 0.055	0.784^{de}±0.027	1.13^{ef} ±0.4931
Glomus dimorphicum	4.715^{i} ± 0.007	0.976^{fg}±0.057	3.35^{gh} 0.7021
Glomus tubaeformis	5.124^{j} ± 0.076	1.060^{g} ± 0.026	4.70^{hi} ± .0571
Glomus fasciculatum	5.912^{m} ± 0.102	1.519^{j} ± 0.060	9.12^{i}± 0.9011
Glomus mosseae	5.454^{k} ± 0.050	1.365^{i} ± 0.021	5.90^{i} ± 1.011
Glomus macrocarpum	4.131^{f} ± 0.049	0.690^{cd}±0.027	9.86^{de} ± 1.106
Scutellospora heterogamma	3.496^{c} ± 0.037	0.525^{bc}±0.024	7.82^{bc} ± 0.585
Sclerocystis pachycaulis	3.651^{d} ± 0.053	0.623^{bcd}±0.035	8.39^{cde} ±0.8712
Gluconacetobacter diazotrophicus	3.234^{b} ± 0.015	0.783^{de}±0.103	7.48^{n} ± 1.3681
+ Entrophospora infrequens	4.383^{g} ± 0.080	1.040^{g} ±0.081	1.72^{ef} ±0.6801
+ Gigaspora albida	5.156^{j} ± 0.206	1.550^{j} ± 0.045	8.82^{j} ± 1.4931
+ Glomus albida	4.802^{i} ± 0.187	1.394^{i} ± 0.070	5.69^{j} ± 0.9642
+ Glomus dimorphicum	5.649^{L} ± 0.028	1.739^{k}± 0.044	0.59^{k} ± 1.7692
+ Glomus tubaeformis	5.943^{m} ± 0.040	1.853^{L}± 0.102	2.39^{L} ±0.6553
+ Glomus fasciculatum	7.20^{o} ± 0.061	2.396^{n}± 0.085	2.09^{o} ±2.0662
+ Glomus mosseae	6.776^{n} ± 0.025	2.186^{m} ±0.072	4.55^{m} ±0.3781
+ Glomus macrocarpum	4.586^{h} ± 0.060	1.175^{h} ±0.080	3.69^{gh}± .953
+ Scutellospora heterogamma	3.802^{e} ± 0.080	0.817^{e} ± 0.007	9.86^{de} ± 1.106
+ Sclerocystis pachycaulis	4.106^{f} ± 0.075	0.922^{f} ± 0.066	10.13^{de} ±1.059
LSD(P< 0.05)	1093.77**	283.26**	194.35**

± Standard deviation .Means followed by a common alphabet within a column are not significantly different at 5 % level of DMRT.

*Significant
**Highly significant

Table 4. Effect of AM fungi & *Gluconacetobacter diazotrophicus* inoculation on P, Soluble sugars, Chlorophyll content of Sorghum bicolor grown in sterile soil-sand mixture at 35 DAI .

Treatments	P(mg g dry wt-^{1})	Soluble sugars (mg g dry wt-^{1})	Chl (mg g fr leaf-^{1})
Control	6.01^{a} ± 0.817	12.40^{a} ±0.058	1.220^{a} ± 0.147
Entrophospora infrequens	10.00^{c} ± 0.236	17.50^{e} ±0.250	1.937^{bcde} ± 0.105
Gigaspora albida	13.50^{g} ± 0.331	19.90^{hi} ±0.156	3.555^{g} ± 0.409
Glomus albida	12.40^{ef} ±0.285	19.20^{ef} ±0.213	2.446^{ef} ± 0.267
Glomus dimorphicum	14.50^{h} ±0.389	20.50^{ij} ±0.064	4.159^{hi} ± 0.355
Glomus tubaeformis	16.00^{i} ± 0.264	21.10^{jk} ±0.185	4.283^{ij} ± 0.518
Glomus fasciculatum	21.00^{n} ± 0.476	22.30^{m} ±0.171	4.518^{ij} ± 0.290
Glomus mosseae	18.10^{jk} ±0.498	21.60^{klm}±0.16	4.353^{ij} ± 0.508
Glomus macrocarpum	11.00^{d} ± 0.314	018.60^{fg} ± 0.287	2.161^{abcd} ± 0.138
Scutellospora heterogamma	7.30^{b} ± 0.235	15.20^{c} ± 0.288	1.480^{ab} ± 0.085
Sclerocystis pachycaulis	9.00^{c} ± 0.264	16.20^{d} ± 0.275	1.617^{abc} ± 0.103
Gluconacetobacter diazotrophicus	8.10^{b} ± 0.246	13.80^{b} ±0.212	1.745^{abcd} ± 0.409
+ Entrophospora infrequens	16.30^{i} ± 0.071	18.70^{fg}±0.043	2.432^{ef} ± 0.146
+ Gigaspora albida	19.20^{kl} ±0.052	20.60^{ijk}±0.065	4.431^{ij} ± 0.531
+ Glomus albida	18.70^{kl} ±0.043	19.90^{hi} ±0.047	3.657^{gh} ± 0.407
+ Glomus dimorphicum	19.90^{Lm}±0.04	21.30^{jkl}±0.045	4.707^{ij} ± 0.355
+ Glomus tubaeformis	720.60^{mn}±0.065	22.20^{l}m±0.050	4.680^{ij} ± 0.407
+ Glomus fasciculatum	25.40^{o} ± 0.692	25.10^{o} ± 0.165	5.727^{k} ± 0.407
+ Glomus mosseae	21.30^{n} ± 0.045	23.40^{n} ± 0.156	4.955^{j} ± 0.576
+ Glomus macrocarpum	17.80^{j} ± 0.065	19.20^{gh} ±0.052	2.858^{f} ± 0.354
+ Scutellospora heterogamma	12.06^{de} ±0.226	16.30^{d} ± 0.071	2.146^{cde} ± 0.172
+ Sclerocystis pachycaulis	13.30^{fg} ±0.481	17.80^{fg} ±0.065	2.247^{de} ± 0.309
LSD(P< 0.05)	247.40	102.32	51.15**

± Standard deviation .Means followed by a common alphabet within a column are not significantly different at 5 % level of DMRT.

*Significant
**Highly significant

4.Discussion

Nitrogen and Phosphorus are the major inputs for the production of cereals. However, the escalating cost of chemical fertilizers forced us to exploit alternate source of fertilizers- biofertilizers. The endophytic diazotrophs proliferate inside roots, stem and leaves and fix N efficiently exploiting on environment where there is little competition for nutrients and low oxygen prevailed [27]. In this study *Saccharum officinarum* plant species from Madurai districts of Tamil Nadu were chosen for isolation of *Gluconacetobacter diazotrophicus*.

Arbuscular Mycorrhizal fungal spores were isolated from rhizosphere of *S.bicolor* grown in different localities of southern Tamil nadu using wet sieving and decanting technique and identified as *Entrophosphora infrequens, Gigaspora albida*, *Glomus albida, Glomus dimorphicum, Glomus tubaeformis, Glomus fasciculatum, Glomus mosseae, Glomus macrocarpum,* Scutellospora *heterogamma* and *Sclerocystis pachycaulis.* Although there is no strict host specificity [28] , individual sp. of AM fungi vary in their potential to promote plant growth [29] . Both inter and intraspecific differences in the efficiency of AM fungi in terms of plant growth have been reported in many instances [30,31,32] Among the ten species of AM fungi were colonized on plant roots and produced vesicular infection and not produced arbuscular infection. The notable absence of arbuscules may be due to the short study period, since arbuscules generally degenerate within 14 days. Also, it has been shown that colonization of non-host roots with intercellular development of hyphae, often form vesicles but not arbuscules [33]. The wide variation in per cent AM infection reported here clearly indicates that *G.fasciculatum* forms a preferential association with *S.bicolor.* Similar preferential association has also been reported in Trifoliate organ [34].The increase of AM infection observed when AM fungi were coinoculated with *G.diazotrophicus* varied for each of the tested fungus. A similar trend was observed with enhancement of mycorrhizal root colonization in dual inoculated sweet potato, sugarcane and sweet sorghum plants was reported [14] when *G.diazotrophicus* was coinoculated with the acid-tolerant AM fungus *Glomus clarum* [35] .

Significant increase in fresh and dry matter yield was observed in dual inoculated plants over other treatments viz uninoculated control, *G.diazotrophicus* inoculated and *G.fasciculatum* inoculated plants. Similar increase in plant growth was observed in *S.bicolor* inoculated with *G.diazotrophicus* + *G.fasciculatum* [36] . Production of growth hormones by diazotrophs [37, 38] plays a vital role in enhancing the growth of grasses. It has also been shown that *G.diazotrophicus* is beneficial to sugarcane through production of growth promoting factors [39]. Early study from this laboratory has also shown that IAA production by *G.diazotrophicus* correlated well with the induction of plant growth [40] .

The nitrogen content of *S.bicolor* increased considerably upon inoculation with *G.diazotrophicus*. The N15 incorporation studies in sugarcane have also demonstrated the potential for nitrogen fixation in *G.diazotrophicus-sugarcane* interaction [41]. Maximum N content observed in plants inoculated with *G.fasciculatum* + *G.diazotrophicus*, could be the result of better nitrogen fixation and uptake of N from the soil. The enhanced level of P in dual inoculated plants reported here could be the result of uptake of P from soil. The increase in plant biomass in *S.bicolor* upon dual inoculation was associated with increased nitrogen and phosphorus content. Investigations have shown that the growth and yield increase of legumes inoculated with AM fungi and rhizobia is due to enhanced N and P uptake [42,43,,44 ,45].

The chlorophyll content increased significantly in *G.fasciculatum* + *G.diazotrophicus* inoculated plants. This increase bears a positive correlation with the increase in soluble sugars. The promotion of chlorophyll formation in dual inoculated plants may presumably reflect more photosynthesis to meet the carbon requirements of AM fungi and *G.diazotrophicus*, since both the microsymbionts depend on the host plant for their carbon source [46, 47]

5.Conclusion

The present study revealed that *G.diazotrophicus* + *G.fasciculatum* form a preferential association with *S.bicolor.* Our results suggested the need for screening microbial compatibility and efficiency prior to field application.

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6.References

[1] Bonciarelli F. 1989. Fondamenti di Agronomia Generale. Edegricole. Bologna. 1989; 2: 1-3.

[2] Raman N, Mahadevan A. Mycorrhizal research – a priority in Agriculture. In Concepts in Mycorrhizal Research [K.G. Mukerji (ed.)], 1996; 2: 41-75.

[3] Jeffries P. Use of mycorrhizae in agriculture. Crit.Rev.Biotech. 1987; 5: 319-348.

[4] Kennedy IR, Tchan YT. Biological nitrogen in non-leguminous field crops: Recent advances. Plant Soil. 1992; 141: 93-118.

[5] Cavalcante VA, Dobereiner J. A new acid-tolerant nitrogen fixing bacterium associated with sugarcane. Plant Soil. 1988; 108: 23-31.

[6] Jimnez-Salgado T, Fuentez-Ramirez LE, Tapia-Hernandez A, Miguel A, Esparza M, Maritinez-Romero E, Caballero-Mellado J. Coffea Arabica L., a new host plant for Acetobacter diazotrophicus and isolation of other nitrogen fixing Acetobacteria. Env.Microbiol. 1997; 63: 3676-3683.

[7] Loganathan P, Sunitha R, Parida AK, Nair S. Isolation and characterization of two genetically distant groups of Acetobacter diazotrophicus from a new host plant Eleusine corocana L. Appl. Microbiol. 1999; 87: 167-172.

[8] Hernandez AT, Bustillos-Cristales MR, Jimnez-Salgado T, Cabellero-Mellado J, Fuentez-Ramirez LE. Natural endophytic occurrence of Acetobacter diazotrophicus in pineapple plants. Microb.Ecol. 2000; 39: 49-55.

[9] Loganathan P, Nair S. Crop-specific endophytic colonization by a novel, salt-tolerant, Nitrogen fixing and phosphate –solubilizing Gluconacetobacter sp. From wild rice. Biotechnol.Lett. 2003; 25, 497-501.

[10] Teixeira KRS, Stephan MP, Dobereiner J. Physiological studies of Saccharobacter nitrocaptans a new acid tolerant nitrogen fixing bacteria.4th international symposium on nitrogen fixation with non legumes, Rio de, Janeiro, Final program abstract, 1987; p-149.

[11] Bagyaraj DJ, Menge JA. Interaction between a VAM and Acetobacter and their effects on rhizosphere microflora and plant growth. New phytol. 1978; 80: 567-573.

[12] Boddey RM, Urquiaga S., Reis V, Dobereiner J. Biological nitrogen fixation associated with sugar cane. Plant and Soil. 1991; 137: 111-117.

[13] Dhillion S. Dual inoculation of pretransplant stage Oryza sativa L. plants with indigenous vesicular-arbuscular mycorrhizal fungi and fluorescent Psudomonas spp. Biol.Fertil. Soils. 1992; 13: 147-151.

[14] Paula MA, Reis VM, Dobereiner J. Interactions of Glomus clarum with Acetobacter diazotrophicus in infection of sweet potato (Ipomoea batatas), sugarcane (Saccharum spp), and sweet sorghum (Sorghum vulgare). Biol. Fertil.Soils. 1991; 11: 111-115.

[15] Paula MA, Urquiaga S, Siqueira JO, Dobereiner J. Synergistic effects of vesicular-arbuscular mycorrhizal fungi and diazotrophic bacteria on nutrition and growth of sweet potato (Ipomea batatas). Biol.Fertil.Soils. 1992; 14: 61-66.

[16] Jackson ML. Soil chemical analysis. Prentice Hall, New Delhi. 1971; 2: 1-5.

[17] Muthukumar T, Udaiyan K, Manian S. Vesicular-arbuscular mycorrhiza in tropical sedges of southern India. Biol.Fertil.Soils. 1996; 22: 96-100.

[18] Schenck NC, Perez Y. Manual for the identification of VA mycorrhizal fungi, 2nd ed. INVAM. University of Florida, Gianesville, 1990. 2: 241.

[19] Koske RE, Gemma JN. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research. 1989; 92: 488-505.

[20] McGonigle TP, Miller MH, Evans DG, Fairchid GL, Swan JA. A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 1990; 115: 495-501.

[21] Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. California Agricultural Experimental station circular University of Californi, 1950; 347: 12-15.

[22] Bergersen FJ. Measurement of nitrogen fixation by direct means. In: Methods for evaluating nitrogen fixation. F.J. Bergersen, ed. Wiley, Chichester, New York. 1980; 3: 65-110.

[23] Bartlett GR. Phosphorus assay in column chromatography. J. Biol.Chem. 1959; 234: 446-468.

[24] Mooris DL. Quantitative determination of carbohydrate with dry wood anthrone reagent. Science. 1948; 107: 254-255.

[25] Arnon DI. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidases in Beta vulgaris. Plant physiology. 24: 1-15.

[26] Duncan DB. Multiple range and multiple f-tests. Biometrics. 1955; 11: 1-42.

[27] James EK,Olivares FL. Infection of sugarcane and other graminaceous plants by endophytic diazotrophs. Crit. Rev. In. Plant.Sci. 1997; 17: 77-119.

[28] Johnsen NC, Copealed PI, Crookston RK, Pfleger FL. Mycorrhizae: a possible explanation for yield decline associated with continues cropping. Agron, J. 1992; 84: 387-390.

[29] Abbot LK, Robson R. Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. New Phytol . 1978; 81: 575-587.

[30] Harley JL, Smith SE. Mycorrhizal symbiosis. Academic press, New York. 1983.

[31] Sieverding E. Vesicular-arbuscular mycorrhiza management in tropical agro ecosystems. Technical co operation, Federal Rebublic of Germany, 1991; 8805: 462-465.

[32] Ruiz-Lozano JM, Azcon R. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water stress. Physiologia Plantarum. 1995; 95: 472-478.

[33] Giovannetti M, Sbrana C. Meeting a non-host: behavior of AM fungi. Mycorrhiza. 1998; 8: 123-130.

[34] Vinayak K, Bagyaraj DJ. Selection of efficient VA mycorrhizal fungi for Trifoliate organe. Biol.Agric.Hortic. 1990; 6: 305-311.

[35] Giovanetti M. Some anatomic feautures and spore germination of the vesicular-arbuscular endophyte Glomus clarum Nicolson and Schenck. Annali di Microbiologia. 1981; 31: 103-107.

[36] Isopi R, Fabbri P, Delgallo M, Puppi G. Dual inoculation of Sorghum bicolor (L.) Monech ssp with vesicular arbuscular mycorrhizas and Acetobacter diazotrophicus. Symbiosis. 1995; 18: 43-55.

[37] Fuentes-Ramirez LE, Jimnez-Salgado T, Abarca-Ocampo IR, Caballero-Mellado J. Acetobacter diazotrophicus an indole acetic acid producing bacterium isolated from sugarcane cultivars of Mexico. Plant Soil. 1993; 154: 145-150.

[38] Okon Y, Labandera-Gonzalez CA. Agronomic applications of Azospirillum and evaluation of twenty years world wide field inoulation. Soil.Biol. Biochem. 1994; 26: 1591-1601.

[39] Lee S, Reth A, Meletzus D, Sevilla M, Kennedy C. Characterization of a major cluster of nif, fix and associated genes in a sugarcane endophyte Acetobacter diazotrophicus. J.Bacteriol. 2000; 182: 7088-7091.

[40] Kumarasamy V. Studies on diazotrophs associated with grasses and their rhizosphere in southern Tamil Nadu. Ph.D., thesis, Dept.of. Botany, Thiagarajar College, Madurai-9, Tamil Nadu, India. 2002.

[41] Sevilla M, Kennedy C. Genetic analysis of nitrogen fixation and plant-growth stimulating properties of Acetobacter diazotrophicus, an endophyte of sugarcane. In: Nitrogen fixation in bacteria: Molecular cell biology. 1998; 14: 564-569.

[42] Manjunath A, Bagyaraj DJ, Gowda HSG. Dual inoculation with VA mycorrhiza and Rhizobium is beneficial to Leucaena. Plant Soil. 1984; 78: 445-448.

[43] Pocovsky RS, Fuller G, Stafford AE. Nutrient and growth interactions in soybean colonized with Glomus fasciculatum and Rhizobium japonicum. Plant Soil. 1986; 92: 37-45.

[44] Azcon R, Rubia R, Barea JM. Selective interactions between different speices of mycorrhizal fungi and Rhizobium meliloti strains and their effects on growth, nitrogen fixation and nutrition of Medicago sativa. New phytol. 1991; 117: 399-404.

[45] Xavier LJC, Germida A. Response of lentil to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy. Soil.Biol.Biochem. 2002; 34: 181-188.

[46] Tester M, Smith SE, Smith FG, Walker NA. Effect of photon irradiance on the growth of shoots and roots, on the rate of intiation of mycorrhizal infection and on the growth of infections units in Trifolium subterraneum (L). New Phytol. 1986; 103: 375-390.

[47] Sivaprasad P, Rai PV. Mechanisms of enhanced nodulation in vesicular arbuscular mycorrhizal in Pigeon pea, and Cajanus cajan (L). Agric.Rus.J. 1987; 25: 99-102.

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