Growth of Brachiaria brizantha *seedlings in response to the application of mycorrhizal fungi and diazotrophic bacteria*

Mónica Guadalupe Lozano-Contreras, F. Rivas-Pantoja y J. E. Castillo-Huchim

Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias
Campo Experimental Mocochá, km 25, Antigua carretera Mérida-Motul, Mocochá, Yucatán, México
E-mail: lozano.monica@inifap.gob.mx

ABSTRACT

A study was conducted to evaluate the growth of seedlings of Insurgente pasture (*Brachiaria brizantha*), treated with mycorrhizal fungi and pseudomonads as simple or associated inocula. Under net house conditions the following treatments were evaluated: T1: *Glomus mosseae* strain 1; T2: *G. mosseae* strain 2; T3: *G. mosseae* strain 3; T4: *Gigaspora albida* strain 4; T5: *Gigaspora* sp. strain 5; T6: *Glomus coremioides* strain 6; T7: *Rhizophagus intraradices* strain INIFAP; T8: bacterial *Pseudomonas* sp. 2709 INIFAP; T9: mixture of T7 and T8; T10: mixture of T1 to T7; T11: mixture of T1 to T8; T12: control without inoculation and T13: control without inoculation, fertilized with the formula 120-80-00 of NPK. The quantified variables were: total (aerial and root) biomass production per plant (g DM/plant^{-1}), root volume (cm^3/plant^{-1}), absolute leaf emergence rate (number of leaves week^{-1}), absolute growth rate in height (cm week^{-1}) and root length (cm plant^{-1}). The evaluations were conducted since seedling emergence started until they reached 54,6 cm of average height. The design was completely randomized, with 10 repetitions per treatment. The data were subject to a variance analysis through a general linear model, with bifactorial arrangement 13 (inoculants) x 2 (soil treatments). The plants inoculated with *G. mosseae* strain 3 and with the mixture of mycorrhizae and *Pseudomonas* sp. (T11) had higher total biomass production ($P<0,05$), as compared with *B. brizantha* Insurgente plants without inoculation and with those that were not inoculated and were treated with double nitrogen-phosphorus fertilization rate. The results showed the inoculum-host specificity in plants of *B. brizantha* pasture cv. Insurgente and proved the benefits of facultative mutualism for the growth of this forage species.

Key words: *Brachiaria brizantha*, mycorrhizae, *Pseudomonas*

INTRODUCTION

In Yucatán, México, most tropical soils dedicated to livestock production show fertility limitations for the optimum development of pastures (Pech, Santos and Montes, 2002). In addition, livestock producers who manage pasturelands extensively and without technification cause severe damage, due to overgrazing and paddock burning; which brings about erosion, nutrient lixiviation, soil compaction and desertification. Finally, the accelerated loss of soil fertility unfavorably influences biomass availability for livestock feeding (Sánchez, Hernández and Ruz, 2011).

A technological choice to reduce the impact of soil poverty is the use of biofertilizers, also known as plant growth promoting organisms. Diazotrophic bacteria (soil bacterial strains capable of colonizing the roots and the rhizospheric environment) and arbuscular mycorrhizal fungi (AMF) perform functions that benefit the soil and plant growth, because they fix nitrogen, solubilize minerals, produce plant growth promoters, control pathogens biologically and phytoremediate contaminated soils, among others (Javot, Pumplin and Harrison, 2007; Saldajeno, Chandanie, Kubota and Hyakumachi, 2008; Maldonado, Rivera, Izquierdo and Palma, 2010; Kelemu *et al*., 2011; Mena, Olalde, Fernández, Jerez and Serrato, 2011; Bécquer *et al*., 2012).

In tropical forage grasses, strain specificity, type of host plant and soil conditions seem to be critical factors that determine plant response.

Garrido, Cárdenas, Bonilla and Baldani (2010) state that, under water stress conditions, increasing the population of diazotrophic bacteria in environments where pastures of the *Brachiaria* genus are cultivated may be useful for the survival and productivity of these grasses. In addition, they suggest to develop studies that involve bacteria and AMF strains as essential requisite to identify inoculants which allow the plants to express their maximum productive potential.

The objective of this work was to evaluate the growth of *Brachiaria brizantha* cv. Insurgente in response to the application of AMF and *Pseudomonas* sp., as simple or associated inoculants.

MATERIALS AND METHODS

The study lasted for five months (September, 2010-January, 2011) and was conducted under net house conditions on lands of the experimental field Mocochá-INIFAP, Yucatán, México. The seeds of *B. brizantha* cv. Insurgente were inoculated and distributed in the following treatments: : T1: *Glomus mosseae* strain 1; T2: *G. mosseae* strain 2; T3: *G. mosseae* strain 3; T4: *Gigaspora albida* strain 4; T5: *Gigaspora* sp. strain 5; T6: *G. coremioides* strain 6; T7: *Rhizophagus intraradices* strain INIFAP; T8: bacterial *Pseudomonas* sp. 2709 INIFAP; T9: mixture of T7 and T8; T10: mixture of T1 to T7; T11: mixture of T1 to T8; T12: control without inoculation and T13: control without inoculation, fertilized with 120-80-00 of NPK kg ha^{-1}. The mycorrhizal strains were provided by Dr. María de los Ángeles Peña Río, researcher from the National Institute of Forestry, Agricultural and Livestock Research (INIFAP). These strains are part of the project of national biofertilizer collection of México, which is located in the C. E. Bajío-INIFAP, in Celaya, Guanajuato.

The inoculated seeds were planted in black polyethylene bags; which contained 5 kg of non-disinfected (ND) natural soil (calcaric Cambisol of average texture, with pH 7,8 and 26 % organic matter) and 5 kg of natural soil chemically disinfected (D) with metam-sodium (N-sodium methyldithiocarbamate); at the moment of emergence a selection was made and one plant was left per bag. With the exception of T13, the others received 60-40-0 kg of NPK ha^{-1}. The nitrogen-phosphorus fertilization was performed 10 weeks after seeding. The quantified variables were: total (aerial and root) biomass production of the plant (g DM/$plant^{-1}$), root volume (cm^3/$plant^{-1}$), absolute leaf emergence rate (number of leaves $week^{-1}$), absolute growth rate in height (cm $week^{-1}$) and root length (cm $plant^{-1}$). The evaluations were conducted since seedling emergence until they reached 54,6 cm of average height. The data expressed in plant weight were transformed for their analysis to log + 1 (Lentner and Bishop, 1993), and were subject to a variance analysis through the general linear model, with bifactorial arrangement 13 (inoculants) x 2 (soil treatments), in a completely randomized block design, with 10 repetitions per treatment. The least square means were compared through paired t-tests, at 0,05 of probability; both analyses were made with the SAS software (2012) version 9.2.

RESULTS AND DISCUSSION

Total biomass production. The aerial and root dry matter biomass responded to the interaction between the inoculants and the soil disinfection management. At 22 weeks after sowing, the plants on non-disinfected soil grew similarly ($P>0,05$), indistinctly from the administered treatment. In contrast, on disinfected soils, the pasture treated with *G. mosseae* strain 3 (T3) and with the mixture of fungi and bacteria (T11) had a significant accumulation ($P<0,05$) of biomass, which was higher than that of the control without inoculation and that of the plants which received double nitrogen-phosphorus fertilization (table 1).

On disinfected soil, the absence of native colonization allowed to identify, at least, *G. mosseae* strain 3 (T3) as an inoculant with high mutualistic specificity for the Insurgente pasture. In addition, this strain is likely to have contributed in an important way for T11 to propitiate high total biomass production. Nevertheless, the nature of the multiple association of fungi and bacteria of T11 did not allow to discern the degree of participation of each component in the symbiosis. The native colonization of microbiota in the non-disinfected soils (Nogales, 2005; Sivila and Angulo, 2006) may explain the similar forage yields in this pasture ($P>0,05$), which were observed through the applied inoculants. This colonization permitted the utilization of mineral resources, because the accumulation of dry matter in the plants –dosed with 60 and 40 kg/ha^{-1} of nitrogen and phosphorus– was similar to the plants treated with double fertilization. Salamanca (1999) observed that the native mycorrhizal strains showed higher affinity and efficiency in the pastures *Brachiaria decumbens* and *B. dictyoneura* than those recorded with introduced strains. This author also indicated that these two forage species responded better to inoculation than the pasture *Panicum maximum*, and that the Glomus genus stood out among the mycorrhizae due to its affinity with both *Brachiaria* species.

Table 1. Total (aerial and root) biomass production in plants of Insurgente pasture.

No.	Treatment	Total biomass (g DM/plant^{-1} ± SE)	
		D	ND
1	*Glomus mosseae* strain 1	9,7 ± 3,6^{BCa}	9,3 ± 1,8^{Aa}
2	*Glomus mosseae* strain 2	6,3 ± 2,4^{Ca}	6,7 ± 1,9^{Aa}
3	*Glomus mosseae* strain 3	25,6 ± 15,7^{Aa}	7,8 ± 2,4^{Ab}
4	*Gigaspora albida* strain 4	10,1 ± 2,9^{BCa}	5,5 ± 0,7^{Aa}
5	*Gigaspora* sp. strain 5	9,7 ± 2,5^{BCa}	5,0 ± 1,1^{Aa}
6	*Glomus coremioides* strain 6	1,8 ± 0,5^{Ca}	4,7 ± 0,8^{Aa}
7	*Rhizophagus intraradices* strain INIFAP	3,1 ± 1,4^{Ca}	9,2 ± 1,9^{Aa}
8	*Bacterial Pseudomonas* sp. 2709 INIFAP	1,3 ± 0,9^{Ca}	10,0 ± 3,8^{Aa}
9	Mixture of T7 and T8	6,1 ± 1,7^{Ca}	7,9 ± 2,9^{Aa}
10	Mixture of T1 to T7	3,4 ± 1,1^{Ca}	5,8 ± 1,2^{Aa}
11	Mixture of T1 to T8	18,8 ± 4,6^{ABa}	9,1 ± 1,5^{Ab}
12	Non inoculated control	6,6 ± 3,5^{Ca}	8,7 ± 2,7^{Aa}
13	Non inoculated control fertilized with 120-80-00	2,2 ± 0,7^{Ca}	9,7 ± 2,9^{Aa}

D: disinfected soil, ND: non-disinfected soil
a,b Averages followed by the same capital letter within each column and small letters between rows for each variable, are similar (P>0,05).

Root volume and length. The root volume of the Insurgente plants showed interaction between the inoculants and soil management, while the root length did not have important changes as response to these treatments (table 2).

In the non-disinfected soil, the artificial application of inoculants in the grass did not generate (P>0,05) a positive response in the development of root volume, which maybe means that the indigenous microbiota allowed an efficacious utilization of the mineral resources of the substratum used in the plants with these treatments. Rajan, Reddy and Bagyaraj (2000) reported that the plants which are inoculated with mycorrhizae grow better than the

Table 2. Root volume and length of Insurgente pasture plants.

No.	Treatment	Root volume (cm^3 ± SE)		Root length (cm ± SE)
		D	SD	
1	*Glomus mosseae* strain 1	16,5 ± 6,5^{Ba}	11,8 ± 3,2^{Aa}	34,8 ± 2,7^{a}
2	*Glomus mosseae* strain 2	7,6 ± 3,7^{BCDa}	8,4 ± 3,3^{Aa}	28,7 ± 2,9^{a}
3	*Glomus mosseae* strain 3	11,8 ± 3,8^{BCDa}	9,8 ± 3,4^{Aa}	33,1 ± 2,9^{a}
4	*Gigaspora albida* strain 4	12,2 ± 3,8^{BCa}	6,6 ± 1,0^{Aa}	36,9 ± 3,2^{a}
5	*Gigaspora* sp. strain 5	14,2 ± 4,9^{Ba}	5,7 ± 1,6^{Aa}	34,5 ± 3,4^{a}
6	*Glomus coremioides* strain 6	1,4 ± 0,5^{Da}	5,5 ± 1,5^{Aa}	34,1 ± 3,7^{a}
7	*Rhizophagus intraradices* strain INIFAP	3,4 ± 1,3^{CDa}	12,6 ± 4,2^{Aa}	36,5 ± 3,3^{a}
8	*Bacterial Pseudomonas* sp. 2709 INIFAP	1,6 ± 1,1^{Db}	14,8 ± 6,4^{Aa}	27,7 ± 3,1^{a}
9	Mixture of T7 and T8	7,3 ± 2,2^{BCDa}	10,9 ± 4,4^{Aa}	32,3 ± 3,3^{a}
10	Mixture of T1 to T7	3,6 ± 1,1^{CDa}	8,9 ± 2,1^{Aa}	34,9 ± 3,0^{a}
11	Mixture of T1 to T8	28,6 ± 7,9^{Aa}	12,5 ± 2,6^{Ab}	36,8 ± 2,0^{a}
12	Non inoculated control	8,0 ± 4,0^{BCDa}	13,2 ± 5,0^{Aa}	39,2 ± 2,7^{a}
13	Non inoculated control fertilized with 120-80-00	3,3 ± 1,1^{CDa}	10,1 ± 3,6^{Aa}	34,8 ± 3,5^{a}

D: disinfected soil, ND: non-disinfected soil.
a,b Averages followed by the same capital letter within each column and small letters between rows for each variable, are similar (P>0,05).

non-inoculated ones on non-sterile soils, due to an increase in mineral nutrition through the hyphae, which help to explore a higher soil volume.

In contrast, in disinfected soils the inoculation with mycorrhizae and bacteria (T11) was observed to promote increases of root volume in 258 % and 767 % over the one registered in non-inoculated plants and in non-inoculated ones but with double nitrogen-phosphorus fertilization, respectively. This same treatment (T11) stimulated in more than double the root volume (28,6 cm^3) of the Insurgente pasture when compared with its homologue on non-disinfected soils (12,5 cm^3). On the other hand, the inoculation with Pseudomonas sp. in the pasture cultivated on non-disinfected soils caused an important increase ($P<0,05$) in root volume (14,8 *vs.* 1,6 cm^3), which could have been originated by a synergic interaction of this inoculant with the indigenous microbiota. In certain plants, the inoculation with *Pseudomonas* in the presence of mycorrhizae in the rhizosphere has caused substantial increases in the root volume of the studied species (Wilson and Harnett, 1998). Studies conducted by Garrido *et al.* (2010) report that diazotrophic bacteria, such as *Pseudomonas* sp., may show selectivity to the crop and even of varieties within the same species and soil type. It is essential to stimulate in the Insurgente pasture higher root volume, because it to shows high and positive correlation ($r = 0,93$) with the plant forage production (table 3).

The root length of the pasture did not respond in this case to the applied treatments (table 2). This could have occurred due to restrictions of vertical space for the free underground elongation, because some species of the *Brachiaria* genus have deep roots which allow them to survive during long drought periods (Olivera, Machado and Pozo, 2006).

Absolute emergence rate. The leaf emergence rate in the Insurgente pasture significantly varied among the applied inoculants (table 4).

Table 3. Correlation matrix among vegetative variables of the Insurgente pasture influenced by the inoculants and soil management, under net house conditions.

Variable	X1	X2	X3	X4	X5
X1 Total biomass	1,00				
X2 Root length	0,27***	1,00			
X3 Root volume	0,93***	0,28***	1,00		
X4 AGR in height	0,53***	0,47***	0,54***	1,00	
X5 Leaf emergence	0,88***	0,33***	0,79***	0,62***	1,00

* P<,05; ** P<,01; *** P<,0001

Table 4. Absolute leaf emergence rate and plant height of Insurgente pasture.

No.	Treatment	Absolute rate: Emergence (leaves $week^{-1} \pm SE$)	Absolute rate: Height (cm $week^{-1} \pm SE$)
1	*Glomus mosseae* strain 1	$1,2 \pm 0,2^{ab}$	$2,5 \pm 0,2^{a}$
2	*Glomus mosseae* strain 2	$1,0 \pm 0,2^{abc}$	$2,0 \pm 0,2^{a}$
3	*Glomus mosseae* strain 3	$1,4 \pm 0,4^{a}$	$2,4 \pm 0,3^{a}$
4	*Gigaspora albida* strain 4	$1,0 \pm 0,1^{abc}$	$2,4 \pm 0,2^{a}$
5	*Gigaspora* sp. strain 5	$1,1 \pm 0,1^{abc}$	$2,4 \pm 0,2^{a}$
6	*Glomus coremioides* strain 6	$0,7 \pm 0,1^{c}$	$1,9 \pm 0,1^{a}$
7	*Rhizophagus intraradices* strain INIFAP	$0,9 \pm 0,1^{bc}$	$2,2 \pm 0,2^{a}$
8	*Bacterial Pseudomonas* sp. 2709 INIFAP	$0,7 \pm 0,1^{c}$	$2,0 \pm 0,3^{a}$
9	Mixture of T7 and T8	$0,9 \pm 0,1^{bc}$	$2,3 \pm 0,2^{a}$
10	Mixture of T1 to T7	$0,8 \pm 0,1^{bc}$	$2,0 \pm 0,2^{a}$
11	Mixture of T1 to T8	$1,4 \pm 0,2^{a}$	$2,8 \pm 0,2^{a}$
12	Non inoculated control	$1,1 \pm 0,2^{abc}$	$2,2 \pm 0,2^{a}$
13	Non inoculated control fertilized with 120-80-00	$0,8 \pm 0,1^{bc}$	$2,3 \pm 0,2^{a}$

a,b Averages followed by the same small letter within each column or variable are similar ($P>0,05$).

From all the evaluated treatments, the inoculants of *G. mosseae* strain 3 (T3) and the mixture o fungi and bacteria (T11) were the only ones that stood out, because they caused higher ($P<0,05$) leaf emergence per week (1,4 leaves $week^{-1}$) when compared with T6, T7, T8, T9, T10 and T13, which recorded between 0,7 and 0,9 leaves $week^{-1}$. Some mycorrhizae of the *Glomus* genus have stood out for their beneficial effects on the development of different plant species (Roveda and Polo, 2007; Baños et al., 2008; Díaz, Aguirre and Díaz, 2013); nevertheless, all the treatments had a weekly leaf production similar to that recorded in the non-inoculated control (T12).

The growth rate in height was not significantly affected ($P>0,05$) by the applied treatments. Parada, Jaén, Becerril and García (2001) reported that the inoculation with *G. mosseae* and the fertilization in *Manikara sapota* L. plants did not show significant increases in plant height; however, total dry weight increased in an important way, which was similar to the report in the Insurgente pasture.

It is concluded that the AMF G. mosseae strain 3 (T3), in absence of the competition of indigenous microbiota, promotes higher forage yield in *Brachiaria brizantha* cv. Insurgente, when compared with those plants without artificial inoculation or that receive double nitrogen-phosphorus fertilization.

Received: February 8, 2012
Accepted: April 10, 2013

VI EDICIÓN DE LA CONFERENCIA CIENTÍFICA INTERNACIONAL SOBRE DESARROLLO AGROPECUARIO Y SOSTENIBILIDAD "AGROCENTRO' 2014"

Estimados colegas:

La Facultad de Ciencias Agropecuarias de la Universidad Central "Marta Abreu" de Las Villas, su claustro y el comité organizador convocan a la VI Edición de la Conferencia Científica Internacional sobre Desarrollo Agropecuario y Sostenibilidad "AGROCENTRO' 2014" a desarrollarse entre los días 9 al 11 de abril del mismo año; la cual propiciará un fructífero intercambio entre especialistas de las más diversas partes del mundo en temas de gran actualidad y trascendencia, que serán abordados con el mayor rigor científico-académico y con una visión de futuro que permita enfrentar de manera ventajosa las problemáticas del actual siglo.

AGROCENTRO estimulará el intercambio entre profesionales, científicos, técnicos, productores, empresarios, representantes gubernamentales, organismos internacionales y público en general, interesados en investigar y promover las temáticas y socializar resultados.

El Comité Organizador de AGROCENTRO les reitera la invitación a presentar sus contribuciones profesionales o prácticas, con la garantía de que alcanzaremos los objetivos comunes en un clima de amistad y solidaridad.

Queda de Ud. fraternalmente,
Dr. C. Luis Antonio Barranco Olivera
Presidente Comité Organizador
AGROCENTRO' 2014

Para mayor información dirigirse a:
http://agrocentro.uclv.edu.cu/

Dirección: Carretera a Camajuaní km 5, Santa Clara, Villa Clara. CP: 54 830.

E-mail: agrocentro@uclv.edu.cu; yulygr@uclv.edu.cu

Fax: (53)(42) 281608

Teléfono: (53)(42) 281692