Response of *Melinis minutiflora* to inoculation with arbuscular mycorrhizal fungi in an Inceptisol of Colombia

Respuesta de *Melinis minutiflora* a la inoculación con hongos micorrícico arbusculares en un Inceptisol de Colombia

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Abstract

The effects of five inocula of arbuscular mycorrhizal fungi (AMF) on the grass *Melinis minutiflora* Beauv. were investigated under greenhouse conditions at the International Center for Tropical Agriculture (CIAT), Cali, Colombia, with the aim of selecting the most efficient AMF inocula. Non-disinfected (ND) and disinfected (D) substrates were studied. Inocula were: *Kuklospora colombiana*, *Gigaspora margarita*, *Glomus manihotis* and a mixture of those three species of AMF with and without sterilization. Yield parameters were aerial and radical biomass, root length, mycorrhizal colonization and N, P, K, Ca, and Mg concentrations in the aerial biomass. Pots measuring 13.5 x 8.0 x 14.0 cm were used as experimental units. Inceptisol soil, with low nutrient availability, previously sieved and mixed with sand was used as substrate. *Gi. margarita* and *Gl. manihotis* inocula showed the best results in the accumulation of aerial and root biomass, root length, mycorrhizal colonization and concentration of elements in the aerial biomass, while *Ku. colombiana* presented inhibitory effects on the variables evaluated. ND substrate condition increased accumulation of aerial and radical biomass and P concentration in the aerial biomass, and also stimulated root length of *M. minutiflora*. Aerial biomass had higher concentrations of N, K, Ca, and Mg in D substrate. *Mellinis minutiflora* is a promising grass species for rehabilitation of degraded soils in combination with arbuscular mycorrhizal fungi inoculation.

Key words: *Kuklospora colombiana*, *Gigaspora margarita*, *Glomus manihotis*, *Melinis minutiflora*, vesicular arbuscular micorrhizae.

Resumen

En un invernadero del Centro Internacional de Agricultura Tropical, CIAT, Cali (Colombia) se evaluó la aplicación de cinco inóculos de hongos micorrícico

arbusculares, HMA: *Kuklospora colombiana*, *Gigaspora margarita*, *Glomus manihotis* y la mezcla de estos con y sin esterilización, en sustrato sin desinfectar (SD) y desinfectado (D) sobre variables de rendimiento (biomasa aérea y radical, longitud radical), colonización por HMA y concentración foliar de nutrientes en la gramínea *Melinis minutiflora* Beauv., con el objetivo de seleccionar los inóculos más eficientes. Se utilizaron como unidades experimentales materos de 13.5 x 16.0 x 14.0 cm. El sustrato empleado fue suelo procedente de un Inceptisol con baja disponibilidad de nutrientes, tamizado y mezclado con arena. Los inóculos de *Gi. margarita* y *Gl. manihotis* presentaron los mejores resultados en la acumulación de biomasa aérea y radical, longitud radical, porcentaje de colonización micorrícica y concentración de elementos. *Kuklospora colombiana* presentó efectos inhibitorios sobre las variables evaluadas. La condición del sustrato SD favoreció la acumulación de biomasa aérea y radical y la concentración de fósforo (P) en la biomasa aérea, además, estimuló la longitud radical de *M. minutiflora*. La concentración de N, K, Ca y Mg en la biomasa aérea fue mayor en el sustrato D. Los resultados muestran que *Mellinis minutiflora* con inoculación HMA es promisoria para la recuperación de suelos degradados.

Palabra clave: *Kuklospora colombiana*, *Gigaspora margarita*, *Glomus manihotis*, *Melinis minutiflora*, Inceptisol, micorrizas arbusculares vesiculares.

Introduction

During the process of degraded area restoration, both native and introduced arbuscular mycorrhizal fungi (AMF) combined with vegetation cover may contribute to enhancing beneficial impact (Silveira, 1992). However, the effects are variable, due to the functional diversity presented by different AMF species in this action (Brundett, 2000; Marschner, 1995), and suggest the existence of a certain degree of selectivity between AMF and host plant under particular soil conditions (Caldeira et al., 1983). This has been termed ´discriminatory ability´ of the AMF by Siqueira & Franco (1988).

The Program for Soil Management at the International Center for Tropical Agriculture (CIAT) has evaluated and explored a number of plant species, and soil management practices for degraded area restoration, and to control hillside erosion in the Municipality of Caldono, Cauca Department, Colombia. Studies have been made of the role played by fallows, the effect of AMF host plants, and the influence of external AMF mycelium of the physical properties of the soil (Torres, 2000).

Melinis minutiflora originates from Africa, and is now found in South and Central America, and in the Caribbean, as well as parts of India (Cornell, 2008). It was introduced to Colombia in 1906 (Pérez, 1996), and is known by the common name of yaraguá´ grass. The mycotrophic condition of the species has been registered (Gomide & Zometa, 1978), and Reyes (2001) confirmed this for conditions in the Department of Cauca (Colombia), where this grass constitutes naturalized pastures (Filipe, 1997). The FAO (2008) considers it a valuable species due to its easy establishment, productive capacity, reasonable nutritive value, and its use in soil conservation on steep hillsides with poor soils. Zárate (2007) found the association between the root system of this grass and the external mycelium of the AMF to have an important role in the soil structure. These conditions highlight both the commercial and the environmental importance of the species, and the need for its inclusion in plans for soil management and conservation.

Considering the need to study the potential of individual species of AMF in order to select specific inoculants for restoration and soil protection plans (Dodd, 1994), the Soil Management Program of CIAT proposed this study, with the aim of selecting the most efficient AMF inoculants for maximizing performance of *M. minutiflora*. The effect of inoculating three AMF species in the aerial and root biomass was evaluated over a period of 90 days. The behavior of the AMF was analyzed through the percentage of mycorrhizal colonization, and its effect on nutrient concentration (N, P, K, Ca y Mg) in aerial biomass.

Materials and methods

The study was carried out in the greenhouse at CIAT (Cali, Valle del Cauca, Colombia) between September 2004 and August 2005. An Inceptisol (IGAC, 1979) was used: pH = 5.2, 16.7% of MO, N-total = 6575 ppm, P-Bray II = 0.8 ppm, K = 0.31, Ca = 1.89, Mg = 0.74, Al = 0.52 meq/100g from the locality of Pescador, Caldono Municipality (2° 48’ N, 76° 33’ O y a 1500 m.a.s.l.), Cauca Department, Colombia (CIAT, 2000).

The seeds of *M. minutiflora* used in the study were collected from natural pastures in the same locality, disinfected (sodium hypochlorite 10% for 5 min) and pre-germinated in sterilized sand before being planted in pots. The AMF inocula used was obtained from the mycorrhizal collection at CIAT. The pots used as experimental units (E.U.) measured 13.5 x 16.0 x 14.0 cm. The substrate was obtained from a mix of soil sieved through a mesh of 2 mm plus sand in the proportion 2:1 (p/p) (Thomas et al., 1993), and disinfected for 15 days 60 g/m^3 of Basamid (tetrahydro-3,5- dimethyl-2H-1,3,5-tiadizine-2-tiona), according to Reyes (2001).

Once disinfected, the substrate was placed in the pots to a level of 2/3 capacity, with subsequent inoculation with the AMF (700 spores for each E.U.), and the pots filled to capacity. The substrate was watered to field capacity (22%), and on the following day four plants of three weeks of age were planted in each E.U.

The experimental design used was that of divided plots with four replicas. The principal plot resulted from a 2 x 5 factorial, with two substrate conditions: not disinifected (SD), and disinfected (D) and five AMF inoculums: *Kuklospora colombiana* (KCLB) Oehl & Sieverding (2006), *Gigaspora margarita* (GMRG) W.N. Becker & I. R. Hall (1976), *Glomus manihotis* (LMNH) R.H. Howeler, Siever & N.C. Schenck (1984), a mix of the three species without sterilizing (Mix) and a sterilized mix (O). The subplots were the sampling times (1, 2 & 3 corresponding to 30, 60 and 90 days respectively).

After 30, 60 and 90 days post planting the following parameters were measured: root length (RL, expressed as meters per experimental unit, m/EU) after separation and washing of the roots, and measured using a Comair Root Length Scanner (Commonwealth Aircraft Corp. Ltd, Melbourne, Australia); Aerial (AB) and root (RB) biomass were evaluated gravimetrically after drying at 60 °C for 48 h, with results expressed as grams per E.U., g/UE. Mycorrhizal colonization was estimated (expressed as % colonization) using: 1. A root staining method according to Sieverding (1983); and 2. Quantification of the percentage of colonization by AMF, using the method of Allen & Allen (Sieverding, 1983). After 90 days the concentration of the following nutrients in the aerial biomass (AB) was determined: N (semimicro Kjeldahl, digestion with H_2SO_4); P (digestion with H_2SO_4); K; Ca; and Mg (digestion with nitro-perchlorate). Data were analyzed in the program SAS (SAS Institute Inc. 1998-2000), with tests for normality, ANOVA, Duncan test and Correlation analysis.

Results and Discussion

Effect of inoculation on biomass production

The analysis of variance (Box 1) showed highly significant differences in AB and RB for the effect of inoculation with AMF, substrate condition, evaluation time and interactions. The mean for AB increased slowly over the evaluation time after 30 days. The individual factors in the AB showed that non-disinfected substrate (ND) favored the accumulation of AB in *M. minutiflor* by 20% with respect to disinfected substrate (D). The greatest accumulation of AB occurred in plants inoculated with GMRG (*Gi. margarita*) with 2.8 g/EU, and the least in KCLB (*Ku. colombiana*) with 1.5 g/EU ($P < 0.05$) (Figure 1A).

Cuadro 1. Análisis de varianza de biomasa aérea y radical, longitud radical y colonización micorrícica en *M. minutiflora*.

Fuente de variación	Gl	Cuadrados medios			
		Biomasa aérea (g/UE)	Biomasa Radical (g/UE)	Longitud Radical (m/UE^{-1})	Colonización micorrícica (%)
Repetición (R)	3	1.536*	0.827*	2128.916 ns	203.632 ns
Condición de sustrato (S)	1	5.883**	6.878**	0.382 ns	2887.868*
Inóculo (I)	4	7.464**	3.034**	4939.661*	2732.446**
S x I	4	2.854**	1.046**	5873.341*	846.411**
Tiempo (T)	2	146.235**	51.532**	197533.120**	889.656**
S x T	2	1.379**	1.646**	5371.399**	454.075*
I x T	8	2.486**	1.143**	2363.455*	194.603 ns
S x I x T	8	0.908**	0.413**	3297.050**	524.465**
Promedio		2.150	1.233	100.575	38.539
Coeficiente de Variación		17.793	21.543	30.314	29.412

*significativo (P < 0.05), ** altamente significativo (P < 0.01), ns: no significativo.

In the non-disinfected substrate, the mean accumulation of RB increased by 50%, with GMRG presenting the greatest value, 1.7 g/UE; and, over the study period, the variable increased to 2.4 g/EU in the third sample (P < 0.05) (Figure 1B). The positive and highly significant correlation (P < 0.01) of the AB and RB confirmed that over the study period the increases in AB were accompanied by increases in RB (r = 0.86, 0.92 & 0.94 at samplings 1, 2 & 3 respectively).

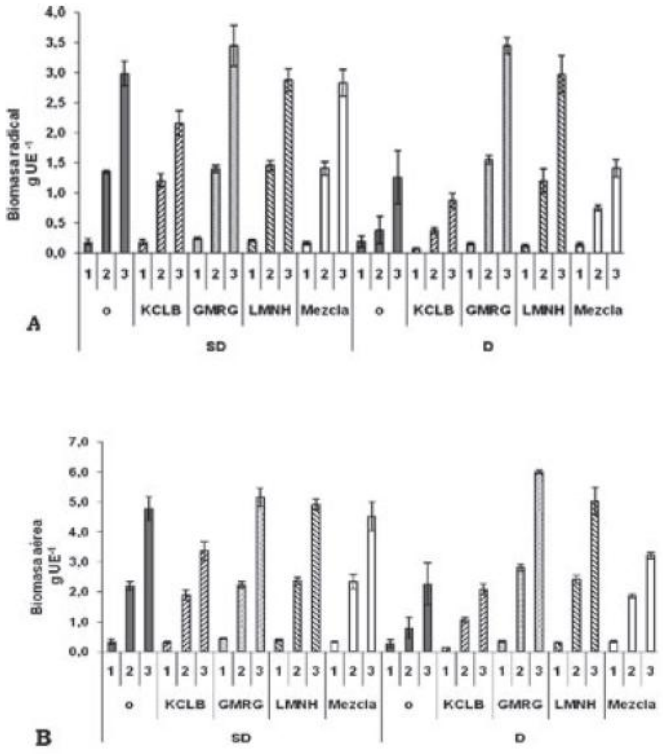

Figura 1. A = Biomasa aérea y B = Biomasa radical de M. *minutiflora* resultado de la interacción sustrato, inóculo de HMA y tiempo de evaluación. Barras indican error estándar. P < 0.05.

Effect on root length

Significant differences were seen in root length (RL) for the AMF inocula, and the interaction inocula–substrate condition. Highly significant differences were seen in relation to the evaluation time, and the interaction evaluation time–substrate condition–AMF inocula (Box 1). Individual factors showed that RL increased with time, and that the inocula of LMNH and GMRG in the disinfected substrate (D), stimulated the root growth, reaching a final measurement of 256.9 & 247.4 m/EU respectively ($P < 0.05$) (Figure 2). In contrast, in the substrate ND, the native AMF (ND+o) were those that favored the increase in root length (193.2 m/EU).

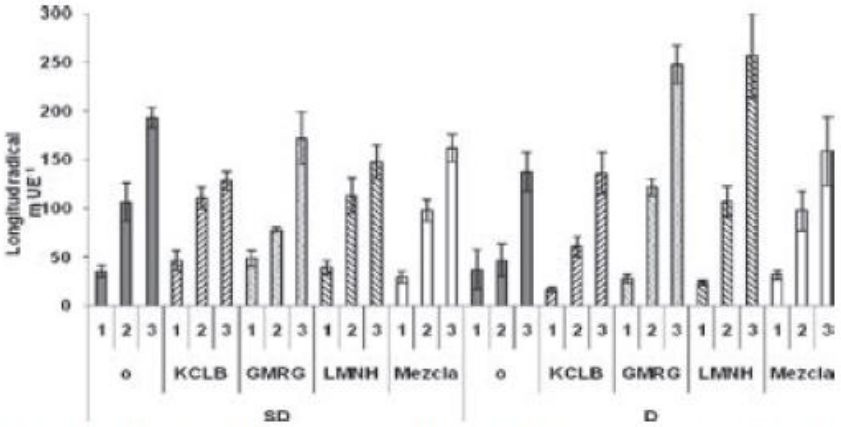

Figura 2. Efecto del sustrato, inóculo de HMA y tiempo de evaluación en la longitud radical de *M. minutiflora*. Barras indican error estándar. $P < 0.05$.

Effect on nutrition of *M. minutiflora*

Substrate condition, as well as influencing the AB, RB and the RL, affected the nutrient concentration (Box 2). For the AMF inocula evaluated, the concentration of N, K, Ca and Mg in the AB was greatest in D substrate, and that of P in the ND substrate. The effects of the treatments in the P concentration in the AB presented a positive and significant correlation ($P < 0.05$) with the mycorrhizal colonization (COL) ($r = 0.32$) and this, in turn, was significantly ($P < 0.05$) correlated with AB and RB in the second ($r = 0.41, 0.38$) and the third ($r = 0.37, 0.39$) sampling. On the other hand, the content of N and Mg presented a significant negative correlation with colonization ($r = -0.51$ & -0.54, N & Mg respectively).

Cuadro 2. Concentración de nutrientes (mg/g) en la biomasa aérea (BA) de *M. minutiflora* noventa días después de la siembra.

Tratamiento	mg/g					g/UE
	N	P	K	Ca	Mg	(BA)
SD + O	10.1	0.4	11.7	1.7	1.5	4.8
SD + KCLB	12.1	0.7	12.8	2.0	1.7	3.4
SD + GMRG	9.8	0.4	11.6	1.4	1.3	5.2
SD + LMNH	8.9	0.8	13.4	1.9	1.4	4.9
SD + Mezcla	11.0	0.6	14.5	1.6	1.5	4.5
D + O	21.2	0.4	14.1	1.9	2.2	2.3
D + KCLB	24.9	0.6	18.2	2.5	2.0	2.1
D + GMRG	14.9	0.4	15.9	1.8	1.5	6.0
D + LMNH	16.4	0.4	18.0	2.1	1.6	5.0
D + Mezcla	21.4	0.4	20.3	2.2	1.9	3.2

SD: sin desinfectar, D: desinfectado. KCLB: *Kuklospora colombiana*, GMRG: *Gigaspora margarita*, LMNH: *Glomus manihotis*, Mezcla: mezcla de HMA anteriores y O: mezcla de HMA esterilizados.

Effect on mycorrhizal colonization

The percentage of COL presented significant differences for the substrate condition and highly significant differences for inocula, sampling time, and the interactions substrate-inoculum and substrate-inoculum-sampling time (Box 1). The ND substrate stimulated the mycorrhizal colonization for all the inocula evaluated. However, LMNH expressed the greatest mycorrhizal potential in both substrate conditions. The process of disinfection applied to the soil reduced almost completely the activity of the native AMF (D + o) (Box 3).

Cuadro 3. Porcentaje de colonización micorrícica (COL) en *M. minutiflora*. Promedio general de los tres tiempos de muestreo.

sustrato	COL (%), según inóculo de HMA				
	O	KCLB	GMRG	LMNH	Mezcla
SD	37	39	44	49	47
D	9	26	38	52	43

SD: sin desinfectar, D: desinfectado. KCLB: *Kuklospora colombiana*, GMRG: *Gigaspora margarita*, LMNH: *Glomus manihotis*, Mezcla: mezcla de HMA anteriores y O: mezcla de HMA esterilizados.

Discussion

From the values registered for the AB and the RB it was possible to establish the following general action sequence of the myco-symbionts under the non-disinfected substrate conditions: RG > LMNH >Mix > o >KCLB; and Disinfected: GMRG > LMNH > O >Mix > KCLB. The AB and RB indicate that in the disinfected substrate the potential of the individual AMF inocula is particularly expressed, and in the ND substrate, that of the interaction processes between the inoculated and the native AMF.

Thus, GMRG presented the best results with respect to its ability to stimulate the accumulation of biomass in *M. minutiflora*. In contrast, KCLB in both evaluated substrates presented an inhibitory effect in the accumulation of both aerial and root biomass, presenting values lower than those of the treatment with disinfected substrate inoculated with a sterilized inoculum (D + O), which acts as the absolute control treatment. Negative effects on the accumulation of biomass have also been seen in *B. decumbens* in plants inoculated with AMF from the genus *Glomus* (Sosa et al., 2006). The results showed evidence of how the activity of the native AMF overcomes the antagonistic or competitive effects that result from a mix of the three AMF species (SD + Mix vs. D + Mix), whose response in the D substrate was inhibitory.

The accumulation of biomass in *M. minutiflora* for all inocula evaluated was greater in the aerial part, a result in accordance with those of Filipe (1997) for the same species under field conditions. However, these findings differ from those found by Reyes (2001) for this host. The contrast in the proportion of biomass distribution among the aerial and root portions of the plant may have its origin in the presence of different species and / or communities of AMF (Silveira, 1992), and the response of the host to those changes in particular soil conditions.

The response of RL showed a tendency similar to that of AB and RB, with respect to the inocula that stimulated the variable (Figure 2). Thus, GMRG and LMNH stimulated the length of the root system in *M. minutiflora* and KCLB presented an inhibitory response. Additionally, highly significant positive correlations were found ($P < 0.01$) between AB and RB during the study ($r = 0.71, 0.70, 0.58$ & $0.81, 0.70, 0.58$ for times 1, 2 & 3 for AB and RB respectively).

The concentrations of N, P, K, Ca and Mg found in the aerial biomass of *M. minutiflora* were within the values previously registered for the species (Primavesi, 1982; Gomide & Zometa, 1978; Caldeira et al., 1983; Filipe, 1997). Based on the accumulation of AB, it was seen that the treatments with a value >3.2 g/E.U. presented the following relationship between the evaluated elements: K > N > Ca > Mg > P; when the value was equal to or less than this, the relationship was: N > K > Ca > Mg > P (Box 2). Laredo (1985) and Reyes (2001) found that K > N when the plants were more than 40 days old, and the inverse relationship at younger ages. The results showed that the investment was more associated with the accumulation of AB than with age.

The greatest concentration of P in the ND substrate is concordant with the findings of Schweiger et al. (2000), who compared plants growing in fumigated and non-fumigated soils, and indicates the importance of the interaction of the effects of AMF with the activity of the soil microbial community enhanced by the mycorrhizal effect (Sánchez, 2007), even more when the low soil fertility employed in this study

is taken into consideration. With respect to the responses seen in the D substrate, it is important to mention that sterilization process may cause changes in te availability of N, Mn and K (Rusell y Rusell, 1964; Sánchez, 1999; Torres, 2000), and may affect the response of the AMF and the host plant.

Melinis minutiflora showed a marked contrast between the concentration of elements in the AB, and the accumulation of biomass for the evaluated inocula (Box 2). This is evident in the highly significant negative correlation ($P < 0.01$) between the concentrations of N, Ca and Mg, and that of AB ($r = 0.65, -0.55, -0.80$ with N, Ca and Mg respectively). Primavesi (1982) and Siqueira and Franco (1988) explained this aspect of the phenomenon as the effect of dilution when there is an accelerated growth in the plant. On the other hand, Smith and Read (1997) maintain that the increase in the concentrations of nutrients and the negative response in the growth of the plants with mycorrhizae is direct evidence of plants limited by C, more than by nutrients. Primavesi (1982) confirm that the high levels of nutrients in the plant tissue can not necessarily be taken as a sign of optimal nutrition. The results obtained differ from those registered by Caldeira et al. (1983) who showed that the AMF specie that induced the greatest accumulation of AB in *M. minutiflora*, also increase nutrient concentration.

The results suggest that it is not possible to relate in a direct manner the COL and the effect of symbiosis. While LCLB did not present lower levels of colonization, the responses to inoculated treatments where inhibitory. In contrast the inoculum 'O' showed lower percentage of colonization, and its results were intermediate values.

Long term studies are required in order to evaluate other types of effects, for example, those non-nutritional effects associated with the presence of the mycorrhizal symbiosis that may have a positive impact in the establishment of the host species. The colonization percentages recorded for *M. minutiflora* were similar to those found by Posada (2006) in pastures of *Brachiaria* sp.. in field conditions, but less than those found by Caldeira et al. (1983) for *M. minutiflora* in soils in Brazil.

Conclusions

- The non-disinfected substrate favored the accumulation in aerial and root biomass and the concentration of P in the AB, as well as stimulating root length in *M. minutiflora.*
- *Kuklospora colombiana* presented inhibitory effects in the evaluated variables.
- Considering the results in the AB, RB, RL, COL and mineral concentrations, the inoculums of *Gi. margarita* (GMRG) followed by *Gl. manihotis* (LMNH) may be suggested for *M. minutiflora*, as a component of rehabilitation plans

for pastures in the zone of Pescador, Cauca, or in places with similar inceptisol soil characteristics. However, the effect of the native AMF should not be underestimated.

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