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SELECTION OF ARBUSCULAR MYCORRHIZAL FUNGI EFFICIENT STRAINS FOR GUINEA GRASS (*Megathyrsus maximus* CV. LIKONI)

Selección de cepas eficientes de hongos micorrízicos arbusculares para el pasto guinea (*Megathyrsus maximus* cv. Likoni)

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ABSTRACT. In order to select the most efficient strains of arbuscular mycorrhizal fungi (AMF) to improve the nutritional status and productivity of Guinea grass, the following research was carried out on a Lixiviated Ferralitic Red soil. It was conducted at the Cattle Production Enterprise "Niña Bonita", located in the Bauta municipality, Artemisa province, Cuba. In it *Funneliformis mosseae*, *Glomus cubense* and *Rhizophagus intraradices* AMF strains plus a control without inoculation were evaluated, in a latin square design. A solid mycorrhizal inoculant formulated with each of the strains was used, containing 35 spores per gram of substrate; which were evenly distributed over the freshly cut grass, with a manual backpack sprayer loaded with a suspension of the AMF mycorrhizal inoculant and water at a 1:10 ratio, to reach a dose of 20 kg ha^{-1} of inoculum. It was found that the inoculation of *G. cubense* produced a higher frequency and intensity of mycorrhizal colonization, a higher number of spores in the rhizosphere and the tallest plants, higher concentrations of N, P and K in the biomass of the aerial part and the highest yield of pasture. The effect of this strain remained in the pasture up to 270 days after inoculation. It is concluded that *G. cubense* was the most efficient strain to improve the nutritional status and increase the productivity of Guinea grass established on the above-mentioned soil.

Key words: *glomus*, inoculation, nutrition

RESUMEN. Con el objetivo de seleccionar las cepas de hongos micorrízicos arbusculares (HMA) más eficientes para mejorar el estado nutricional y la productividad del pasto guinea, se llevó a cabo la investigación sobre un suelo Ferralítico Rojo Lixiviado. El mismo se realizó en la Empresa Pecuaria Genética "Niña Bonita", ubicada en el municipio Bauta, provincia Artemisa, Cuba. En él se evaluaron las cepas de HMA *Funneliformis mosseae*, *Glomus cubense* y *Rhizophagus intraradices* más un testigo sin inocular, en un diseño cuadrado latino. Para la aplicación de los HMA se utilizó un inoculante micorrízico sólido formulado con cada una de ellas, que contenían 35 esporas por gramo de sustrato; los cuales se distribuyeron de forma uniforme sobre el pasto recién segado, con una mochila manual cargada con una suspensión de inoculante micorrízico y agua en una relación 1:10, hasta entregar una dosis de 20 kg ha^{-1} de inoculante. Se pudo constatar que con la inoculación de *G. cubense* se alcanzó la mayor frecuencia e intensidad de la colonización micorrízica, el mayor número de esporas en la rizosfera; así como la mayor altura de las plantas, las concentraciones más altas de N, P y K en la biomasa de la parte aérea y el mayor rendimiento del pasto. El efecto de esta cepa se mantuvo en el pasto hasta 270 días después de su inoculación. Se concluye que la misma resultó ser la más eficiente para mejorar el estado nutricional e incrementar la productividad del pasto guinea establecido en el suelo anteriormente mencionado.

Palabras clave: *glomus*, inoculación, nutrición

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INTRODUCTION

Livestock in Cuba requires sustainable development, in which increases in production are based on economic efficiency and the substitution of concentrates and other imported foods, for pastures and forages.

In order to achieve this, it is essential to obtain high volumes of biomass with sufficient quality and few cultural requirements, thus satisfying the nutritional requirements of the animals (1, 2).

The low fertility of soils dedicated to livestock and the difficulties to have sufficient amount of fertilizers to improve yields of these crops, because their high prices in the international market limit the yields and nutritive value of the biomass that consumes livestock and consequently reduces the productivity of these agroecosystems (3, 4).

The management of arbuscular mycorrhizal associations is a way to improve the productivity and nutritive value of the biomass and at the same time, to reduce the use of fertilizers in the pastures. The benefits of arbuscular mycorrhizal fungi (AMF) in grassland agroecosystems are closely linked to the increase in soil volume that can be exploited by roots and, in fact, to increased efficiency of nutrient utilization (5, 6).

When there are low amounts of mycorrhizal propagules in the grassland or the AMF resident populations are not able to establish an effective symbiosis, inoculation with AMF strains can provide considerable benefits (7). However, due to the diversity of meadow species and exploitation regimes to which these crops are subjected; as well as by the edaphoclimatic conditions in which the grasses are cultivated, it is necessary to carry out studies in different agroecosystems with the purpose of selecting the most efficient strains for these environments. Taking into account the above, this research was carried out with the objective of selecting more efficient AMF strains to improve the nutritional status and yield of guinea grass (Megathyrsus maximus cv. Likoni).

MATERIALS AND METHODS

The experiment was carried out in the micro-station of Pastures and Forages of the Genetic Livestock Company "Niña Bonita", located in Bauta municipality, Artemisa province, on a Ferralitic Red Lixiviated soil (8), whose main chemical characteristics are presented in Table I.

The pH of the soil was slightly acidic, with average contents of organic matter and exchangeable potassium; very low calcium (Ca^{2+}) and sodium (Na^+) content, as well as low assimilable phosphorus (P_2O_5) and exchangeable magnesium (Mg^{2+}) content. Base exchange capacity (CEC) was also low. For the soil analysis, the methods established in the soil and plant tissue laboratory of the Department of Biofertilizers and Plant Nutrition of the National Institute of Agricultural Sciences (INCA) (9) were used.

In a Latin square design, four treatments were evaluated: a control and the inoculation of three AMAM-2 strains: Funneliformis mosseae (Nicol. & Gerd.) Walker & Schüßler (10); INCAM-4: Glomus cubense (Y. Rodr. & Dalpé) (11) and INCAM-11: Rhizoglomus intraradices (Schenck & Smith) Walker & Schüßler (10). The plots constituted the experimental unit and had a total area of 21 m².

The experiment was carried out from June 2011 to February 2012, in a grazing area with an area of 1 ha planted Likoni guinea over the last five years under dry conditions. For the application of the treatments a solid inoculant produced in the INCA was used, with a concentration of 35 spore g^{-1} of substrate; after grazing and decompression of the soil surface with a trident, to guarantee the descent of the spores and their greater contact with the radical system of the plants. This was applied at a rate of 20 kg ha^{-1}. For this, a suspension of solid mycorrhizal inoculant and water in a ratio of 1:10 was prepared, which was applied with a manual backpack and distributed on the grass at the beginning of the experiment, coinciding with the beginning of the rainy season. No fertilizers were applied.

Two cuts were made, one at 70 and the other at 270 days after the treatments were applied, coinciding with the rainy and little rainy season, respectively. The height of the plants was measured at the time of each cut, for which ten individuals were taken within the area of calculation of each plot.

[A] Paneque, V. M. y Calaña, J. M. "La fertilización de los cultivos. Aspectos teórico-prácticos para su recomendación". En: *Curso de postgrado*, Ed. Instituto Nacional de Ciencias Agrícolas, San José de las Lajas, La Habana, 2001, p. 29

Table I. Chemical characteristics of soils (depth: 0-20 cm)

pH H_2O	OM (%)	P_2O_5 (mg 100 g^{-1})	Ca^{2+}	Mg^{2+}	Na^+	K^+	CCB
			(cmol$_c$ kg^{-1})				
6,4	3,34	2,0	8,9	2,1	0,19	0,35	11,54
(0,2)	(0,33)	(0,6)	(0,8)	(0,3)	(0,02)	(0,03)	(0,76)

OM: organic matter, CCB: Base exchange capability
Values in parentheses indicate confidence intervals (α=0,05)

The green mass (GM) of the aerial part of all the plants that occupied the plots was harvested. A 200 g sample was then weighed and measured to determine the percentage of dry mass (DM) and N, P and K concentrations of aerial biomass. The yield of DM was calculated from the yield of GM and the percent of DM (9).

In the cut, five subsamples of roots and soil of the rhizosphere were taken from each plot to a depth of 0-20 cm, using a metal cylinder 5 cm in diameter and 20 cm in height, following the protocol used for the determination of mycorrhizal structures in pastures (12). These were homogenized to form a sample composed of plot, from which 1 g of roots were extracted for staining and clarification (13). The indicators that were evaluated were: frequency of mycorrhizal colonization, visual density or colonization intensity according to the methodology described in the Manual of Procedures (14) and the number of spores in the rhizosphere (15), as modified by other authors[B].

The efficiency index (EI) of the inoculated AMF strains and the degree of their participation in pasture nutrition were also determined. The IE was calculated using the following formula (16):

$$IE = \frac{\text{Yield. DM tte inoculated} - \text{Yield. DM control}}{\text{Yield. DM control}} \times 100$$

where

Rend=Yield. MS=DM of inoculated tnt = yield DM (t ha^{-1}) of the inoculated treatment Yield. DM control = yield DM (t ha^{-1}) of the control.

The following formula (17) was used to calculate the participation of AMF strains in pasture nutrition.

$$\text{Participación (\%)} = \frac{\text{Conc. N, P, K biom aérea tto inoc.} - \text{Conc. N, P, K biom aérea testigo}}{\text{Conc. N, P, K biom. aérea testigo}} \times 100$$

where:

Conc. N, P, K Aerial biomass of inoc tnt. = Concentration of N,P or K (%) of the aerial biomass of the inoculated treatment.

Conc. N, P, K aerial biomass. inoc tnt. = Concentration of N, P or K (%) in the aerial biomass of the control treatment.

Data were processed by analysis of variance according to the experimental design used, and the means were compared by Duncan's test p <0,05 (18) or the confidence interval at α = 0,05 (19), after verified that they complied with the adjustment of normal distribution and homogeneity of variances. Statistical software SPSS® version 11.5 was used for Microsoft® Windows® (20).

[B] Herrera, R. A.; Ferrer, R. L. y Furralzola, E. *Estrategia de funcionamiento de las micorrizas VA en un bosque tropical. Biodiversidad en Iberoamérica: Ecosistemas, evolución y procesos sociales*. (ed. Monasterio M.), Programa Iberoamericano de Ciencia y tecnología para el desarrollo. Subprograma XII, Diversidad Biológica, 1995, Mérida, México

RESULTS AND DISCUSSION

Table II shows the influence of AMF strain inoculation on biomass height and yield (t DM ha^{-1}), as well as the efficiency index of the strains, which expresses, in percentage terms, their effect on the increase of pasture productivity with respect to the uninoculated treatment (control). At 70 days after the treatments were applied (date corresponding to the rainy season), all strains of AMF increased the height and yield of the biomass in relation to the control that was not inoculated; however, the greatest effect was obtained with *G. cubense*, which differed significantly from the rest of the treatments. This strain also showed the highest efficiency index.

At 270 days, (rainy season), no effect of F. mosseae and R. intraradices was found on growth or productivity of the grass, since the height and yield of the biomass that were obtained with the inoculation of both strains were similar to those observed in the uninoculated control, and this was also reflected in the low efficiency indexes that reached both strains. Only with *G. cubense* were observed increases in both variables and a higher efficiency index, showing not only its greater effectiveness, but also a greater permanence in the pasture.

Table II. Effect of treatments on the height and yield of pasture biomass and efficiency indexes of AMF strains

Treatments	70 days			270 days		
	Height (cm)	DM (t ha^{-1})	IE (%)	Height (cm)	DM (t ha^{-1})	IE (%)
Control	71,3 c	2,53 c	-	34,5 b	1,75 b	-
G. cubense	112,5 a	3,95 a	56,1	53,9 a	2,51 a	43,4
F. mosseae	89,7 b	3,19 b	26,1	35,1 b	1,79 b	2,3
R. intraradices	91,2 b	3,13 b	23,7	34,7 b	1,82 b	4,0
ES	3,5**	0,43**	-	2,3**	0,28**	-

AMF strains: *Glomus cubense*, *Funneliformis mosseae*, *Rhizophagus intraradices*

70 days: rainy season, 270 days: slight rainy season. Efficiency index (EI), dry mass (DM), standard error (SE)

** Means with different letters in the same column differ from each other, according to Duncan's test (p <0.05)

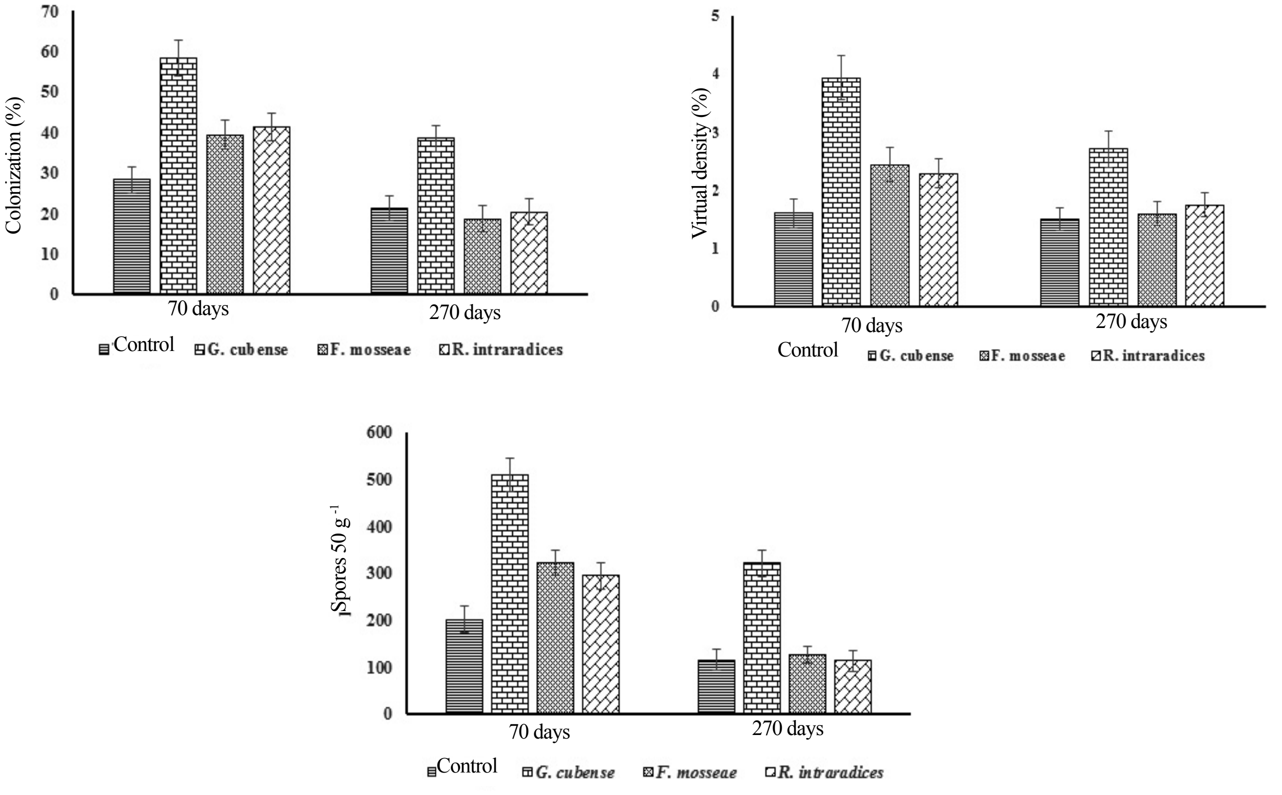
In all treatments, the absolute values of the measured variables were greater than 70 days, with respect to the subsequent sampling.

The effect of the treatments on the mycorrhizal structures was evaluated through the indicators of mycorrhizal colonization and visual density, which reflect the occupation level of the host plant root by the mycorrhizal fungus and the colonization intensity, respectively, as well as by the number of spores in 50 g of soil. As can be seen in Figure 1, at 70 days of treatment, all strains of AMF increased colonization levels, visual density and number of spores in the rhizosphere; although the highest values of these variables were reached with *G. cubense*, which differed significantly from the rest. This behavior was very similar to that observed in the evaluation of the height and the biomass yield.

At 270 days, both F. mosseae and R.intraradices showed colonization percentages, visual density and number of spores similar to those observed in the control treatment, which reflected the level of radical occupation of the resident AMF, indicating the disappearance of the inoculation effect of both strains on the mycorrhizal structures of the grass.

Although the influence of *G. cubense* persisted to this date, the values of these variables were significantly lower than those reached at 70 days, although they remained higher than in the rest of the treatments.

The effect of AMF strains on concentrations of primary macronutrients in aerial grass biomass is presented in Table III. At 70 days post inoculation all strains were observed to increase N, P and K concentrations; however, the highest values of these nutrients were reached with the *G. cubense* strain. AMF strains also had a differentiated behavior in time, as in the biomass yield and the mycorrhizal variables, at 270 days after the treatments were applied only with the inoculation of *G. cubense*, a significant increase of the concentrations of these nutrients in the biomass was achieved. Likewise, *G. cubense* had a greater contribution in the nitrogen, phosphorus and potassium nutrition of the pasture (Figure 2). Both at 70 and 270 days, this strain produced the greatest increases in the concentrations of these nutrients in the biomass in relation to the uninoculated control; they ranged between 27 and 33 %.



The vertical bars show the confidence interval of the mean. Confidence intervals that overlap each other do not differ significantly (α = 0.05)
AMF strains: *Glomus cubense*, *Funneliformis mosseae*, *Rhizophagus intraradices*

Figure 1. The inoculation effect of the AMF strains on the fungal variables of the pasture

Table III. Inoculation effect of AMF strains on concentrations (%) of primary macronutrients in aerial pasture biomass

Treatments	70 days			270 days		
	N	P	K	N	P	K
Control	1,07 c	0,16 c	1,10 c	1,19 b	0,18 b	1,21 b
G. cubense	1,41 a	0,22 a	1,47 a	1,52 a	0,23 a	1,56 a
F. mosseae	1,29 b	0,19 b	1,29 b	1,21 b	0,17 b	1,23 b
R. intraradices	1,31 b	0,19 b	1,31 b	1,20 b	0,18 b	1,25 b
ES	0,04**	0,01**	0,03**	0,03**	0,01**	0,02**

AMF strains: *Glomus cubense*, *Funneliformis mosseae*, *Rhizophagus intraradices*, standard error (ES)

** Means with different letters in the same column differ from each other according to Duncan test (p<0,05)

While F. mosseae and R. intraradices only managed to increase the tenors of N, P and K between 17 and 21 % at 70 and at 270 days, they had practically no influence on grass nutrition.

When analyzing in an integral way the results obtained in this experiment, it was observed that the response of the grass to the inoculation of AMF depended on the strain effectiveness; as well as to the existence of different degrees of functional compatibility between the host plant and the fungus; A fact that is in line with what was observed by other researchers when evaluating the effect of the introduction of AMF species on forage grasses (21, 22).

In this sense, it has been suggested that although there is no evidence of strict fungal-plant specificity, not all AMF species colonize with the same intensity and efficiency the different plant species.

The existence of different degrees of functional compatibility in the symbiosis as a result of environmental influences on the genotypic expression of both symbionts was demonstrated (23, 24).

Also, the soil influence on the inoculation effectiveness cannot be ruled out, since the studies carried out in Cuba on different crops and edaphic conditions have demonstrated the existence of a high compatibility between soil type-efficient strain of AMF, as well as a low specificity of the latter with the host plant, in the response of the cultures to the application of these microorganisms (25).

In a study carried out on the inoculation influence with AMF strains on Brachiaria grasses cultivated in a Ferralitic Red Leachate Soil, results similar to those of this experiment were obtained; Since it was demonstrated that *G. cubense* was the most efficient to improve the yield and the nutritional state of the grasses, corroborating in addition the existence of the high compatibility type-efficient strain of soil mentioned previously.

The effect of *G. cubense* on the increase of pasture biomass yield was related to the improvement of its nutritional status. This was evidenced both by the higher concentration of N, P and K in the aerial biomass that was obtained as a result of inoculation; as well as, due to its greater participation in the increase of these elements, in relation to the rest of the evaluated strains (26, 27).

In relation to the participation of AMF strains in grass nutrition, it was observed that both at 70 and

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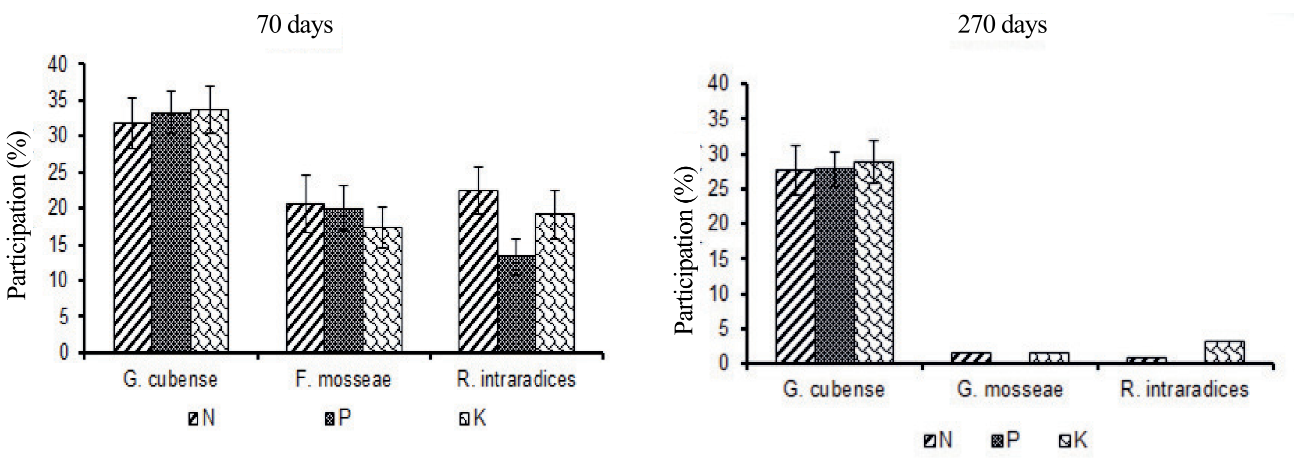

The vertical bars show the confidence interval of the mean. Confidence intervals that overlap each other do not differ significantly (α = 0.05)
AMF strains: *Glomus cubense*, *Funneliformis mosseae*, *Rhizophagus intraradices*

Figure 2. Participation of AMF strains in the increase of N, P and K concentrations in the biomass pasture area

270 days after inoculation, *G. cubense* contributed very similarly, in quantitative terms, to the increase in concentrations of N, P and K in the aerial part biomass of the pasture. This behavior seems to be closely related to the strain effect on the increase of nutrient utilization of the soil, and with the needs of the grass, since this was cultivated in a soil with low tenors of assimilable P and exchangeable K, as well as with tenors of N which are judged by their content of organic matter, are insufficient for fodder grasses (28, 29).

In this sense, it has been suggested that mycorrhizal symbiosis, rather than favoring the absorption of one or another element, behaves as a mechanism that allows plants to obtain their nutritional requirements, depending on their own needs and the availability of them on the soil (30, 31).

CONCLUSIONS

G. cubense proved to be the most efficient AMF strain to increase mycorrhizal structures, yield and nutrient concentrations in the biomass of the aerial part of Likoni guinea grass. Its effect on the pasture was maintained at least up to 270 days after inoculation.

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