



EFFECTIVENESS OF AMF STRAINS ON CASSAVA CROP (*Manihot esculenta* Crantz) IN TWO TYPES OF SOILS

Efectividad de cepas de HMA en el cultivo de la yuca (*Manihot esculenta* Crantz) en dos tipos de suelos

José P. João¹✉, Alberto Espinosa Cuellar², Luís Ruiz Martínez², Jaime Simó González² and Ramón Rivera Espinosa³

ABSTRACT. This study was carried out with the objective of evaluating the effectiveness of inoculating arbuscular mycorrhizal fungal strains (AMF) on commercial clones of cassava crop (*Manihot esculenta* Crantz) and its dependence on the type of soil. Thus, the effectiveness of three AMF strains, *Glomus cubense*, *Rizophagus intraradices* and *Funneliformis mosseae*, was compared in Carbonated Loose Brown and Lixiviated Red Ferralitic soils. In the first soil, six commercial clones were used at a randomized block design with factorial arrangement, four replications and repeated for two years. In the second soil, AMF strains were just evaluated in 'CMC-40' clone at randomized block design with four repetitions and conducted for two years. Cassava crop showed a high response to AMF strain inoculation. Strain effectiveness was different in either soil, so that *R. intraradices* behaved as the efficient AMF strain in Carbonated Loose Brown soils with pH 7,0 and *G. cubense* in Lixiviated Red Ferralitic soils with pH about 6,5. Although some clones showed compatibility for either strain, they did not stop establishing an effective symbiosis with the efficient strain, in such a way that compatibilities among clones do not change the criteria to recommend AMF strains per soil. Efficient strains per type of soil obtained for cassava crop coincide with the ones previously found for different crops.

Key words: arbuscular mycorrhizal fungi, inoculation, soils

RESUMEN. Con el objetivo de evaluar la efectividad de la inoculación de cepas de hongos micorrízicos arbusculares (HMA) en clones comerciales de yuca (*Manihot esculenta* Crantz) y su dependencia con el tipo de suelo, se comparó la efectividad de tres cepas de HMA, *Glomus cubense*, *Rizophagus intraradices* y *Funneliformis mosseae*, tanto en suelo Pardo Mullido Carbonatado como en Ferralítico Rojo Lixiviado. En el primer suelo, se utilizaron seis clones comerciales en un diseño de bloques al azar con arreglo factorial, cuatro réplicas y repetido durante dos años. En el segundo suelo, solo se evaluaron las cepas de HMA en el clon 'CMC-40' en un diseño de bloques al azar con cuatro repeticiones y ejecutado en dos años. El cultivo de la yuca presentó una alta respuesta a la inoculación de cepas de HMA. La efectividad de las cepas fue diferente en uno y otro suelo, de forma tal que *R. intraradices* se comportó como la cepa eficiente de HMA en el suelo Pardo Mullido Carbonatado con pH de 7,4 y *G. cubense* en el Ferralítico Rojo Lixiviado con pH alrededor de 6,5. Si bien algunos clones presentaron una compatibilidad por una u otra cepa, no dejaron igualmente de establecer una simbiosis efectiva con la cepa eficiente, de forma tal que las compatibilidades de los clones no cambian los criterios de recomendación de cepas de HMA por suelo. Las cepas eficientes por tipo de suelo obtenidas para la yuca coinciden con las encontradas anteriormente para diferentes cultivos.

Palabras clave: hongos micorrízicos arbusculares, inoculación, suelos

INTRODUCTION

The cultivation of cassava (*Manihot esculenta* Crantz) is widely spread in the tropics and in a large

group of countries and soil types, used not only as a food source human beings^{A, B} and animals (1, 2), but even with proven efficiency for generating alcohol

¹ Universidad "José Eduardo dos Santos", Angola.

² Instituto de Investigaciones de Viandas Tropicales (INIVIT), Santo Domingo, provincia de Villa Clara, Cuba

³ Instituto Nacional Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32700.

✉ zecapedro2003@yahoo.com.br

^A FAO. *FAOSTAT* [en línea]. 2013, [Consultado: 17 de enero de 2015], Disponible en: <<http://faostat3.fao.org/home/index.html>>.

^B Centro Internacional de Agricultura Tropical (CIAT). *La Promesa de la Agricultura Tropical Hecha Realidad* [en línea]. Informe Anual CIAT 2012-2013, Inst. Centro Internacional de Agricultura Tropical (CIAT), Colombia, 2013, p. 47, ISSN 2145-1311, [Consultado: 10 de diciembre de 2015], Disponible en: <http://ciat.cgiar.org/wp-content/uploads/2013/06/informe_anual_2012.pdf>.

(3, 4), all of which guarantees its economic importance. Cuba is no exception and annually 150 000 ha are planted and an increase in the crop surface are expected to in the coming years (5).

While it is a rustic culture, high yields require the application of mineral fertilizers or organic fertilizers and the use of optimal doses can be obtained between 40 and 60 t ha⁻¹ yr⁻¹ commercial real estate (6); however, in Cuba generally it is not fertilized nor watered^C, does all of which lead to low yields 7 t ha⁻¹ yr⁻¹.

Moreover, cassava is a crop with high mycorrhizal dependency (2, 7) and in Cuba the works of inoculation using arbuscular mycorrhizal fungi (AMF) strains started in this crop in Brown soils overstepped Carbonate, which allowed to establish the effectiveness inoculation, ensuring high yields and decreased fertilizer requirements^D; However, high doses of inoculant recommended (7) and then used (50 g plant⁻¹) did not allow their use in agricultural practice.

Subsequently, it was possible to standardize the method of applying the inoculant by way of coating the tips of the stakes, in doses of 10 kg ha⁻¹ and total coating at a dose of 20 kg ha⁻¹ (8), whose doses relatively low not only ensured the effectiveness, but their application to production scale (9) made feasible.

Also, in some cassava producing countries in Africa (10, 11) and Colombia (11), have been reported on a pilot scale positive results to AMF strains inoculation, without considering the recommendation criteria of AMF efficient strains by soil type (12), which have been found in Cuba for a wide range of crops and soil types.

The work of AMF strains recommendation^D were performed with a single clone, while in Cuba are currently available from several commercial clones (13) and it is currently a task to evaluate if product variability among commercial clones are maintained both the response to inoculation of AMF strains, as the existence, at least of an AMF strain per soil type (12), with which any of the clones establishes an effective symbiosis and maintains therefore the regularity of low specificity of efficient AMF strain with clones in this case.

There is also no prior experimental information on the application efficiency of the different strains of AMF to this crop in Ferrallitic Red soils, which cover about 720 000 ha of agricultural land area (14); also among AMF strains that were initially evaluated^D was not used *G cubense*. (15), a strain with high effectiveness for different crops in such soil (16).

From the above considerations and taking into account the importance of soil Brown loose Carbonated and Red Ferrallitic Lixiviated soils in Cuban agriculture, as well as the recognized the culture mycorrhizal dependency, two studies were developed to evaluate the effectiveness of inoculating AMF strains in commercial clones of cassava (*Manihot esculenta* Crantz) and its dependence on the type of soil and extend the Red Ferrallitic ones, the efficient management criteria of AMF strains in this crop.

MATERIALS AND METHODS

The experiments were performed on two types of soil, a) Brown loose Carbonated (17), classified internationally as Feozem endocarbonated (18) during the years 2012 and 2013 in areas of the Research Institute of Tropical Viands (INIVIT) located in the municipality of Santo Domingo, Villa Clara province, to 22° 35' north latitude and 80° 18' west longitude and 40 m s. n. m.; b) Red Ferrallitic Leachate (17), also classified as Ferralic Nitisol, Rodic, Lixic, Eutric (18) during the years 2013 and 2014, in the experimental areas of the National Institute of Agricultural Sciences (INCA), located in San Jose, las Lajas, Mayabeque province, to de 23° 01' north latitude and 82° 08' west longitude and 120 m s. n. m.

The main chemical characteristics of these soils and content of AMF spores "resident" in them are presented in Table I.

Brown loose carbonated soils presented values of pH-H₂O slightly alkaline, with low phosphorus content and means available potassium and high contents of Ca and Mg exchangeable with high CIC about 50 c mol kg⁻¹, being so generally, values representative of this type of soil. Organic matter presented low percentages of 2,0 %.

"Residents" spores of AMF in the initial conditions were very low and less than 30 to 50 g, similar to the contents obtained by other authors in these same soils (8, 20) and possibly associated with intensive farming and systematic fertilization that these soils have received.

^C Oficina Nacional de Estadística e Información. *Sector agropecuario. Indicadores seleccionados* [en línea]. 2014. [Consultado: 10 de diciembre de 2015]. Disponible en: <http://www.one.cu/publicaciones/05agropecuario/ppalesindsectoragrop/ppales_inddic13.pdf>.

^D Ruiz, L. *Efectividad de las asociaciones micorrizicas en raíces y tubérculos en dos tipos de suelos*. Tesis de Doctorado, Universidad Agraria de La Habana-Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba, 2001, 101 p.

Table I. Some chemical characteristics of the soils at the experiment beginning (*) and amount AMF "resident" spores (0-20 cm depth)

Types of soils	pH H ₂ O	MO (%)	P (mg kg ⁻¹)	Na ⁺	K ⁺ (cmol _c kg ⁻¹)	Ca ²⁺	Mg ⁺	Spores # 50 g ⁻¹
Ferralitic Red leachate	6,6	3,20	341,2	0,12	0,25	11,9	2,4	130
Brown Fluffy Carbonated	7,4	2,0	1,75	0,35	0,46	32,0	4,2	28

*Mean values of 8 samplings (four in each year)

Analytical techniques used^E (19): pH-H₂O, potentiometry with extraction 1: 2,5; organic matter (OM), wet digestion according Walkey and Black; P (assimilable), colorimetry with extraction 1:25 with H₂SO₄ 0,025 M; exchangeable cations, titration with 1 M NH₄Ac extraction. Spores AMF, decanting-wet method modified

Annual rainfall during the experimental period in the area of Fluffy Brown Carbonated soil was 1626,4 mm in 2012 and 1519,1 mm in 2013, above the average for the last 25 years (1349 mm). The annual average temperatures were 24,1 and 24,6 °C, respectively, and similar to historical 25 years (24,3 °C).

Red Ferralitic Leachate soil presented neutral pH with contents of exchangeable Ca and Mg in the range of 11,9 and 2,6 cmol kg⁻¹ respectively, which are typical for these soils. The contents of available phosphorus were high and strong previous applications related to mineral fertilizers. The residents were low and possibly spores associated with previous fertilizer applications and high availability of phosphorus in the soil.

The organic matter contents were high for this type of Ferralitic soil located 120 m s. n. m. and with a system of annual average temperatures over the last 25 years of 24,2 °C.

The annual rainfall in 2013 corresponded to a rainy year, 1709,9 mm and 1414,2 mm in 2014, slightly below the historical average of the last 25 years (1591,8 mm), which allow consider this location as an area under adequate rainfall. The annual average temperatures were 24,2 and 23,8 °C in 2013 and 2014 respectively, with little variation among the years and similar to the average of the last 25 years.

AMF STRAINS EXPERIMENT AND CASSAVA CLONES IN FLUFFY BROWN CARBONATED SOILS

The behavior of different AMF strains was tested against six clones of *Manihot esculenta* Crantz, in a randomized block design factorial arrangement and four replications. A factor of six levels, where AMF strains included, namely 0 % NPK, 25 % NPK, 25 % NPK + *Funneliformis mosseae* (21), 25 % NPK + *Glomus cubense* (15), 25 % NPK + *Rizophagus*

intraradices (21) and 100% NPK; B factor, consisting of clones, also composed of six levels and the C factor by two levels, corresponding to the repetition in two years.

The key to identifying the AMF strains in the National Institute of Agricultural Sciences of Cuba collection corresponds to INCAM-2, INCAM-4 and INCAM-11, respectively. In all cases, the content of spore in the inocula ranged from 25 to 30 spores g⁻¹ soil and they were supplied by the Department of Biofertilizers and Plant Nutrition of this institution.

The dose of fertilizer used in conjunction with the implementation of the AMF strains was based on previous results^D, which established that in the presence of efficient strain inoculation for the soil condition, the requirements of mineral fertilizers for proper mycorrhizal operation were 25 % of the mineral fertilizer dose (NPK), used to obtain high yields. Doses of 150, 60 and 200 kg were used as 100 % NPK ha⁻¹ of N, P₂O₅ and K₂O, respectively.

Cassava clones used were 'INIVIT E 80+1', 'CMC-40', 'INIVIT and 93-4', 'CEMSA 74-6329', 'CEMSA 74-725' and 'Señorita', belonging to the collection of INIVIT and supplied by the institution, which are considered commercial clones for cultivation in the country (13). The planting (13) and the planting date of each clone are presented in Table II.

The plots were composed of 32 plants, of which 12 were evaluated, performing the harvest at 12 months of planted clones. The experiment was repeated twice.

EXPERIMENT OF AMF STRAINS IN FERRALITIC RED LEACHATE SOILS

The behavior of three AMF strains and a control treatment in a randomized block design with five replicates was evaluated. The clone used was the 'CMC-40'. AMF strains used were the same as assessed in the above described experiment and with similar characteristics.

^EHerrera, 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. ser. Monasterio M.), (ser. Diversidad Biológica), Programa Iberoamericano de Ciencia y tecnología para el desarrollo, Subprograma XII, Mérida, 1995.

Table II. Clones used frames and planting dates in the experiment in Carbonated Brown fluffy soil

Clons	Planting frames (m)	Date of planting
'INIVIT E 80+1'	0,9 x 0,90	January 15
'CMC-40'	0,9 x 1,0	January 15
INIVIT Y 93-4'	0,9 x 1,0	January 15
'CEMSA 74-6329'	0,9 x 1,0	January 15
'CEMSA 74-725'	0,9 x 0,90	January 15
'Señorita'	0,9 x 1,0	January 15

The experiment was repeated twice. The first began on February 26th, 2013 and none of the treatments received mineral fertilizers. In the second one, which was planted on February 5th, 2014, all treatments were 50, 20 and 75 kg ha⁻¹ of N, P₂O₅ and K₂O respectively, which corresponds to the recommendation of mineral fertilizers optimal dose to achieve an effective mycorrhization of crop in this soil type (16).

The planting frame was 0,90 x 1,2 m and plots were 32 plants, which were evaluated 12. The harvest took place in both replicates at nine months of planting.

COMMON ACTIVITIES

In all cases the cultural care were implemented according to the technical instructions of the culture (13), applying irrigation in planting and subsequently with a ten-year frequency, until the rainy stage in different years stabilized.

Inoculation of AMF strains was applied by means of coating both ends of cassava plantlets (8), in amounts of 1,16 g stake⁻¹ of mycorrhizal inoculant and just before planting.

EVALUATIONS AND DONE DETERMINATIONS

The initial count of AMF spores was obtained from four soil samples taken prior to planting each experiment. For extraction proceeded according to initial protocol amendment^E of Gerdemann and Nicholson; subsequently they were washed with distilled water and poured into petri dish, for counting using stereo microscope 700x (Stemi 2000-C).

In the case of experiment on Red Ferralitic soil and in the second year, sampling was performed to determine AMF spores in the rhizosphere of plants of each plot to the depth of 0-20 cm. This sampling was carried out when the plants were 5½ months.

In both experiments and calculation plants of each treatment were taken, 4½ months and planted cassava, composite samples per plot to assess the percentage of mycorrhizal colonization, except for the first year of the experiment on Ferralitic Red in this sampling was performed.

200 mg of roots were used in each sample, which were dried in an oven at 70 °C to constant mass to be

dried (22). The evaluation was performed in stereo microscope 70x (Stemi 2000-C) and then the intercepts method (23) was used for determination.

The experiments were harvested manually using plants of each plot. The harvest was quantified separately commercial and noncommercial roots and fresh mass in kg plot⁻¹ were determined. The results were expressed in t ha⁻¹ of commercial real estate, according to the planting of each clone.

STATISTICAL PROCESSING

For the experiment in Fluffy Brown Carbonated soils, as interest was assessed for each clone the application effect of the different AMF strains, we proceeded to split meaningful interaction higher order, so that for each clone evaluated the factor level effect in strains was included.

In the experiment in Ferralitic Red Leachate soils, randomized block with four treatments, analysis of variance double for each of the years of work classification was performed. In all cases the docimación among treatments was performed by Duncan test at P≤0,05.

RESULTS

EFFECTIVENESS EXPERIMENT OF AMF IN MANY CLONES ON CARBONATED BROWN FLUFFY SOIL

The statistical analysis of the variables, yield and percentage of mycorrhizal colonization showed significant interaction of the factors (P≤0,05), but while performance interaction A factor (strains) x B (clones) x C (repeat) was significant in the case of the percentage of colonization, the interaction of third order was not and the corresponding factors A x B was significant (Table III).

This led to differences in how to present the unfolding of the interaction (Tables IV and V). In all clones showed a significant yield response to mineral fertilization (Table IV) and high yields were found in treatments receiving 100 % of fertilization (14), ranging between 40 and 50 t ha⁻¹, depending on the clone and away from this behavior the clon'CEMSA 74-6329 'that did not reach the 30 t ha⁻¹.

Also, a differentiated response of clone performance to inoculation with AMF strains (Table IV), in such a way was found that in any of the clones and years, inoculation with *R. intraradices* strain in the presence of fertilization with 25 % NPK, also originated high yields, always significantly higher than those obtained with the homologous treatment uninoculated (25 % NPK) and similar yields to the treatment received the highest doses of fertilizers except clone 'CEMSA 74-6329', in which the application of

R. intraradices originated even higher yields which received higher doses of fertilization.

In some of the clones as CEMSA 74-725 and INIVIT E 80+1, it was also presented, high compatibility with the strain *G. Cubense*, which was also extended to *F. mosseae* in clone CEMSA 74-725, of such that inoculating any of them, a significant and similar response to that obtained by inoculation with *R. intraradices* is found.

Table III. Experiment on Brown Fluffy Carbonated soil. Significance of the different terms of interaction and factors for ANOVA of the variables: yield and percentage of mycorrhizal colonization

Origin	Yield t ha ⁻¹ Signification	Colonization % Signification
Corrected model	0,000	0,000
Intersection	0,000	0,000
A x B x C	0,000	0,979
A x B	0,000	0,000
A x C	0,080	0,004
B x C	0,000	0,771
A	0,000	0,000
B	0,000	0,000
C	0,136	0,868
(replicates x year)	0,394	0,526

Factor A (include AMF strain); Factor B (Clons); Factor C (Years)

Table IV. Effect of treatments on yield, (t ha⁻¹), cleavage of A x B x C interaction: of level effect of A factor (includes strains) for each combination B (clones) x C (years)

Factor B (levels)	Factor C (levels)	Factor A (levels)					
		0 % NPK	25 % NPK	100 % NPK	<i>R. intra.*</i>	<i>G. cub.*</i>	<i>F. mos.*</i>
'INIVIT E 80+1'	year 1	36,02 c	38,02 b	42,01 a	41,93 a	41,45 a	38,91 b
	year 2	37,18 d	41,37 c	45,23 ab	46,90 a	46,30 a	44,24 b
'CMC-40'	year 1	37,72 c	39,09 c	46,03 a	44,92 a	42,15 b	41,37 b
	year 2	35,91 c	42,32 b	48,18 a	47,18 a	43,93 b	43,23 b
'INIVIT Y 93-4'	year 1	40,35 d	43,19 c	46,41 ab	47,64 a	45,00 bc	43,59 c
	year 2	43,19 c	44,77 c	50,77 ab	51,75 a	48,62 b	48,91 b
'CEMSA 74-6329'	year 1	25,66 c	28,73 b	29,67 b	33,60 a	30,40 b	28,50 b
	year 2	23,09 c	25,86 b	26,70 a	28,30 a	26,03 b	25,65 b
'CEMSA 74-725'	year 1	26,27 c	31,53 b	44,16 a	44,41 a	43,03 a	42,58 a
	year 2	25,90 c	28,38 b	39,74 a	38,47 a	38,72 a	38,32 a
'Señorita'	year 1	35,80 d	41,36 c	47,51 a	47,57 a	43,03 c	44,53 bc
	year 2	34,20 d	37,70 c	42,76 a	42,07 a	39,50 b	38,90 bc
Es x				0,588**			

R. intra. (*R. intraradices*); *G. cub.* (*G. cubense*); *F. mos.* (*F. mosseae*). The docimation was performed by the test of Duncan P≤0,05

* These treatments always received a 25 % NPK fertilization

Table V. Percentage of mycorrhizal colonization. Cleavage of A interaction (includes strains) x B (clones): effect of A factor levels per each clone

Factor B (levels)	Factor A (levels)					
	0 % NPK	25 % NPK	100 % NPK	<i>R. intra.*</i>	<i>G. cub.*</i>	<i>F. mos.*</i>
'INIVIT E 80+1'	12,50 e	15,63 d	15,75 d	73,5 a	68,25 b	62,88 c
'CMC-40'	12,13 e	15,88 d	16,00 d	73,88 a	68,88 b	63,25 c
'INIVIT Y 93-4'	14,38 e	16,63 d	16,38 d	75,50 a	70,13 b	65,13 c
'CEMSA 74-6329'	10,13 e	15,00 d	14,75 d	69,75 a	63,88 b	60,38 c
'CEMSA 74-725'	10,50 e	15,38 d	15,13 d	71,63 a	64,75 b	61,63 c
'Señorita'	11,88 e	15,75 d	15,38 d	73,00 a	66,38 b	62,00 c
Media general	11,92	15,71	15,57	72,88	67,05	62,55
ES \bar{x}				0,27**		

R. intra. (*R. intraradices*); *G. cub.* (*G. cubense*); *F. mos.* (*F. mosseae*). The docimation was performed by the test of Duncan P≤0,05

* These treatments always received a 25 % NPK fertilization

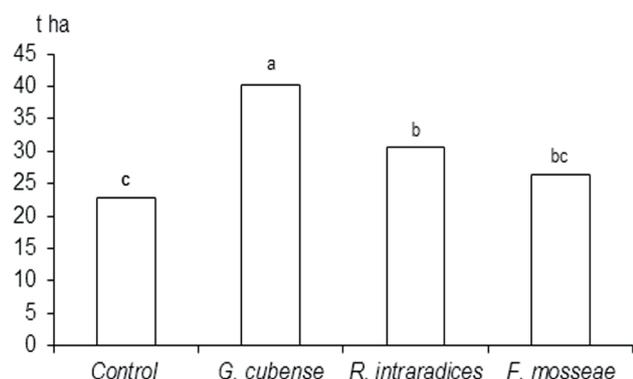
In the other clones *R. intraradices* strain always presented a higher and significant effect ($P \leq 0,05$), differing from those obtained by the other strains. The differential effects of the strains were maintained in either year, making clear the reproducibility of the found effects.

Also, a significant response of AMF strains inoculation on the percentage of mycorrhizal colonization was found (Table V), presenting no effects of years. This variable always existed significant differences between the effects caused by the application of each of the strains for any of the clones, so that is ordered as follows: *R. intraradices* > *G. Cubense* > *F. mosseae* and all higher than those found in non-inoculated treatments significantly. With *R. Intraradices* highest values were reached between 70 and 75 % colonization.

Uninoculated treatments always had low values, between 10 and 15 %, with a tendency to have similar percentages between the two fertilized treatments, which were slightly higher than those found in the unfertilized treatment.

EXPERIMENTS OF COMPARISON PF AMF STRAINS ON FERRALITIC RED LEACHATE SOIL

The results were similar in both years, always with a significant response to inoculation with the strain *G. Cubense*, which differed significantly from other treatments. In the first year in which background fertilizer (Figure 1) were not applied, the response was much greater and the order of 73 % compared to control treatment. In the same year, the application of *R. intraradices* was also significantly superior to the control treatment, although no differences with the application of *F. mosseae*.



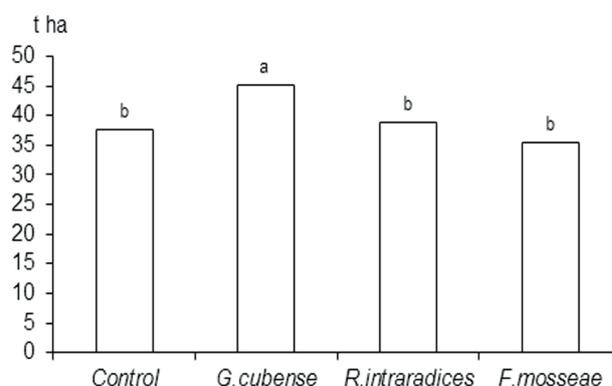
No fertilizers were applied in any tratamiento. ES x = 1.15
Different letters mean significant differences according to Duncan test $p \leq 0,05$

Figure 1. Influence of AMF strains inoculation on the clone 'CMC-40' performance in Ferralitic Red leachate. Year 2013

In the second year the answer to *G. Cubense* was lower than in the first (Figure 2), although with significant increases between 15 and 28 %, related to the rest of treatments and significant differences ($P < 0,05$) were not presented among obtained yields with the other strains and control treatment.

With the application of *G. Cubense*, yields of both experiments were high and the order of 40-45 t ha⁻¹.

Regarding the mycorrhizal colonization percentage (Table VI), significant differences ($P < 0,01$) among treatments, as well as higher values and similar to each other with applications *G. Cubense*. and *R. intraradices* were found. The application of *F. mosseae* treatment did not differ from control.



All treatments received 50, 20 and 75 kg ha⁻¹ of N, P₂O₅ y K₂O ES x= 0,30**

Different letters imply significant differences according to Duncan's test $p \leq 0,05$

Figure 2. Influence of AMF strain inoculation on the performance of CMC-40 clone in Ferralitic Red Leachate. Year 2014

Table VI. AMF inoculation influence on the mycorrhizal colonization and number of spores percentages in the Red Ferralitic Leachate soil. Year 2014

	Total colonization	Spore numbers
	%	# g ⁻¹
Control	33,1 b	10,0 c
<i>G. cubense</i>	44,4 a	14,3 a
<i>R. intraradices</i>	47,5 a	13,6 ab
<i>F. mosseae</i>	28,8 a	12,0 bc
ES \bar{x} =	1,92**	0,49**

Different letters lead to significant differences between treatments $P \leq 0,05$, according to Duncan test at $P \leq 0,05$

Regarding the amount of mycorrhizal spores (Table VI), the effects were similar; values were higher and no difference each other by the application of *G. cubense* and *R. intraradices*, being both greater than the control treatment. While the application differed significantly *G. Cubense* spores obtained with *F. mosseae*, inoculation of *R. intraradices* was no

different. Moreover, the application of *F. mosseae* not different from the number of spores observed in the control treatment.

DISCUSSION

Manihot esculenta is a highly mycotroph crop^D (6, 7) and its results corroborate it also indicating the application effectiveness of AMF efficient strains by way of coating the tips of propagules (8).

Under the conditions of Fluffy Brown Carbonated soils, a strong response to the application of *R. intraradices* in the presence of 25 % of the recommended high yields fertilization was found (13), achieving satisfactory yields in any of the clones, expanding the results for clones' Señorita^D and CMC 40 (8).

The results highlighted in the differential response to inoculation strains in different AMF clones. While there were two types of clones; one in which always the best results were obtained with *R. intraradices* and others in which any of the strains caused similar and significant effects on uninoculated treatment, the results do not change the *R. intraradices* recommendation as AMF efficient strain in these conditions^D (12) and whose application is always the greatest effects will be obtained in any of the clones.

Other authors^D have found compatibility of 'Señorita' clone with *G. manihotis* strain in two soil conditions, which did not prevent this clone was also effectively partner with AMF strains recommended for inoculation of mycorrhizal crops in each of the two soil conditions studied.

That is, the existence of compatibility between some of the clones and AMF strains did not prevent equally effective partnerships for any of the clones with the strain that works satisfactorily and, in general, be established in this condition the soil. With a broader scope, integrating 39 experiments comparing AMF strains in different crops and soils, this important behavior was obtained (16).

The behavior of mycorrhizal colonization percentage was different since in this case always higher percentages were found in any of the clones with the *R. intraradices* application. This behavior was different from that obtained by others^{D, F}, who reported that the strains presented compatibilities

with clones or varieties; these were expressed not only in performance but also indicators of mycorrhizal functioning, with no differences among the colonization percentages obtained with efficient strains and compatible ones.

In soils Ferralitic Red Leachate soils, but also response to mycorrhizal inoculation was found, was *G. Cubense* which always presented the greatest impact, both in performance and in the percentages of mycorrhizal colonization and number of spores.

The differences found between the response magnitude to the application of *G. Cubense*, in both years appear to be associated with the differentiated application of mineral fertilizer background in the two years.

The smallest increase in the yield obtained by inoculation in the second year, associated with high yields with the fertilized reference treatment and average rates of colonization obtained in the treatments inoculated with *G. Cubense*, seem to indicate that the amounts of mineral fertilizers applied were higher than that required optimal inoculated culture and, therefore, the percentage of mycorrhizal colonization was not so high, behavior that has been reported in this crop and other^D (12).

It should be noted that the amounts of applied fertilizer had been previously reported in this same soil type, as suitable for effective mycorrhizal operation (24), being clear then the need to recommend doses of fertilizer for crops inoculated according to specific availability of nutrients in the soil (12).

While previously *G. Cubense* has been recommended as AMF efficient strain for crops dependent on mycorrhization in this soil condition (24), there were no specific experimental reports for the cultivation of cassava in these soil conditions and therefore with these results is included in complying with such regularity.

The differences between the percentages of colonization of witnesses and other treatments in a soil condition, with higher percentages in the Ferralitic Red Leachate soil, appear to be a result of higher initial amounts of "resident" spores in these soils.

The achieved information notes the determinism of edaphic factor in the effectiveness and behavior of AMF strains (12, 16) in the group of AMF studied strains

Recently noted the importance of soil pH as one of its properties that are more related to the variation in effectiveness of the inoculated strains (16), possibly is in keeping with the importance of soil reaction on the occurrence and distribution of AMF strains in different agro-ecosystems.

^F González, P. J. *Manejo efectivo de la simbiosis micorrizica arbuscular vía inoculación y la fertilización mineral en pastos del género Brachiaria* [en línea]. [Tesis de Doctorado], Universidad Agraria de La Habana-Instituto Nacional de Ciencias Agrícolas, Mayabeque, Cuba, 2014, 128 p., [Consultado: 10 de enero de 2015], Disponible en: <<http://www.inca.edu.cu/redmicorizas/docs/posgrados/resumen/2.pdf>>.

Under the conditions of the soils studied, with near neutral pH or slightly basic, low effectiveness of the *F. mosseae* strain (16) is reported, which is corroborated in these works, when the strain showed the lowest effectiveness of three studied; but nevertheless; this becomes efficient strain Ferruginous Nodular Gley soil with pH 4,7 (16).

Although previously it has been reported the existence of a low specificity in AMF efficient strain with crops (12), the fact that *R. intraradices* is recommended as efficient AMF strain for crops in soils Brown Fluffy Carbonated, also it established an effective mycorrhization with any of the commercial clones, which has a high practical value for use in production of mycorrhizal inoculants scale in this crop.

The significant change in effectiveness of AMF strains depending on the soil, the very existence of a low specificity AMF efficient strain -culture and therefore the recommendations of efficient strains per soil type regardless of the culture (16) permit assume that in other soil conditions that cassava clones are planted, they also establish mycorrhizal effective associations with AMF efficient strain recommended for these soil conditions, regardless of compatibilities of any of the clones are presented with either AMF strain.

The results also confirm the application method effectiveness of mycorrhizal inoculant, via inoculation of the tips of propagules (8) for different cassava clones used.

CONCLUSIONS

- ◆ It is confirmed that the cassava has a high response to inoculation with AMF efficient strains.
- ◆ The effectiveness of the strains is different among soils, so that *R. intraradices* behaves as AMF efficient strain in a Brown Fluffy Carbonated soil with pH 7,4 and *G. cubense* in Red Ferrallitic leachate soil pH about 6,5.
- ◆ Compatibilities that presented some clones with any AMF strain did not prevent also providing an equally effective symbiosis with the efficient strain.
- ◆ Recommendations for efficient strains per soil type match those previously found in different cultures.

BIBLIOGRAPHY

1. Buitrago, A. J. "Dry Cassava Root and Foliage Meal for Poultry, Swine and Ruminants" [en línea]. En: ed. Howeler R. H., *The Cassava Handbook. A Reference Manual based on the Asian Regional Cassava Training Course, held in Thailand*, edit. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 2011, pp. 665-692, [Consultado: 10 de diciembre de 2015], Disponible en: <http://ciat-library.ciat.cgiar.org/Articulos_Ciat/biblioteca/The%20Cassava%20Handbook%202011.pdf>.
2. Howeler, R. H. "Cassava Leaf Production for Animal Feeding" [en línea]. En: ed. Howeler R. H., *The Cassava Handbook. A Reference Manual based on the Asian Regional Cassava Training Course, held in Thailand*, edit. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 2011, pp. 665-692, [Consultado: 10 de diciembre de 2015], Disponible en: <http://ciat-library.ciat.cgiar.org/Articulos_Ciat/biblioteca/The%20Cassava%20Handbook%202011.pdf>.
3. Ospina, P. B.; Gallego, C. S.; Ospina, P. H. y Gil, J. L. "Use of Cassava for Small-scale Ethanol Production with Value-added By-products" [en línea]. En: ed. Howeler R. H., *The Cassava Handbook. A Reference Manual based on the Asian Regional Cassava Training Course, held in Thailand*, edit. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 2011, pp. 665-692, [Consultado: 10 de diciembre de 2015], Disponible en: <http://ciat-library.ciat.cgiar.org/Articulos_Ciat/biblioteca/The%20Cassava%20Handbook%202011.pdf>.
4. Aguilera, D. M. "La yuca en el Caribe colombiano: De cultivo ancestral a agroindustrial". *Aguaita. Revista del Observatorio del Caribe Colombiano*, no. 24, diciembre de 2012, pp. 64-99, ISSN 0124-0722.
5. Pérez, L. J.; Peña, T. E.; Llauger, R. R.; Rodríguez, N. A. y Rodríguez, M. S. *Proyección estratégica hasta el 2015. Programa Integral de los Cultivos Varios*. 1.a ed., edit. Liliana, La Habana, Cuba, 2010, 95 p., ISBN 978-959-7111-55-9.
6. Howeler, R. *Sustainable soil and crop management of cassava in Asia*. vol. 389, edit. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 2014, 280 p., ISBN 978-958-694-125-9.
7. Sieverding, E. *Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems*. edit. Bremer, 1991, 402 p., ISBN 978-3-88085-462-8.
8. Ruiz, L. A.; Simó, J. y Rivera, R. "Nuevo método para la inoculación micorrízica del cultivo de la yuca (*Manihot esculenta* Crantz)". *Cultivos Tropicales*, vol. 31, no. 3, septiembre de 2010, pp. 15-20, ISSN 0258-5936.
9. Rivera, E. R.; Fundora, S. L. R.; Calderón, P. A.; Martín, C. J. V.; Marrero, C. Y.; Martínez, L. R.; Simó, G. J.; Riera, N. M. y Joao, J. P. "La efectividad del biofertilizante EcoMic® en el cultivo de la yuca. Resultados de las campañas de extensiones con productores". *Cultivos Tropicales*, vol. 33, no. 1, marzo de 2012, pp. 5-10, ISSN 0258-5936.
10. Okon, I. E. "Field response of two cassava genotypes inoculated with arbuscular mycorrhizal fungus to *Gliricidia sepium* mulch in a Tropical Alfisol". *Botany Research International*, vol. 4, no. 1, 2011, pp. 04-08, ISSN 2221-3635.

11. Ceballos, I.; Ruiz, M.; Fernández, C.; Peña, R.; Rodríguez, A. y Sanders, I. R. "The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava". *PLoS One*, vol. 8, no. 8, 2013, p. 70633, ISSN 1932-6203, DOI 10.1371/journal.pone.0070633.
12. Rivera, R.; Fernández, F.; Fernández, K.; Ruiz, L.; Sánchez, C. y Riera, M. "Avances in the management of effective arbuscular mycorrhizal symbiosis in tropical ecosystems". En: *Mycorrhizae in Crop Production*, edit. Haworth Food & Agricultural Products Press, Binghamton, 10 de julio de 2006, pp. 151-196, ISBN 978-1-56022-307-8.
13. Rodríguez, M. S. R.; García, G. M. G.; Montiel, M. F.; Martínez, L. R. y Tejón, A. M. "Instructivo Técnico para la producción de semillas de yuca (*Manihot esculenta* Crantz)". En: *Instructivo técnico para la producción de semillas de viandas*, edit. Instituto de Investigaciones de Viandas Tropicales, La Habana, Cuba, 2012, pp. 9-30, ISBN 978-959-295-006-1.
14. Dirección Nacional de Suelos y Fertilizantes. *Mapa de Series de Suelos*. [1:50 000], edit. Científico Técnica, La Habana, Cuba, 1985.
15. Rodríguez, Y.; Dalpé, Y.; Séguin, S.; Fernández, K.; Fernández, F. y Rivera, R. A. "*Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba". *Mycotaxon*, vol. 118, no. 1, 5 de enero de 2012, pp. 337-347, ISSN 0093-4666, 2154-8889, DOI 10.5248/118.337.
16. Rivera, R.; Nápoles, M. C. y Espinosa, A. *Manejo conjunto e impacto de biofertilizantes micorrízicos y otros bioproductos en la producción agrícola de diferentes cultivos*. Anexo informe anual del megaproyecto, no. P131LH0010003, 2013, p. 18, DOI 10.13140/RG.2.1.4115.0565.
17. Hernández, A.; Pérez, J.; Bosch, D. y Castro, N. *Clasificación de los suelos de Cuba 2015*. edit. Ediciones INCA, Mayabeque, Cuba, 2015, 93 p., ISBN 978-959-7023-77-7.
18. IUSS Working Group WRB. *World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps*. (ser. World Soil Reports, no. ser. 106), edit. Food and Agriculture Organization of the United Nations, Rome, 2014, ISBN 978-92-5-108370-3.
19. Paneque, V. M.; Calderón, C. J.; Borges, A.; Hernández, T. y Caruncho, M. *Manual de Técnicas analíticas para Análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos*. edit. Ediciones INCA, La Habana, Cuba, 2010, ISBN 978-959-7023-51-7.
20. Simó González J.; L. Ruiz Martínez, R. Rivera Espinosa. Manejo de la simbiosis micorrízica arbuscular y suministro de nutrientes en plantaciones de banano cultivar 'FHIA-18' sobre suelos Pardos mullidos carbonatados. *Cultivos Tropicales*, vol. 36 no. 4, 2015, pp. 43-54. ISSN 0258-5936.
21. Schüßler, A. y Walker, C. "7 Evolution of the 'Plant-Symbiotic' Fungal Phylum, *Glomeromycota*" [en línea]. En: eds. Pöggeler S. y Wöstemeyer J., *Evolution of Fungi and Fungal-Like Organisms*, edit. Springer Berlin Heidelberg, 2011, pp. 163-185, ISBN 978-3-642-19973-8, [Consultado: 10 de diciembre de 2015], Disponible en: <http://link.springer.com/chapter/10.1007/978-3-642-19974-5_7>.
22. Phillips, J. M. y Hayman, D. S. "Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection". *Transactions of the British Mycological Society*, vol. 55, no. 1, agosto de 1970, pp. 158-185, ISSN 0007-1536, DOI 10.1016/S0007-1536(70)80110-3.
23. Giovannetti, M. y Mosse, B. "An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots". *New Phytologist*, vol. 84, no. 3, 1 de marzo de 1980, pp. 489-500, ISSN 1469-8137, DOI 10.1111/j.1469-8137.1980.tb04556.x.
24. Rivera, R., et al. *Manejo conjunto e impacto de biofertilizantes micorrízicos y otros bioproductos en la producción agrícola de diferentes cultivos*. [Informe Primer Semestre 2015 Proyecto P131LH0010003] Mayabeque: Instituto Nacional Ciencias Agrícolas, 2015. DOI: 10.13140/RG.2.1.2416.3605.

Received: August 10th, 2015

Accepted: November 12th, 2015