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# MYCORRHAL INOCULATION OF PRECEDENTS CROPS: A WAY TO MYCORRHIZE EFFICIENTLY SWEET POTATO (*Ipomoea batatas* LAM.)

Inoculación micorrízica de cultivos precedentes: vía para micorrizar eficientemente el boniato (*Ipomoea batatas* Lam.)

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ABSTRACT. Ipomea batatas L. (Lam) is a culture with high response to the inoculation of efficient strains of arbuscular mycorrhizal fungi; however, the high quantities of inoculants required does not make its application accessible on a productive scale. This research was carried out with the objective of studying the feasibility of using the 'effect of permanence' of the mycorrhizal inoculant applied to the previous crop, as a way to efficiently mycorrhize the sweet potato in succession. Two types of preceding crops were evaluated in the two sowing seasons, in moderately washed Calcaric Cambisol brown soils and repeated for two years. In the rainy season the precedents were Zea mays L. / var. MC-4 and Vigna unguiculata (L.) Walp. / var. Guariba and in the dry season, corn and beans (*Phaseolus vulgaris* / var. BAT-306), in all cases the sweet potato variety 'CEMSA 78-354' was used and planted 30 days after the harvest of the preceding ones. The inoculation was performed with Rhizoglomus intraradices / INCAM-11 strain. In all crops and seasons a positive response was found to mycorrhizal inoculation, guaranteeing high yields of 3,6; 2,5 and 25 to 30 t ha<sup>-1</sup> of corn, beans and sweet potatoes respectively and an efficient mycorrhizal operation in the presence of only 50 % of the amounts of mineral fertilizers and with yields similar to those obtained in the different crops when receiving the 100 % fertilization. With any of the precedents inoculated and in both seasons, a positive effect of permanence of the inoculant was achieved, which allowed to efficiently mycorrhize the sweet potato in succession, not being necessary the direct inoculation of the sweet potato to reach the benefits of an effective mycorrhization.

Key words: beans, AMF, corn, succession

RESUMEN. Ipomea batatas L. (Lam) es un cultivo con alta respuesta a la inoculación de cepas eficientes de hongos micorrízico arbusculares; sin embargo, las altas cantidades de inoculantes que requiere no hacen accesible su aplicación a escala productiva. Esta investigación se realizó con el objetivo de estudiar la factibilidad de utilizar el 'efecto de permanencia' del inoculante micorrízico aplicado al cultivo precedente, como vía para micorrizar eficientemente el boniato en sucesión. Se evaluaron dos tipos de cultivos precedentes en las dos épocas de siembra, en suelos Pardo argénico medianamente lavado y repetidos durante dos años. En la época lluviosa los precedentes fueron Zea mays L. / var. MC-4 y Vigna unguiculata (L.) Walp. / var. Guariba y en la época poco lluviosa, maíz y frijol (Phaseolus vulgaris / var. BAT-306), en todos los casos se utilizó la variedad de boniato 'CEMSA 78-354' y plantada 30 días después de la cosecha de los precedentes. La inoculación se realizó con la cepa de Rhizoglomus intraradices/INCAM-11. En todos los cultivos y épocas se encontró una respuesta positiva a la inoculación micorrízica, garantizando altos rendimientos de 3,6; 2,5 y 25 a 30 t ha<sup>-1</sup> de maíz, frijol y boniato respectivamente y un funcionamiento micorrízico eficiente en presencia de solo el 50 % de las cantidades de fertilizantes minerales y con rendimientos similares a los obtenidos en los diferentes cultivos al recibir el 100 % de la fertilización. Con cualquiera de los precedentes inoculados y en ambas épocas, se logró un positivo efecto de permanencia del inoculante, que permitió micorrizar eficientemente el boniato en sucesión, no siendo necesaria la inoculación directa del boniato para alcanzar los beneficios de una micorrización efectiva.

Palabras clave: frijol, HMA, maíz, sucesión

# INTRODUCTION

There is a worldwide recognition of the importance of mycorrhizal symbiosis in ecosystems, as a mechanism of adaptation of plants to different stressful

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conditions, through increases in the absorption of nutrients and water, improvements in soil aggregates, the effect of bioprotection against some pathogens among other benefits (1-3); However, one of the main challenges to achieve these benefits in agroecosystems is their integration in crop technologies.

In recent years, a large group of positive results has been obtained in Cuba on the management of the arbuscular mycorrhizal symbiosis in agroecosystems, from the existence of inoculants that are applied in low quantities and the knowledge of the bases for an effective management of these (4,5) and integrated not only with mineral fertilizers, but with green and organic fertilizers (4,6,7).

The sweet potato (*Ipomoea batatas* Lam), mycotrophic culture (8), has not been the exception and positive results have been obtained with the inoculation of efficient strains of arbuscular mycorrhizal fungi (AMF), decreasing the amount of fertilizers necessary to obtain high yields (9-11); however, even using the coating of the cuttings (9), high quantities of inoculants (35 kg ha<sup>-1</sup>) are required, which make their use at a productive scale unfeasible.

The use of efficient and generalist strains with plant species (12) as the basis of simple inoculants, together with the effective reproduction of propagules by mycotic cultures inoculated with efficient strains seem to explain the positive effect of permanence of the inoculant applied it has been reported (10,13) and that guarantees an effective mycorrhization of the crop in succession, nevertheless, in these works, the influence of the previous inoculated crop type, nor of the sowing time of these was not studied.

The effect of permanence of the efficient strain applied must depend among others on the reproduction capacity of mycorrhizal propagules by the inoculated culture and a component of this capacity could be the profuse mycorrhizal root system (4).

Therefore, from using as precedents of the sweet potato, mycotic cultures with different types of radical systems and common in Cuban agriculture, the present work was developed with the objectives of: 1) establishing if the effect of permanence was manifested, 2) if the previous crop type had an

influence on this effect 3) if the time of planting the previous crop influenced it.

# MATERIALS AND METHODS

The research was carried out at the Research Institute of Tropical Roots and Tuber Crops (INIVIT) located at 22°35'N, 80018' W and 40 m a.s.l, in Santo Domingo municipality, Villa Clara Province, Cuba, on argenic Brown soil moderately washed (14), also classified as Calcaric Cambisol (15), during the period 2013 to 2015.

The soils in the experimental area presented similar characteristics (Table 1), with a neutral reaction and low values of organic matter possibly associated with continuous cultivation. The contents of available phosphorus were low and those of potassium were medium. The exchangeable calcium and magnesium showed high and typical values of these soils. Mycorrhizal spores were low, similar to those reported in these soils (7,9).

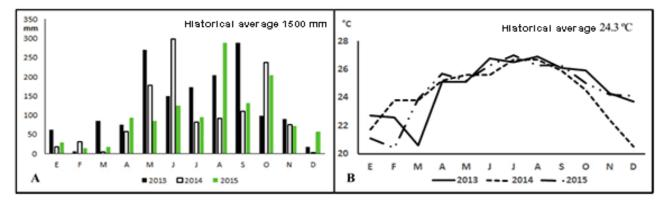
Average monthly precipitation and temperatures in the experimental period (Figure 1) reflected a year 2013 with annual rainfall of 1519 mm, similar to the historical average of 1500 mm year<sup>1</sup> and the other two years with annual rainfall of the order of 80 %; but in all cases around 80% occurred in the period from May to October. Temperatures were very similar between three years with annual averages between 24.3 and 24.6 °C and similar to the historical average of 24.3 °C.

The experiments were developed by planting the preceding crops (Table 2), during the rainy season (May or June) and during the dry season (January or February). In the case of sowing in the rainy season, the preceding crops were maize (*Zea maize* L./var MC-4) and vigna bean (*Vigna unguiculata* (L.) Walp./ Var. Guariba), in the case of the same variety of corn and common bean (*Phaseolus vulgaris*/var. BAT-306) was used in November. The fact of using vigna bean in summer and common bean in January or February was due to the fact that the common bean is not sown as an economic crop in the summer season. In each era, two campaigns were carried out.

Table 1. Initial characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental characterization of the soil, at 0 - 20 cm depth, in the experimental	nental area
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Year	р	Н	Nt	МО	P <sub>2</sub> 0 <sub>5</sub>	K <sub>2</sub> 0	Ca <sup>2+</sup>	$Mg^{2+}$	$K^+$	Spores
Teal	KCl	$H_2O$	(g k	g-1)	mg l	kg-1	(	(cmol <sub>c</sub> kg <sup>-1</sup> )		in 50 g
1	6,1	7,0	1,4	18,6	17,4	20,20	24,15	4,0	0,46	41,5
±IC	±0,10	±0,10	±1,07	±0,14	±0,14	±0,06	±0,01	±0,62	±0,01	±2
2	6,0	6,9	1,5	18,2	17,3	21,25	26,23	4,1	0,43	42
±IC	±0,13	±0,07	±0,62	±0,12	±0,14	±0,02	±0,02	±0,06	±0,02	±2

IC = Confidence interval (1- $\alpha$  = 0.05), Each value is average of 10 composite samples



Data from weather station # 326/INSMET, located at INIVIT (Research Institute of Tropical Root and Tuber Crops) Figure 1. Precipitations (A) and monthly average temperatures (B) during the experimental period

Season		Sowing	Harvest		Plantation	Harvest
Rainy	Corn year 1	10/6/2013	12/10/2013	sweet potato	12/11/2013	15/4/2014
	Vigna year 1	13 7/2013	13/10/2013	sweet potato	12/11/2013	15/4 /2014
	Corn year 2	15/6/2014	17/10/2014	sweet potato	15/11/2014	18/4/2015
	Vigna año 2	15/7/2014	17/10/2014	sweet potato	15/11/2014	18/4 /2015
Little rainy	Corn year 1	17/1/2013	21/5/2013	sweet potato	25/6/2013	30 /11/2013
	Bean year 1	12/2/2013	20/5/2013	sweet potato	25/6/2013	30/11/2013
	Corn year 2	15/1/2014	20/5/2014	sweet potato	22/6/2014	27/11/2014
	Beans year 2	15/2/2014	20/5/2014	sweet potato	22/6/2014	27/11/2014

After the harvest of the preceding crops, after the preparation of the soil and in a period of approximately 30 days, the sweet potato crop (*Ipomea batatas*, Lam / var.'CEMSA 78-354') was planted in the same plots and ensuring that the dates of planting in each season were the same, regardless of the precedent.

As the preceding cultures were different in the epochs and also do not have the same extension of their biological cycle, the experiments did not include the crops, or the time as factors and they were developed as independent experiments. The treatments (Table 3) and the design used in each experiment were similar, randomized blocks of four treatments, which included both the preceding crop stage and the sweet potato stage (factor A), repeated for two years (factor B), with factorial arrangement of 4x2 and with four replicas.

The plantation frames used in the different crops were  $0.9 \times 0.3$  m for corn, the common bean  $0.7 \times 0.2$ m and in the vigna  $0.9 \times 0.1$  m (17-19). In the cultivation of sweet potato the plantation frame was of  $0.9 \times 0.23$  m in the rainy period and  $0.9 \times 0.3$  m in the dry season (20). The plots were constituted by five furrows of six meters long, except for the bean that had six furrows. In each experiment, the preceding cultures and the sweet potato of each treatment were always located in the same plots.

Table 3. Treatments studied in the different experiments executed for both seasons and previous crops

	, <del>p</del> 5	
Treatments	Stage preceding crops <sup>1</sup>	Stage of cultivation of sweet potato <sup>2</sup>
1	50 % NPK+HMA	50 % NP 25 % K + HMA
2	50 % NPK+HMA	50 % NP 25 % K
3	50 % NPK	50 % NP 25 % K
4	100 % NPK	100 % NPK

1: Previous crops in the rainy season: maize (var MC-4) and vigna bean (var Guariba). Previous crops in a dry season: corn (MC-4 variety) and common bean (variety BAT-304). 2: The sweet potato variety used was 'CEMSA 78-354'. AMF strain: R. intraradices (INCAM 11). Fertilizer doses for the previous cultures inoculated and in the sweet potato was based on previous results (10,11,16)

#### NOCULANT AND MYCORRHIZAL INOCULATION

The inoculant was prepared at the National Institute of Agricultural Sciences (INCA), Mayabeque, Cuba, based on the strain of *Rhizoglomus intraradices* (21) (/INCAM 11) and using *Urochloa decumbens* (Hochst. Ex A. Rich) as a host plant. This strain is recommended for its effective behavior in this type of soil (5). The inoculant possessed a titer of 30 spores g<sup>-1</sup>.

The inoculation was carried out via the coating of the seeds. In the case of the grains, seeds were covered with an amount of inoculant equivalent to 10 % of the seed weight (16). In the case of the sweet potato, it was inoculated by coating the lower third of the cutting with an aqueous mixture of 12.5 kg of inoculant per 60 liters of water and applying the equivalent of 35 kg ha<sup>-1</sup> of inoculant (9).

### FERTILIZATION AND CULTURAL ATTENTION

The doses of fertilizers recommended to obtain high yields were identified as 100 % NPK and they were in the corn of 90, 130, 170; in the bean and the vigna of 80, 60, 90 and in the sweet potato of 120, 100, 300 kg ha<sup>-1</sup> of N,  $P_2O_5$  and  $K_2O$  respectively (17-20). The doses for optimal mycorrhizal functioning in the different inoculated precedents were 50 % NPK (16); in the case of the inoculated sweet potato 50 % NP, 25 % K were applied according to previous results (10,11).

The workings to the preceding crops and to the sweet potato were carried out according to the Technical Instructions of these crops (17-20). In the dry season the irrigation for the cultivation of corn was applied a standard of 350 m<sup>3</sup> ha<sup>-1</sup> every seven days until the formation of the cob, then it was increased to 400 m<sup>3</sup> ha<sup>-1</sup> and between 80 and 100 days it was suspended to let the grain ripen and dry (17); In the bean crop a standard of 350 to 480 m<sup>3</sup> ha<sup>-1</sup> was applied every 10 days (18) and in the cultivation of the sweet potato an application of 250-300 m<sup>3</sup> ha<sup>-1</sup> was made every seven days until 45 days and after every 10 days until suspending it 15 days before harvest (20). In the rainy period, irrigation was applied with the same criteria, when rainfall was delayed or did not equal the application norms of each period

### **EVALUATIONS**

**Soil analysis**. Samples of composite soils were taken in the depth of 0-20 cm in each of the replicas of the experiments and at the beginning of these. The determinations and methodologies carried out were: the pH in KCl and  $H_2O$ , with a relation soil-solution of 1: 2.5; total nitrogen (Nt) by the micro-Kjeldahl method; organic matter by the Walkley-Black method,  $P_2O_5$  and  $K_2O$  were performed by the Machiguin method with extraction with (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> solution with pH 9. The interchangeable cations Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup> were made by extraction with AcNH<sub>4</sub> 1N at pH 7.0 (22).

**Total root colonization with AMF** (%).It was carried out 45 days after sowing for corn, vigna and common bean and 90 days after planting for sweet potato. For the total colonization, samples of fine roots were taken from eight plants per plot, located in the central furrows. The roots were stained (23) and the evaluation by the intercepts method (24) was performed in a stereo microscope (Carl Zeiss, Stemi 2000-C/50x).

**Mycorrhizal spores**. They were determined at the beginning and at the time of harvest of each crop. A sample composed of 10 subsamples (0-20 cm) was taken per plot and the extraction of spores was carried out by the wet decantation method (25). The spores were counted with the use of the microscope and were expressed as spores in 50 g of soil.

**Yield**. The plants of three central rows of each plot were harvested, not using the beginning and end of each furrow. The yield at t ha<sup>-1</sup> was estimated and expressed in the grains based on 14 % moisture and in the sweet potato as edible tubers.

**Statistical analysis**. The statistical package of the SPSS-2012 Program was used to perform the ANOVA in each experiment and in the case of significant differences between the means, they were certified according to the Tukey multiple comparison test (p≤ 0.05). For the comparison of the means between different experiments, the confidence intervals p <0.05, estimated from the Es<sub>x</sub> obtained for each variable in each experiment, were used by the formula  $Z_{1-\alpha}^*$  Es<sub>x</sub>, where  $Z_1 = 1.96$ .

# RESULTS

In the statistical analyzes performed on each of the variables in the different experiments, the treatment interactions per year were not significant at p <0.05 (Table 4), so that in all cases; only the results of the treatment factor will be presented.

#### Table 4. P value of Interactions treatments x years for each of the variables evaluated in the different experiments

Stage	Crop	Yield (t ha <sup>-1</sup> )	Colonization (%)	Spores AMF 50 g soil <sup>-1</sup>
Rainy	Corn	0,48	0,329	0,125
	Sweet potato	0,230	0,606	0,282
	Vigna	0,993	0,905	0,765
	Sweet potato	0,994	0,600	0,189
Little rainy	Corn	0,602	0,863	0,314
	Sweet potato	0,583	0,476	0,108
	Bean	0,989	0,456	0,280
	Sweet potato	0,255	0,987	0,215

The identification of the stage corresponds to the sowing of the preceding one. The cultivation of sweet potato always follows the previous one used

# INOCULATION OF THE PRECEDING CROPS IN THE RAINY SEASON (TABLE 5)

Previous non-inoculated cultures responded positively to fertilization with higher yields (p <0.05) in treatments that received 100 % NPK. Inoculation with the efficient strain of AMF led to significant increases in yield, percentage of total colonization and number of spores in both corn and vigna, in relation to the uninoculated homolog treatment (50 % NPK), with significant differences between them in each of the variables.

The treatments inoculated in both precedents that received 50 % of the doses of recommended fertilizers presented high yields and similar to the non-inoculated treatments that received the doses of complete fertilization (100 % NPK).

Also, they reached high percentages of mycorrhizal colonization and although in the case of the vigna were close to 60 %, those of corn were higher (p < 0.05). In a similar way, although the spores increased between 8 and 15 times as a result of the inoculation of the previous ones, the values obtained with the corn were higher (p < 0.05).

The cultivation of the sweet potato responded in a similar way to the previous ones, since in the absence of inoculation the highest yields (p < 0.05) were obtained with the highest fertilizer doses and also presented a positive response to the inoculation, with yields and indicators higher mycorrhizal (p<0.05) to homologs (50 % NPK) that were never inoculated and with similar performances to the non-inoculated treatment that received the optimal dose of fertilization (100 % NPK). Also, whenever the precedents were inoculated, no significant differences were found between the yields and indicators of the mycorrhizal functioning of the sweet potato, whether or not it April-June

was inoculated, indicating that a positive effect of permanence of the inoculant applied to any of the preceding ones was obtained in this crop.

The positive effect of permanence of the inoculant applied, can also be observed in the significant increases obtained (p < 0.05) in the different variables evaluated in the sweet potato crop, between the treatments of the previous inoculated and sweet potato without inoculating in relation to the homologue who received the same fertilization, but who was never inoculated

# INOCULATION OF PREVIOUS CROPS DURING DRY SEASON (TABLE 6)

In a similar way to that obtained when precedents were sown in the rainy season and under conditions of response to fertilization, a significant response (p < 0.05) was also found to the inoculation with the efficient AMF strain with increases in the yield, percentage of total colonization and number of spores in both corn and beans, in relation to the non-inoculated homolog treatment (50 % NPK) and presenting the treatments inoculated yields similar to the treatments that received the fertilization doses of 100%.

Although the percentages of mycorrhizal colonization in the inoculated precedents were high, they were higher in corn in relation to beans and a similar behavior was observed in spores, which in this case increased between 7 and 11 times, associated with the highest increases in inoculated corn; However, in this period the values of both indicators of mycorrhizal functioning found in maize were lower than when this crop was planted in the rainy season, although no significant differences were found in the yield of maize sown in both seasons.

Table 5. Effect of the previous crop inocu	lated and sown in the rainy period in the permanence effect
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		Rainy season				Sweet p	otato/little rainy	period
Cultivar	Treatments	Yield t ha-1	Total colonization (%)	Spores in 50 g soil	Treatments	Yield t ha-1	Total colonization (%)	Spores in 50 g soil
	HMA+ 50 % NPK	3,62 a ± 0,06	75,6 a ± 1,08	743 a ±1,5	HMA+ 50 % NPK	$26,32 a \pm 0,49$	63 a ± 0,98	567 a ± 4,0
Corn	HMA +50 % NPK	3,63 a ± 0,06	74,4 a ± 1,08	741 a ± 1,5	50 % NPK	25,69 a ± 0,49	62,1 a ± 0,98	566 a ± 4,0
	50 % NPK	$2{,}95\text{ b}\pm0{,}06$	$12,5 b \pm 1,08$	$71,5 b \pm 1,5$	50 % NPK	$21,83 \text{ b} \pm ,49$	$10,1 \text{ b} \pm 0,98$	$63 b \pm 4,0$
	100% NPK	3,64 a ± 0,06	9,3 c $\pm$ 1,08	$62,8 c \pm 1,5$	100% NPK	25,9 a ± 0,49	$8,5 c \pm 0,98$	$54 c \pm 4,0$
	$\mathrm{Es}_{\overline{x}}$	0,03*	0,55*	0,75*	$\mathrm{Es}_{\overline{x}}$	0,25*	0,5*	1,96*
	HMA+ 50 % NPK	2,64 a ± 0,06	54,6 a $\pm$ 0,98	444 a ±3,52	HMA+ 50 % NPK	25,1 a $\pm$ 0,55	56,3 a ± 1,04	420 a ± 3,9
Viana	HMA +50 % NPK	2,62 a ± 0,06	54,1 a $\pm$ 0,98	439 a ±3,52	50 % NPK	24,34 a $\pm$ 0,55	54,6 a ± 1,04	419 a ± 3,9
Vigna	50 % NPK	2,02 b $\pm$ 0,06	$11,9 \text{ b} \pm 0,98$	$95 b \pm 3,52$	50 % NPK	21,48 b $\pm$ 0,55	$10,6 b \pm 1,04$	$52 b \pm 3,9$
	100% NPK	2,61 c $\pm$ 0,06	$8,9 \text{ b} \pm 0,98$	$87 c \pm 3,52$	100% NPK	25,38 a ± 0,55	$8,1 c \pm 1,04$	$46 b \pm 3,0$
	$\mathrm{Es}_{\overline{x}}$	0,03*	0,5*	1,8*	$\mathrm{Es}_{\overline{x}}$	0,28*	0,53*	1,94*

Averages with unequal letters in the same column of each experiment, differ by Tukey (HSD) for  $p < 0.05 \pm Confidence$  intervals from  $Z_{1-\alpha} * Es_{v}$ , being  $Z_1 = 1,96$ 

		Ι	little rainy period	1		Sweet potato / rainy period			
Cultivar	Treatments	Yield t ha <sup>-1</sup>	Total colonization (%)	Spores in 50 g soil	Treatments	Yield t ha <sup>-1</sup>	Total colonization (%)	Spores in 50 g suelo	
	HMA+ 50 % NPK	3,55a ± 0,02	65,7 a ± 0,86	560 a ± 2,35	HMA+ 50 % NPK	$30,52 a \pm 0,70$	$65,5 a \pm 0,84$	629 a ± 4,1	
Corn	HMA +50 % NPK	$3,56 a \pm 0,02$	$65,4 a \pm 0,86$	562 a ± 2,35	50 % NPK	29,91a± 0,70	$65,0 a \pm 0,84$	628 a ± 4,1	
	50 % NPK	$3{,}35\text{ b}\pm0{,}02$	$11,7 b \pm 0,86$	$102 b \pm 2,35$	50 % NPK	27,06 b $\pm$ 0,70	$11,5 b \pm 0,84$	$71 b \pm 4,1$	
	100% NPK	$3,55 c \pm 0,02$	9,4 c $\pm$ 0,86	92 c $\pm$ 2,35	100% NPK	$30,55 a \pm 0,70$	9,4 c $\pm$ 0,84	$61 c \pm 4,1$	
	$\mathrm{Es}_{x}$	0,01*	0,44*	1,2*	$\mathrm{Es}_{\overline{x}}$	0,36*	0,43*	2,05*	
	HMA+ 50 % NPK	2,57 a±0,18	55,1 a ± 0,69	376 a ± 2,94	HMA+ 50 % NPK	28,4 a $\pm$ 0,69	62,4 a ± 0,73	620 a ± 3,95	
D	HMA +50 % NPK	2,56 a ± 0,18	56,1 a $\pm$ 0,69	377 a ± 2,94	50 % NPK	$28,\!31a\pm0,\!69$	$61,4 a \pm 0,73$	519 a ± 3,95	
Bean	50 % NPK	$2,33b \pm 0,18$	11,1 b ± 0,69	$60 \text{ b} \pm 2{,}94$	50 % NPK	24,3 b $\pm$ 0,69	$10,8 b \pm 0,73$	$55 b \pm 3,95$	
	100% NPK	2,58 a ± 0,18	8,8 c $\pm$ 0,69	$53 c \pm 2,94$	100% NPK	28,85 a ± 0,69	$8,4 c \pm 0,73$	50 b ± 3,95	
	$\mathrm{Es}_{\overline{x}}$	0,09*	0,35*	1,5*	$\mathrm{Es}_{\overline{x}}$	0,35*	0,37*	2,02*	

Table 6. Effect of the previous crop inoculated and sown in the dry season in the effect of permanence

Averages with unequal letters in the same column of each experiment, differ by Tukey (HSD) for  $p < 0.05 \pm Confidence$  intervals from  $Z_{1-a}^* = Es_{v}^-$ , being  $Z_1 = 1,96$ 

Likewise, sweet potato cultivation responded significantly to the mycorrhizal inoculation performed in both previous cultures, with similar mycorrhizal yields and indicators (p <0.05) between the treatments with inoculated precedents and that were differentiated in the inoculation of the sweet potato and significantly higher (p<0.05) to homologs that received the same fertilization, but were never inoculated. Similarly, the inoculated treatments (50 % NPK) presented similar performances to the treatment that received the optimal dose of fertilization (100 % NPK). Therefore, the positive effect of permanence of the applied inoculant was also reached at this time with any of the preceding

## DISCUSSION

The results showed the positive effect of the inoculation with the efficient strain of *R. intraradices* /INCAM-11, reaching in all the crops high levels of yield and of colonization percentages in the presence of lower doses of fertilizers, indicative of an effective inoculation of agreement with the results previously reported by several authors when inoculating efficient strains of AMF in different cultures (4,6,26-28) and specifically with the sweet potato (11,29,30).

Likewise, it was found with the different preceding crops used and in both working seasons, a positive effect of permanence of the inoculant applied to the previous crop, effectively achieving mycorrhization of the sweet potato crop and guaranteeing the beneficial effects associated with this effective mycorrhizal functioning. In Cuba, although the inoculation permanence effect on crops in succession had already been reported in carbonated Brown soils (9.31) and in other types of soils (13.32.33), it had not been possible to evaluate the importance of the sowing time of the previous inoculated culture, nor of comparing the effectiveness of several preceding crops dependent on mycorrhization, but with differences in the root systems, in this case profuse systems such as corn and less developed as in the bean-type legumes and vigna and that according to previous results (4) should differ in their ability to reproduce spores and possibly mycorrhizal propagules in general.

However, although the quantities of spores reached by the inoculated maize were higher respectively than those reached with the vigna and the bean, and in turn in the rainy season the preceding crops also had higher amounts of spores and possibly in all associated cases with the largest root system of this grass in comparison with the legumes and the favorable effect of the precipitations and temperatures in the growth of the crops respectively, a positive and similar effect of permanence was established from any of the different preceding cultures inoculated and in both seasons, establishing its feasibility and indicating that the smallest amounts of spores and presumably of infective roots obtained by the legumes, were sufficient in these conditions to mycorrhize the sweet potato.

Although the spores as evaluated were total and non-specific and are also not the only mycorrhizal propagules, in a similar soil and inoculating *Canavalia ensiformis* as a precedent, a positive permanence effect was reported, valid for mycorrhization of banana and associated with contents of total spores similar to those found in these experiments, when legumes inoculated as precedents were used (31). Also, in experiments with Braquiaria species inoculated in sowing, in two types of soils and with six cuts in the year, direct relationships have been reported between the amounts of total spores found when executing each cut and the permanence of the inoculation in the next growth cycle; amounts <600 spores in 50 g were indicative of the need to reinoculate (27).

Although the existence of a differentiated effectiveness of inoculated AMF strains on the basis of the edaphic environment in which the mycorrhization takes place (5) and of finding a positive effect of permanence of these when inoculated to the crops, it can be assumed that at least one important percentage of the colonized roots of the sweet potato are with the inoculated strain; however, some authors consider that not necessarily the positive response to the inoculation of a strain is strictly due to the direct effect caused by it and they suggest the existence of complex interactions with resident mycorrhizae among the possible causes (34).

The use of molecular techniques to support the effect of permanence has managed to find the inoculated strains colonizing the crop in succession (35), other authors suggest that the presence does not necessarily quantify the participation in the positive effects found (34), therefore although the information obtained establishes a positive effect of permanence, much remains to be done in order to establish the mechanisms by which the inoculation of an efficient strain achieves positive and reproducible responses, including the effect of permanence.

In the experiments carried out, the period between the harvest of the precedents and the plantation of the sweet potato crop was 30 days, which is a period of time that is not commonly exceeded in the succession of the crops being this an important element to have in account for the reproducibility and implementation of these results. From the predictable decrease of mycorrhizal propagules to longer periods between crops, it becomes important to study the presence of this effect in longer periods and if there are then differences between the previous crop type and the seasons.

The results are a solution to use the benefits of the arbuscular mycorrhizal symbiosis via inoculation of efficient strains in the sweet potato, a culture with high dependence, but whose direct inoculation requires high amounts of inoculant. Also, the information suggests that it is a valid approach for other crops that are reproduced from agamic seed plants, such as taro and yam that also require high amounts of inoculants to cover the seeds (9).

## CONCLUSIONS

- The effect of permanence of the mycorrhizal inoculant applied to the preceding crops, guarantees an effective mycorrhization of the cultivation of the sweet potato planted in succession until 30 days after harvests of the preceding crops and allows to achieve the benefits of the symbiosis in the yield, mycorrhizal functioning and decrease of fertilizer quantities to obtain high yields, without the need of direct inoculation to this crop.
- This effect was achieved with different preceding crops, with response to mycorrhizal inoculation and commonly used in Cuban agriculture, such as maize and bean species, planted both in the rainy season and the rainy season, although always in the presence of irrigation and reaching these crops a good growth and development.

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