



# EVALUATION OF DIFFERENT STRAINS OF ARBUSCULAR MYCORRHIZAE IN THE DEVELOPMENT OF RICE PLANT (*Oryza sativa* L.) IN FLOODED SOIL CONDITIONS

## Evaluación de diferentes cepas de micorrizas arbusculares en el desarrollo de plantas de arroz (*Oryza sativa* L.) en condiciones inundadas del suelo

Michel Ruiz Sánchez<sup>1</sup>✉, Yaumara Muñoz Hernández<sup>2</sup>,  
José M. Dell'Amico Rodríguez<sup>1</sup>, Jaime Simó González<sup>3</sup>  
and Juan A. Cabrera Rodríguez<sup>1</sup>

**ABSTRACT.** The arbuscular mycorrhizal fungi are represented in all soils and colonize more than 80 % of terrestrial plants. Their association depends on many edaphoclimatic factors, plant and fungus. In rice cultivation mycorrhizal symbiosis was reported under anaerobic conditions. That is why the research was conducted in order to evaluate the response in rice plants inoculated with different strains of arbuscular mycorrhizal fungi (*Claroideoglossum claroideum*, *Glomus cubense*, *Rhizoglossum intraradices* y *Funneliformis mosseae*) in flooded conditions of Gley Nodular Hidromorphic Ferruginous Petroferric soil, with a water depth of 5 cm in the Scientific Technological Base Unit "Los Palacios". The results demonstrate that rice colonized, even with low percentages due to the presence of the water layer, which affects the establishment of the fungus in the plant. As for height, tillering, dry mass of the aerial part and roots in rice plants increased when there were inoculated with different strains than the control uninoculated observed a symbiotic efficiency higher depending on tillering and dry mass of the aerial part with strains *R. intraradices*, *G. cubense* and *C. claroideum*, but always strains *R. intraradices* and *G. cubense* were streigh among the strains of inoculum of AMF that showed greater effect on plant development. These results suggest the use of these strains in the production of bio-fertilizers for rice cultivation.

**RESUMEN.** Los hongos micorrízico arbusculares están representados en todos los suelos y colonizan a más del 80 % de las plantas terrestres. Su asociación depende de muchos factores edafoclimáticos, de la planta y del hongo. En el cultivo de arroz se informó simbiosis micorrízica en condiciones de anaerobiosis. Es por ello que la investigación se realizó con el objetivo de evaluar la respuesta en plantas de arroz inoculadas con diferentes cepas de hongos micorrízicas arbusculares (*Claroideoglossum claroideum*, *Glomus cubense*, *Rhizoglossum intraradices* y *Funneliformis mosseae*) en condiciones inundadas del suelo Hidromórfico Gley Nodular Ferruginoso Petroférico, con una lámina de agua 5 cm, en la Unidad Científica Tecnológica de Base "Los Palacios". Los resultados obtenidos demuestran que el arroz se coloniza, aunque con porcentajes bajos, debido a la presencia de la lámina de agua, la cual afecta el establecimiento del hongo en la planta. En cuanto a la altura, el ahijamiento, masa seca de la parte aérea y de las raíces en las plantas de arroz se incrementaron cuando se inocularon con las diferentes cepas respecto al testigo no inoculado, observándose una eficiencia simbiótica superior en función del ahijamiento y la masa seca de la parte aérea con las cepas *R. intraradices*, *G. cubense* y *C. claroideum*, aunque siempre las cepas *R. intraradices* y *G. cubense* estuvieron entre las cepas de inóculos de HMA que manifestaron mayor efecto en el desarrollo de las plantas. Estos resultados sugieran el uso de estas cepas en la producción de biofertilizantes para el cultivo del arroz.

**Key words:** mycorrhizal colonization,  
*Rhizoglossum intraradices*, *Glomus cubense*,  
aerobiosis, flooded

**Palabras clave:** colonización micorrízica,  
*Rhizoglossum intraradices*;  
*Glomus cubense*; aerobiosis, inundado

<sup>1</sup> Unidad Científico Tecnológica de Base «Los Palacios», Instituto Nacional de Ciencias Agrícolas, gaveta postal 1, San José de las Lajas, Mayabeque, CP 32 700, Cuba

<sup>2</sup> Universidad de Pinar del Río «Hermanos Saiz Montes de Oca», calle Martí final, # 270 esq. a 27 de noviembre, Pinar del Río, Cuba.

<sup>3</sup> Instituto de Investigaciones de Viandas Tropicales (INIVIT), Apartado 6, Santo Domingo, Villa Clara, CP 53 000. Cuba

✉ mich@inca.edu.cu

## INTRODUCTION

It is very important reduction of water consumption due to high need for the cultivation of rice compared other crops. Globally, the annual amount of water resources for agriculture is declining because of increasing competition from industrial and urban development (1).

Rice cultivation is facing the challenges of global warming, water shortages and other factors limit the ability of farmers to cultivate in flooded conditions. Cuba has had to reduce planting areas by not having enough water in the dam<sup>A</sup>. Despite the low availability of water existing and future looming, continue planting rice under irrigation, ie with a sheet of water, this condition affects anaerobic mycorrhizal association.

However, mycorrhizal symbiosis was reported under anaerobic conditions for the cultivation of paddy rice by different authors (2); however, this association response with the inoculation of different strains of AMF is unknown.

Recently they reported that mycorrhizal colonization in rice plants contributes to their adaptation to anaerobic and aerobic conditions and to changes from a condition to another. Also, they detected the expression of transporters ammonium phosphate and considered markers of the functionality of symbiosis, even if the percentage of mycorrhizal colonization were low (between 8 and 25 %) given the conditions of anaerobiosis (3). These results demonstrated the functionality of the mycorrhizal symbiosis, plus anaerobiosis among other things, cause low percentages of colonization and hinder the plant fungus signaling.

It is important to note that the soil environment is decisive in the selection of efficient strains, because this influences the symbiotic effectiveness and nutrient supply to the plant. Today it is a priority to achieve greater efficiency in the inoculation of these microorganisms in production systems.

Moreover, it was reported that there is a high specificity soil-strain, not plant-strain (4); although found differences when different strains were inoculated in the same crop and soil (5, 6), behavior that makes us think that there is still no clear picture of the causes and conditions that induce differences and even more in the rice cultivation in flooded conditions.

From the above, this study aimed to evaluate the response in rice plants inoculated with different strains of arbuscular mycorrhizal fungi, (AMF) in flooded conditions of Hydromorphic Gley Nodular Ferruginous petroferric soil from Los Palacios, Pinar del Rio province, Cuba.

## MATERIALS AND METHODS

The experiment was performed in conditions of pots, with the commercial cultivar of rice (*Oryza sativa* L.) short cycle INCA LP-5, which was cultured in a Hydromorphic rated Ferruginous Nodular Gley petroferric (7) soil, areas UCTB research "Los Palacios", belonging to the National Institute of Agricultural Sciences (INCA), with slightly acid pH; medium organic matter (OM); phosphorus (P) medium low and potassium (K) (Table).

The rice plants were grown in pots with 7 kg capacity, which were filled with soil from the place mentioned, previously sterilized. For sterilization, the soil was spread over a polyethylene blanket and sprinkled on it a solution of 4 % formaldehyde with a hand sprayer with a capacity of 16 L; immediately after the entire soil was covered with polyethylene blanket for 72 hours, after this period the soil was aired in the shade for seven days and finally proceeded to fill the pots.

The seeds sowing by the method of coating (8) were pretreated with strains of mycorrhizal inocula *Claroideoglossum claroideum* (Cc = 80 spores g<sup>-1</sup> inoculum), *Glomus cubense* (Gc = 126 spores g<sup>-1</sup> inoculum), *Rhizoglossum intraradices* (Ri = 42 spores g<sup>-1</sup> inoculum) and *Funneliformis mosseae* (Fm = 38 spores g<sup>-1</sup> inoculum) from the strain collection of INCA. In each treatment 100 rice seeds which facilitated the coating process and calculating the amount of inoculum needed to be applied are used, from the wealth of propagules of each, so as to ensure approximately the application of 600 spores per pot. 10 seeds in each pot were deposited to a centimeter deep and three days after emergence (DAE), six plants were left per pot.

After planting, all pots were watered to achieve a water depth of 5 cm above the soil surface, durable for a period of 24 hours, at from which they were drained, holding the soil at field capacity until they sprouted two leaves per plant. Subsequently, the water level was restored and remained so until the end of the experiment (50 DAE).

<sup>A</sup> Gonzales, T. "Entorno de la producción de granos a nivel mundial y nacional, retos y perspectivas". En: VI Encuentro ECOARROZ, Ed. Instituto de Investigaciones de grano, 2013.

**Table. Some properties that characterize the fertility of the cultivable horizon (0-0,20 m) of the Hydromorphic Gley Nodular Ferruginous Petroferric soil of the UCTB "Los Palacios"**

Property	Unit	Mean	Median	Standard Error	Variance
pH		6,46	6,50	0,15	0,09
MO	%	2,86	2,80	0,13	0,07
Ca <sup>2+</sup>		6,97	7,01	0,13	0,08
Mg <sup>2+</sup>	cmol kg <sup>-1</sup>	3,11	3,09	0,06	0,01
Na <sup>+</sup>		0,21	0,19	0,02	0,01
K <sup>+</sup>		0,18	0,18	0,02	0,01
P assimilable	mg kg <sup>-1</sup>	46,80	46,00	3,80	5,70

Some properties that characterize the fertility of arable horizon (0 to 0,20 m) above the g Ferruginous Nodular Gley Hydromorphic petroferric soil of UCTB "Los Palacios"

The total amount of nutrients, 0,738 g equivalent of N; 0,301 g of P<sub>2</sub>O<sub>5</sub> and 0,354 g K<sub>2</sub>O per pot, was applied at 20 and 35 DAE, with each time 50 % of the same, using as carriers Urea (46 % N), triple superphosphate (46 % P<sub>2</sub>O<sub>5</sub>) and potassium chloride (60 % K<sub>2</sub>O), respectively.

The treatments consisted of plants that were inoculated with four strains of AMF (Cc, Gc, Ri and Fm) and uninoculated (noMA), following an experimental design of randomized complete block with four replications.

In the greenhouse where they were placed flower pots, daily temperatures average day and night were 29 and 24 °C, respectively, and the relative humidity ranged from 60-70 %, which were measured with a Testoterm (JAPAN, equipment testo® 610).

Two plants were taken by pot at all times, to assess height, tillering, and dry air and root dry mass to 50 DAE. The percentage of colonization and visual density were evaluated at 30, 40 and 50 DAE.

The plant height was measured from the ground surface to the upper end of the longest blade (9). The number of children was determined by counting stems on each soil and a certain amount was deducted the value 1 (mother plant). It is further determined aboveground biomass and root separately and placed in an oven at 70 °C until constant mass. 200 mg of roots of each sample were taken and stained with Trypan blue (10), subsequently intercepts method (11) was used to quantify mycorrhizal colonization. The visual density was determined by evaluating fungal occupation of the fungus in the root of each intercept being allocated to level occupancy rates (12). It is further calculated symbiotic efficiency index based on each of the evaluated variables (13):

$$\text{Symbiotic efficiency index} = \frac{(\text{MA}) - (\text{noMA})}{(\text{noMA})} * 100$$

where:

MA= inoculated plants

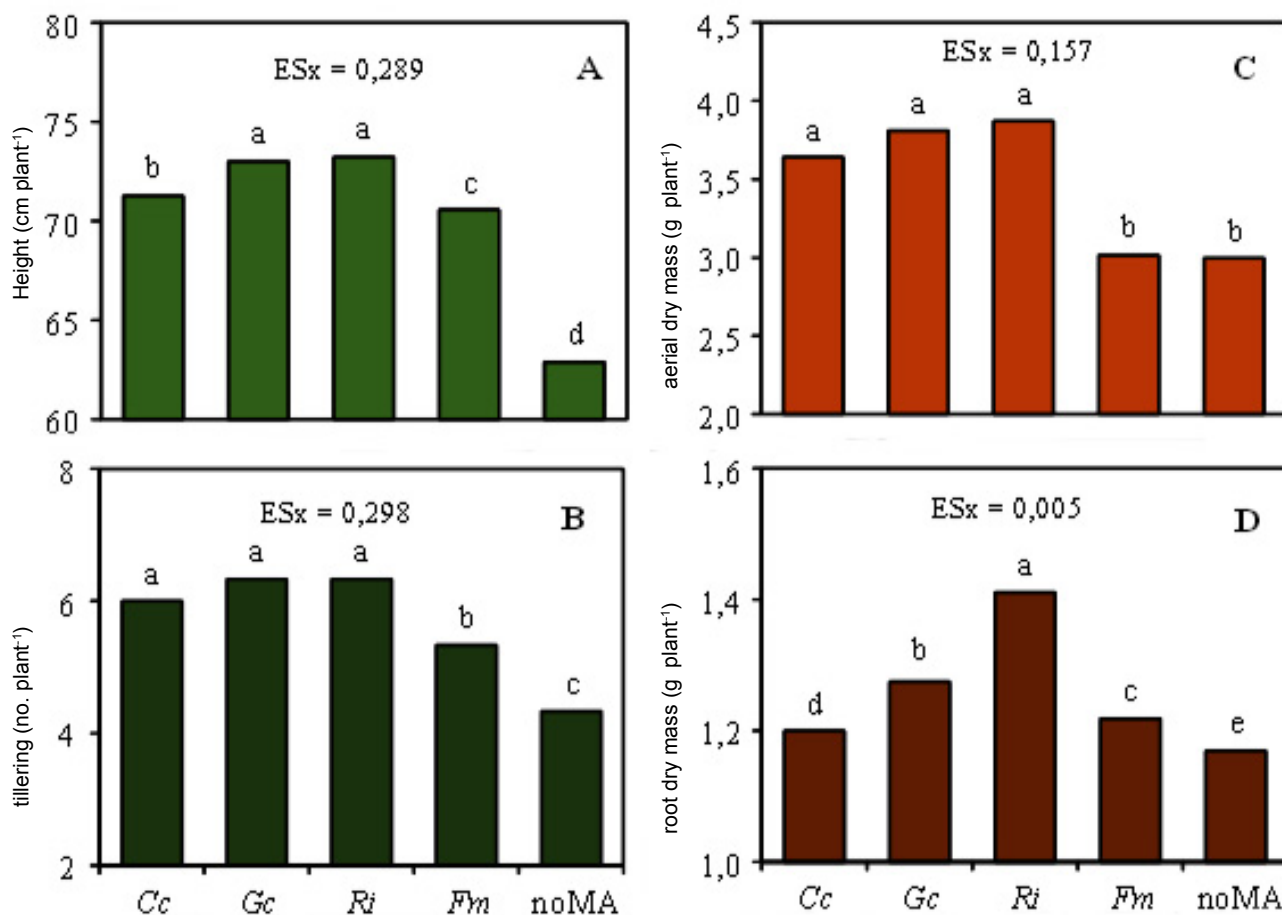
noMA =uninoculated plants.

This research was repeated three times, in November 2009, January 2010 and March 2010. The data obtained from the three replicates were averaged over time for further analysis of variance Double Classification and when there are significant differences, stockings they were compared according to the multiple range test Duncan ( $p \leq 0.05$ ) (14), for which the STATGRAPHICS Plus Program on Windows, version 5.1 (15) was used.

## RESULTS AND DISCUSSION

### DEVELOPMENT OF RICE PLANTS

When analyzing the behavior manifested by the variables height, tillering, air dry mass and root in rice plants at 50 DAE (Figure 1), it was found that, with the exception of air dry mass (Figure 1 C), plants inoculated with AMF reflected the higher magnitudes. The exception was given by the similarity found in dry air mass between plants inoculated with *F. mosseae* and uninoculated plants always *R. intraradices* and *G. Cubense* were among the strains that expressed AMF inocula greater effect on plant development.



Bars with equal letters do not differ significantly ( $p \leq 0.05$ ) according to Duncan's Multiple Range Test

Arbuscular mycorrhizal fungi strains (AMF) *Claroideoglomus claroideum* (Cc) *Glomus cubense* (Gc)  
*Rhizogloium intraradices* (Ri) *Funneliformis mosseae* (Fm) No inoculated plant (noMA)

**Figure 1. Height (H), tillering (TL), aerial dry matter (ADMC) and dry mass of root (DMR) of rice at 50 days after emergence (DAE) without inoculation or inoculated with AMF grown under potted conditions in A Hydromorphic Gley Nodular Ferruginous Petroferric soil**

Regarding the height of the plants, the highest values of this variable were observed in plants inoculated with *R. intraradices* and *G. cubense*, these results correspond (13) with those reported, when inoculating rice plants with different AMF strains (*R. intraradices* and *Glomus cubense*) under similar soil and crop conditions. Height was evaluated at 30, 60 and 90 days after emergence (DAE), with higher values being observed in the inoculated plants and without differences between them, in relation to the uninoculated control.

The conditions under which the experiment was carried out (pots) limited the development of the plants in terms of hake, aerial and root mass, a limitation that became more evident in the capacity of tillering, since the rice plant is capable of emitting more than 20 tillers, when conditions of growth and development are adequate (16, 17). However, there were increases in the number of offspring and in the

aerial and root dry mass, caused by the inoculation of the strains *C. claroideum*, *G. cubense* and *R. intraradices*, with respect to *F. mosseae* and the control. A similar behavior was recently reported (13) in rice plants of the same cultivar and inoculated with HMA, also in pasture (18, 19).

The increase in the development of inoculated plants (height, tillering, aerial dry mass and roots) with respect to uninoculated plants may be due to a possible effect of hormonal growth stimulating production, when the mycorrhizal association is established. Recently, some authors (20, 21) suggest that phytohormones released during colonization may contribute in some way to increased plant growth; although the results in this sense are not totally clear and are sometimes contradictory (18, 20).

The results indicate that at the time of recognition of the symbionts and after the establishment of colonization, there must be a balance in the release and production of hormones, both the AMF, and the plant, which regulate the establishment of colonization and determine a mycorrhizal better performance, which is manifested in the growth and development of the plant.

#### MYCORRHIZAL COLONIZATION AND VISUAL DENSITY

In assessing the percentage of mycorrhizal colonization and visual density, 30 DAE was not observed inside the root fungal occupation in any of the treatments. Various causes may have influenced the fungal occupation 30 DAE. The presence of the sheet of water, lack of oxygen conditions in the soil, which may limit the germination of spores of the fungus.

Coupled with the above, under the conditions of a flooded soil, a dilution effect of exudates signals between the rice plant and AMF is manifested, as in the case of strigolactones, considered hormone signaling in culture (22, 23), which in its chemical structure has a very labile ether bond, which can be easily hydrolyzed in the rhizosphere (24, 25) and therefore, it may affect two stages of establishing the symbiosis: spore germination (26, 27) and the hyphal growth and start the process of colonization (28).

After 30 DAE, colonization of the AMF was observed in rice plants (Figure 2), which may be explained by increased oxygenation of the rhizosphere zone and transport of oxygen from the biomass to the roots, creating more favorable conditions for spore germination and subsequent colonization AMF plants.

At 40 and 50 DAE colonization was found in the roots of plants that were not inoculated with AMF strains due to sterilization to which he was subjected the ground; however, the inoculated plants, mycorrhizal colonization percentages that although low, showed a tendency to increase over time (Figure 2A and B) were noted.

At 40 DAE, mycorrhizal colonization of rice plants inoculated with *R. intraradices* and *C. claroideum* was similar (Figure 2 A); while the 50 DAE (Figure 2 B) no differences were observed in the effect caused by strains *C. claroideum*, *G. Cubense* and *R. intraradices*.

The results showed that after certain period of time, the rice plants are colonized by the AMF, because these are able to carry oxygen to the roots and thus the rhizosphere area is oxygenated (16, 17), creating conditions favorable for germination of spores of AMF and colonization of plants.

The low percentages of mycorrhizal colonization found at 40 and 50 DAE may be due primarily to changes in the architecture of the root in the rice plant, caused by the presence of the sheet of water, dominated roots more aerenchyma tissue, leaving less space cortical tissue (29, 30), in which the tissue mycorrhizal fungi is set to the root (30).

The intensity of fungal occupation (DV) was found inside the roots to the 40 DAE (Figure 2 C) indicated that mycorrhizal colonization was in the early stages of the process of establishing, where still no presence is detected of arbuscules.

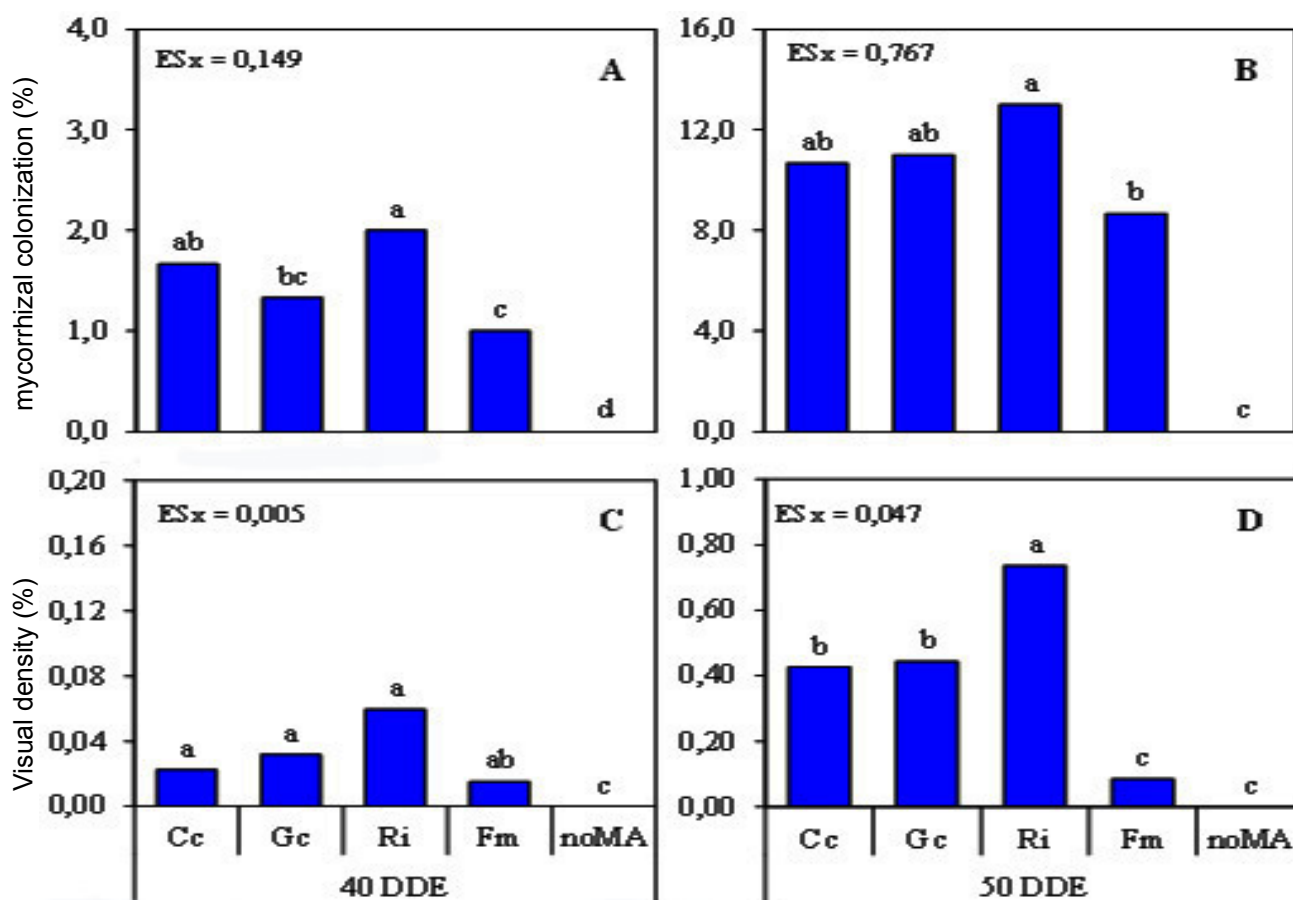
However, DAE 50 (Figure 2D), the intensity of fungal occupation indicated that treatment inoculated with *R. intraradices* strain showed better interaction with the ground, followed by *G. cubense* and *C. claroideum*, results demonstrate the ability of these AMF to adapt to flood conditions, since these strains are not isolated residents of soils dedicated to monoculture rice under these conditions.

The species *R. intraradices* is characterized by a high rate of mycorrhizal colonization and relatively rapid growth and it is therefore often used in agriculture (3). Similarly it happens with the strain of *G. Cubense* in Cuba, which in various soil and climatic conditions has been adapted and has expressed its full potential in symbiosis with crops such as beans (4), grasses (18, 19) and tomato (6).

The results of this research showed that flooding conditions affected the intensity of colonization and fungal occupation, so at transplant at 30 DAE, plants could take the field without being colonized; however, after 30 DAE mycorrhizal colonization was achieved.

Regarding the symbiotic efficiency index depending on the variables evaluated, the highest percentages of efficiency in the number of children per plant (Figure 3) were found. This result is important because the tillering is part of the components of the agricultural yield of rice, since this and the number of stems per plant (emit panicle) represent the same variable, but assessed at different times and defines agricultural output (17, 31).

Always plants inoculated with strain *R. intraradices* and *G. cubense*, were among the highest rate of symbiotic efficiency compared to other treatments based on all variables.



Bars with equal letters do not differ significantly ( $p \leq 0.05$ ) according to Duncan's Multiple Range Test

Arbuscular mycorrhizal fungi strains (AMF) *Claroideoglomus claroideum* (Cc) *Glomus cubense* (Gc)  
*Rhizoglomus intraradices* (Ri) *Funnelformis mosseae* (Fm) Plants no inoculated (noMA)  
 Days after emergency (DAE)

**Figure 2. Mycorrhizal colonization and visual density at 40 DAE (A and B) and 50 DAE (C and D) in rice roots without inoculation or inoculated with AMF cultivated under potted conditions in a Gley Nodular Hydropower Petroferic soil**

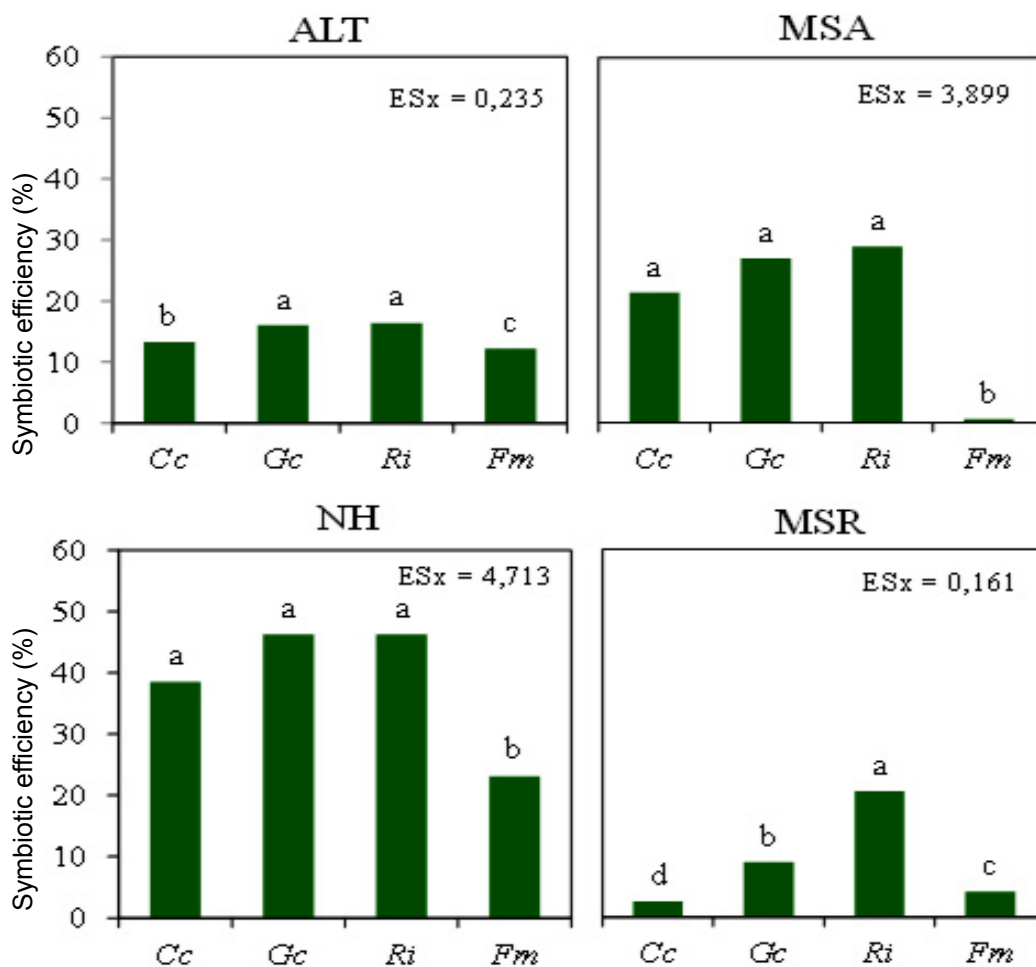
On the other hand, it was found that the plants inoculated with *F. mosseae* were the lowest symbiotic efficiency, with respect to the rest of the inoculated strains in all variables, with the exception of the radical mass that the efficiency of *F. mosseae* was superior to the treatment inoculated with *C. claroideum*.

All this behavior could be attributed to differences in the ability to take nutrients from the soil and transfer them to the plant (32). In addition, it was reported (4) that soil is determinant in mycorrhizal colonization, since AMF are edaphic microorganisms and their physicochemical properties determine the adaptability and establishment capacity, as well as their symbiotic efficiency.

In the research carried out, it is difficult to associate the behavior of the AMF strains with the edaphic medium due to the oxidation - reduction conditions to which the soil was subjected (33), modify the redox potential, pH, nutrient solubilization for plants, among

other properties and, therefore, it becomes complex to associate the behavior of AMF strains with edaphic conditions. These modifications conditioned that three of the AMF strains evaluated showed similar behavior and that the *F. mosseae* strain, described as efficient for acid soil conditions (34), reflected the worse behavior, which was evidenced in a more limited development of the plants, which was corroborated in symbiotic efficiency and visual density.

The above mentioned conditions could allow different AMF strains to exhibit similar behavior, something that would probably not happen if the research were tested on the same soil and with the same species of plant, but modifying the soil water conditions, that is, under conditions of Aerobiosis.



Bars with equal letters do not differ significantly ( $p \leq 0.05$ ) according to Duncan's Multiple Range Test

Cepas de hongos micorrízico arbusculares (AMF) *Claroideoglomus claroideum* (Cc) *Glomus cubense* (Gc)  
*Rhizogloium intraradices* (Ri) *Funnelformis mosseae* (Fm)

**Figure 3. Symbiotic efficiency index according to the variables evaluated: number of tillers per plant (NT); height of plant (HP); Aerial dry mass (ADM) and root (DMR) in rice plants 50 days after emergence inoculated with AMF grown under potting conditions in a Gley Nodular Hydrophobic Petroferric soil**

The behavior in the variables evaluated in the experiment, allowed to consider the *F. mosseae* strain as the least efficient, to show the lowest symbiotic response and to cause a more limited development of the plants, although in general the behavior was superior to the control treatment (noMA), reflecting that the conditions are unfavorable for better mycorrhizal function.

In conclusion, the cultivation condition (anaerobiosis) and the morphological characteristics of the rice plant (root types, aerenchyma tissue, oxygen transport and radical exudates) are determinate for

the establishment and development of mycorrhizal colonization at the early ages of the culture.

For the soil and crop conditions evaluated, the superior symbiotic efficiency was obtained with the *R. intraradices*, *G. cubense* and *C. claroideum* strains, as a function of the increase in aeration and aerial dry mass, although always the strain *R. intraradices* and *G. cubense* were among the AMF strains that showed greater effect on the development of plants.

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