doi: http:// dx.doi.org/10.15446/acag.v64n3.43087 e-ISSN 2323-0118

Arbuscular mycorrhizal symbiosis in rice (*Oryza sativa* L.) plants in flooded and non-flooded conditions

Simbiosis de micorrizas arbusculares en plantas de arroz (*Oryza sativa* L.) en condiciones de inundación y secano

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Rec.: 21.04.2014 Acep.:18.09.2014

Abstract

The research was conducted in the Scientific Technological Base Unit Los Palacios, Cuba, located at 22° 34' 32.73" N and 83° 34' 32.73"14' 11.95" W, with the objective of evaluating the effect of flooding on colonization by arbuscular mycorrhizal fungi during the vegetative phase of rice plants, cultivar INCA LP–5. Two experiments under greenhouse conditions in pots with a capacity of 800 g of soil were performed, both experiments in a completely randomized design. An experiment was performed with *Rhizophagus (=Glomus)* intraradices *(Ri),* where the dynamics of colonization of mycorrhizal symbiosis in flooded and not flooded conditions was evaluated. In the second experiment, we worked with *Glomus cubense (Gc)* and mycorrhizal colonization was evaluated in rice plants grown with different levels of water depth. In both experiments, plant height, the absolute dry weight and growth rate are evaluated. The results showed that the rice plants were colonized from 35 days after germination, both under flooded and non–flooded conditions, with an increasing trend over time. It was found that mycorrhizal colonization decreased in flooded conditions and as the the water lamina was higher, lower values of this indicator were found. Mycorrhizal colonization increased the development of rice plants.

Key words: Mycorrhizal colonization, *Rhizophagus intraradices*, *Glomus cubense*, rice, irrigation.

Resumen

En la Unidad Científico Tecnológica de Base Los Palacios, Cuba, a 22° 34' 32.73" N y 83° 14' 11.95" O, se evaluó el efecto de la inundación sobre la colonización de hongos micorrízicos arbusculares durante la fase vegetativa de plantas de arroz cultivar INCA LP–5. Se realizaron dos experimentos en condiciones de invernadero en macetas con una capacidad de 800 g de suelo, ambos experimentos en un diseño experimental completamente aleatorizado. Uno de ellos se realizó con *Rhizophagus (=Glomus) intraradices (Ri),* donde se evaluó la dinámica de colonización de la simbiosis micorrízica en condiciones de inundación y secano (no inundadas). En el segundo experimento se trabajó con *Glomus cubense (Gc)* y se evaluó la colonización micorrízica en plantas de arroz cultivadas con diferentes alturas de lámina de agua. En ambos experimentos se evaluaron altura de la planta, producción de masa seca y tasa absoluta de crecimiento. Como resultado se encontró que las plantas de arroz fueron colonizadas a partir del día 35 después de la germinación, tanto en condiciones inundadas como no inundadas, con una tendencia al incremento en el tiempo. Se comprobó que la colonización micorrízica disminuyó en condiciones de inundación y a medida que la altura de la lámina de agua fue superior se encontraron valores inferiores de este indicador. La colonización micorrízica incrementó el desarrollo de las plantas de arroz.

Palabras clave: Colonización micorrízica, *Rhizophagus intraradices*, *Glomus cubense*, arroz, inundación, secano.

Introduction

Arbuscular mycorrhizal fungi (AMF) colonize the intraradical tissue of the host plant, where they develop structures characteristic of the symbiosis (arbuscules and vesicles) and extraradical mycelium, which interacts with the ecosystem of the rhizosphere and is responsible for the absorption of nutrients from the soil (Smith and Read, 2008).

The fundamental basis on which the arbuscular mycorrhizal symbiosis is established are nutritious. The plant supplies to the fungus carbon compounds from the photosynthesis, as this contributes to the plant mineral nutrients, especially those less available because of the increased accessibility of the external fungal mycelium to more distant resources in the soil (Barea *et al.*, 2008).

AMF have a marked effect on water relations of the plant and soil, as they modify stomatal conductance, photosynthetic rate, leaf water potential, the concentration of osmolytes, the efficiency of water and nutrient assimilation of the host (Harris–Valle *et al.*, 2009); while the fungal exudates promote the cohesion among soil particles and increase the water retention in the substrate (Rillig and Mummey, 2006). The effects of water in excess in the soil over the AMF have not been widely evaluated, although mycorrhizal association has been found in aquatic plants and flooded areas (Dhillion and Ampornpan, 1992), with exception to those belonging to the *Cyperaceae* and *Juncaceae* families.

Rice has the evolutionary particularity of being semiaquatic and conventionally grows under continuous flooded conditions during the major part of its life cycle. Therefore, has relatively few adaptations to limited water conditions and is extremely sensitive to drought (Kamoshita *et al.*, 2008), it requires high volume of water, and its scarcity negatively affects the plant growth and the grain yield (Vallino *et al.*, 2009). However, around half of the rice growing areas in the world do not have enough water to keep the flooded conditions and the yield is reduced to some extend by drought (Bernier *et al.*, 2008). Having into account this, this research was performed with the goal to evaluate the effects of flooding on the mycorrhizal colonization and development of rice plants during their vegetative stage.

Materials and methods

This research was carried on in the Scientific Technological Base Unit Los Palacios, Cuba, located at 22° 34' 32.73" N and 83° 34' 32.73"14' 11.95" W, that belongs to the National Institute of Agricultural Sciences (INCA). The experiments were established under greenhouse conditions in pots with 800 g of soil, the soil was previously sieved (5 mm diameter) and disinfected with 45 formaline. The soil used was Typical Quartzic Yellow Ferralitic (Hernández *et al.,* 2006) with pH = 7.43, OM = 2.46 %, P_2O_5 = 12.52, K_2O = 9 (cmol/kg), Ca = 10, Mg = 2.71 and Na = 0.57 (ppm), used by farmers for rice production at the riverside of the Guama river in Pinar del Río. During the experimental phase the temperature conditions were between 18 and 27 °C, with and average 22.5 °C and average relative humidity between 74 and 80%.

A rice commercial cultivar INCA LP-5 was used, which has a good agronomical performance under optimal production conditions and as for the AMF strains used: *Rhizophagus intraradices* Schüßler and Walker (2011) and *Glomus cubense* Y. Rodr. and Dalpé, sp. nov., (Rodríguez *et al.*, 2011), with a fungal richness of 40 and 91 spores.g–1 of inoculum, respectively, that were certified by the Mycorrhiza Lab of the National Institute of Agricultural Sciences, Cuba. In each experiment one strain was used, and was selected according to the criteria of use of *R. intraradices* established in previous researched in rice (Vallino *et al.*, 2009) and for *G. cubense* it was due to its high adaptability to different soils in Cuba (Rivera *et al.*, 2003). Inoculation was performed by seed coverage based on 10% of its weight (Fernández *et al.*, 2003). The non-flooded treatments (drought) were kept at field capacity, between 70 and 90% of humidity.

Experiment 1

The dynamics of *R. intraradices* mycorrhizal colonization was evaluated from the time of establishment of the flooded and non-flooded conditions. In the specific case of flooding, the height of the water level did not exceed 5 cm above the soil surface contained in the pot, which was restored daily to compensate for water consumption by the plant and evapotranspiration in the pots. The experiment comprised four treatments and three replicates, for a total of 108 pots, considering nine evaluations between 20 and 60 days after germination (DAG) and before the reproductive phase onset (Jarma *et al.*, 2010), with a frequency of 5 days. The treatments consisted in: plant inoculated with AMF under flooded conditions (MA + Inund), plants inoculated with AMF under non-flooded conditions (MA + noInund), non-inoculated plants under flooded conditions (noMA + Inund) and non-inoculated plants with AM under non-flooded conditions (noMA + noInund).

Experiment 2

It was performed with *G. cubense* to evaluate the effect of flooding in the arbuscular mycorrhizal symbiosis in rice plants inoculated with AMF. The experiment consisted in eight treatments, considering different levels of flooding expressed in height of the water lamina (Table 1) and four replicates. In total 128 pots were used and four evaluations between 35 and 50 days after germination were performed, before the reproductive phase onset (Jarma *et al.*, 2010), with 5 days frequency.

Table 1. Treatments evaluated in the Experiment 2.

No.	Treatments	Label
T1	Plants inoculated with AMF under flooded conditions of 3 cm	MA + 3 cm
T2	Plants inoculated with AMF under flooded conditions of 6 cm	MA + 6 cm
T3	Plants inoculated with AMF under flooded conditions of 9 cm	MA + 9 cm
T4	Plants inoculated with AMF under non-flooded conditions	MA + noInund
T5	Plants not inoculated with AMF under flooded conditions of 3 cm	noMA + 3 cm
T6	Plants not inoculated with AMF under flooded conditions of 6 cm	noMA + 6 cm
T7	Plants not inoculated with AMF under flooded conditions of 9 cm	noMA + 9 cm
T8	Plants not inoculated with AMF under non-flooded conditions	noMA + noInund

Quantification of the mycorrhizal colonization

Once the mycorrhizal symbiosis was established in the plants inoculated with *Ri* and *Gc*, colonization was measured (%). Roots were stained following the method described by Phillips and Hayman (1970) that is based on the use of the trypan blue dye. To determine the mycorrhizal colonization, the grid or intercept method was used, this consists in making a grid at the bottom of a petri dish (1 x 1 cm) followed by observation of the root under a Novel® binocular stereoscopic microscope to count the roots with or without mycorrhiza that were in touch with the vertical lines of the grid, in total 100 roots were recorded (Giovanetti and Mosse, 1980).

Morpho-physiological indicators

These indicators were quantified as follows: (1) Plant height (cm) from the soil surface to the upper end of the longest leaf projected in the direction of the stem; (2) dry matter of the aerial part and root (g), after the samples dried with forced draft of air at 70 °C, until constant mass and measured on a technical digital scale OHUS Adventurer® Pro of 0.01 g precision; and (3) relative growth rate (TRC) (g/g per day), defined from the leaf dry mass in the first and final measurements in both experiments. Calculations were made according to the criteria of Hunt (1982) cited by Barraza *et al.* (2004), modifying the terms of P by M in the equation $(lnM_2\text{-}lnM_1) / (t_2\text{-}t_1)$, where M1 = initial dry mass M2 = final dry mass, t2 - t1 = time interval during the evaluation.

Statistical analysis of data

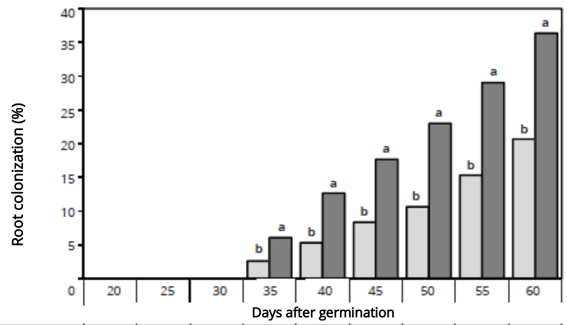
The data of each experiment were process by analysis of variance (ANOVA) ($P \leq 0.05$). Additionally in the Experiment 1 a lineal regression analysis for the height variable was performed. All the analysis were performed with the SPSS software v. 21.0 for Windows.

Results and discussion

Mycorrhizal colonization

Experiment 1. In this study it was found that the non-inoculated plants (NOMA) did not show fungal structures on the roots as a result of the sterilization process to which they were subjected. This was not observed in the plants inoculated with *R. intraradices* in which mycorrhization increased as the time passed and the rice plants grew (Figure 1), a behavior that is related to the interaction between the plant and symbionts (Smith and Read, 2008). Thirty-five days after germination mycorrhizal colonization was observed in plants inoculated with AMF, both under flooded and not flooded conditions, in these last although AMF are strict aerobic microorganisms (Smith and Read, 2008), have ability to establish interaction between symbionts. The highest values were observed in plants grown in non-flooded conditions, which is due to the fact that these conditions favor the germination of spores of the fungus, from a better pre-symbiotic relationship between symbionts (Barea *et al.*, 2008).

The percentages of colonization at 60 DAG increased 18.06 and 30.25% in comparison to the ones of 35 DAG for flooded and non-flooded



	20	25	30	35	40	45	50	55	60
□ MA+Inund	0	0	0	2,61	5,33	8,33	10,67	15,33	20,67
■ MA+noInund	0	0	0	6,09	12,67	17,67	23,00	29,00	36,33
ESx =				0,386	0,316	0,433	0,374	0,200	0,539

Figure 1. Root colonization (%) by *R. intraradices* in rice plants under flooded and non-flooded conditions. Bars with same letters do not differ statistically (P ≤ 0.05).

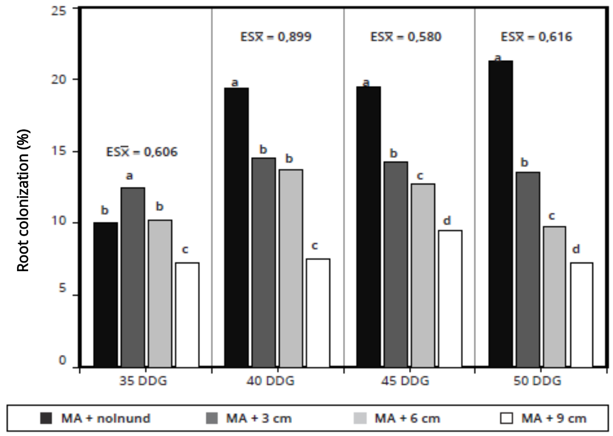


Figure 2. Root colonization (%) by *G. cubense* in rice plants under flooded conditions. Bars with same letters do not differ statistically (P ≤ 0.05). DDG: Days after germination.

conditions, respectively, showing a negative effect of flooding in the mycorrhizal colonization. A possible cause of the low colonization values is the abundance of aerenchyma tissue in the root (Gutjahr *et al.*, 2009) and therefore, the reduction of cortex tissue (Vallino *et al.*, 2014), zone where the symbiosis happens (Smith and Read, 2008; Gutjahr *et al.*, 2009; Vallino *et al.*, 2014). These results are similar to the ones found by Rodríguez *et al.* (2006) with values between 24 and 26 % to 35 DAG in rice cultivars (INCA LP7 and J–104) and an inoculum richness of 250 spores/g of inoculum. Vallino *et al.* (2009) had a similar response with percentages around 14 and 51%, when evaluating 13 rice varieties; Kira and Bouldin (1991) claimed that they observed the formation of air bags in the roots (aerenchyma) because of the degradation of the root cortex that allows oxygen to reach the root tip and, keep them under flooded conditions.

Experiment 2. The colonization percentages in the root by *G. fubense* were different among the flooding levels (Figure 2); similarly there were differences between the evaluation times, which is corroborated by the increase in the colonization between 35 and 50 DAG in the non-flooded treatment, reaching higher values in comparison to the other treatments.

The increased height of the water level affect mycorrhization, with values less than 15%, although in the AM + 3 cm treatment rates higher than those found in other water level heights. This behavior is due to the greater leaf area exposed on the water lamina, which helps transport oxygen from the aerial part to the roots. Solaiman and Hirata (1996) found low percentages of mycorrhizal colonization in the rice crops under flooding conditions with a water lamina of 5 cm.

In the flooded treatments low colonization percentages were found, a behavior that can be determined by the condition of anoxia in the soil as an effect of the flooding (Ismail *et al.*, 2009), which limits the plant-AMF interchange mechanism (Drew, 1997) due to the hydrostatic pressure and the reduction of the porous spaces in the soil which limits the oxygen diffusion and exchange. Nonetheless, the rice plants under flooded conditions are colonized by AMF basically for its ability to transport oxygen to the roots and the area around them (Yoshida, 1981; Snyder and Slaton, 2002; Jarma *et al.*, 2010).

Effect of the mycorrhiza in plant height and DM production

In Figures 3 and 4 the effect of inoculation with AMF and lamina of water in plant height are ob-

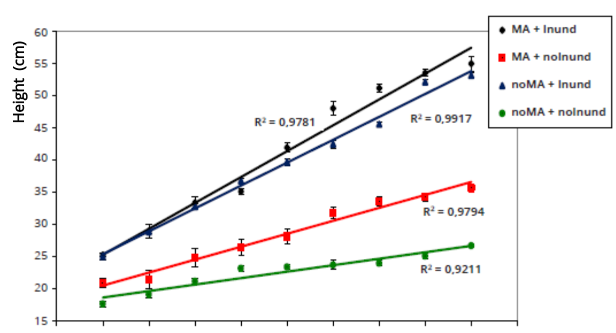


Figure 3. Effect of inoculation with *R. intraradices* in the dynamics of height (cm) in rice plants, experiment 1. R^2: estimation coefficient for the lineal regression. Bars are the standard error of the mean of the treatment in each time. (DDG): Days after germination.

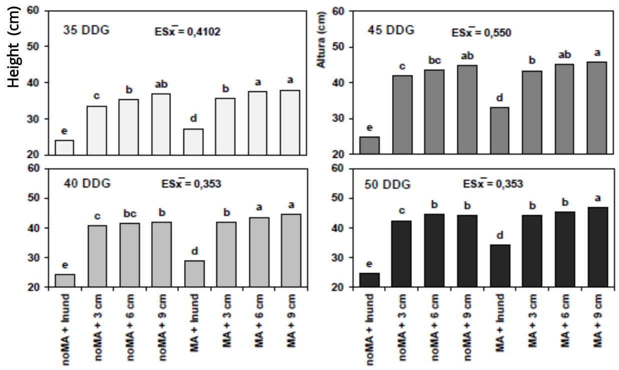


Figure 4. Height (cm) of rice plants inoculated with *G. cubense* (MA) under flooded conditions (Inund) and its control non-flooded (noInund), evaluated at 35, 40, 45 and 50 DDG, experiment 2. Bars with same letters do not differ statistically (P ≤ 0.05).

served, this being higher in inoculated treatments (AM) vs. non-inoculated (NOMA) and flooded vs. non-flooded; these results contrast with those found Hernandez *et al.* (2006) who found no consistent relationship between strains of AMF and rice plant height. Moreover in flooded treatments increased plant growth was observed as the water lamina was higher, regardless of mycorrhizal inoculation.

Reduced growth in plant height of the plants under the non-flooded treatments could be interpreted as an inhibition in cell elongation, since it is more sensitive to reducing turgor than cell division (Panda and Khan, 2004); associated to this physiological response is the genetic component, which determines its adaptability to flood or dry conditions (Muthurajan *et al.,* 2011). Similar results were obtained by Qin *et al.* (2010) on rice in flooded and dry conditions.

In the experiment 1 the dry matter production (DM) of the aerial part at 60 DAG (Table 2) was higher under flooded conditions (P $\leq$ 0.05); whereas the root DM production was higher in the plants inoculated with AMF and cultured under non-flooded conditions. These results agree with the findings of Ruiz–Sánchez *et al.* (2010) in rice crops and suggest a long term

Table 2. Dry matter of the aerial (DMA) and root (DMR) part and the absolute growth rate (TAC) of rice plants at 60 DAG in the Experiment 1.

Treatments	DMA	DMR	TRC
	(g/plant)		(g/g per day)
noMA + Inund	16.416 a*	22.615 b	0.0148 a
noMA + noInund	11.365 c	26.803 b	0.0121 c
MA + Inund	17.540 a	25.086 b	0.0152 a
MA + noInund	13.620 b	34.930 a	0.0127 bc
ESx	1.100	1.479	0.000

* Averages followed by the same letters do not differ statistically (P ≤ 0.05).

effect of the AMF in the plant development. In other crops where AMF have been inoculated, it has been found an increase in comparison to the non-inoculated as for the height and dry matter in maize and sorghum (Mena *et al.*, 2011).

In the experiment 2 the higher values of DM both, for the aerial part and roots, were found in plants with mycorrhiza (Table 3). It is important to state that the increase in water lamina reduced the development of the plants, which can be associated with the oxygen deficiency in the rhizosphere.

In the Table 2 and 3 are also included the values of the relative growth rates for the evaluated treatments in both experiments. A positive effect of mycorrhiza is observed for this parameter, although under flooded conditions there were no differences between the plants with or without mycorrhiza; this is due to the capacity of the AMF to absorb soil nutrients (Smith *et al.*, 2010). A similar result was reported by Gañan (2011) finding that the biomass accumulation was associated with the percentage of mycorrhizal colonization.

Table 3. Dry matter of the aerial (DMA) and root (DMR) part and the absolute growth rate (TAC) of rice plants at 50 DAG in the Experiment 2.

Treatments	DMA	DMR	TRC
	(g/plant)		(g/g per day)
noMA + noInund	5.556 f*	8.495 f	0.0082 b
noMA + 3 cm	10.735 bc	15.825 b	0.0101 a
noMA + 6 cm	10.502 c	14.797 c	0.0101 a
noMA + 9 cm	8.715 d	13.630 d	0.0089 b
MA + noInund	6.665 e	9.583 e	0.0091 ab
MA + 3 cm	12.907 a	17.330 a	0.0093 ab
MA + 6 cm	11.062 b	16.822 a	0.0096 a
MA + 9 cm	10.470 c	15.805 b	0.0092 ab
ESx	0.155	0.196	0.000

* Averages followed by the same letters do not differ statistically (P ≤ 0.05).

Conclusions

In this study, 35 days after germination, the rice plants were colonized with the *R. intraradices* and *G. cubense* strains, both, under flooded and non-flooded conditions, although in the later one the mycorrhization tended to be higher.

The establishment of simbiosis in both conditions had a positive impact in height, plant biomass and the relative growth rate of the plants.

Acknowledgements

This study was financed by the Project CIAC–940142, on the frame of the agreement INTA–AUDEAS–CONADEV.

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