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Application of EcoMic® biofertilizer in technified seedbeds for mechanized transplanting of seed rice

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ABSTRACT

The research was conducted at "Los Palacios" Scientific and Technological Base Unit, in February and March 2020, with the aim of evaluating the effect of EcoMic® biofertilizer application in a technified seedbed for mechanized transplanting of seed rice. The biofertilizer was applied at a dose of 6 % based on seed mass on four rice cultivars (INCA LP-7, Roana, Guillemar and José). Sowing was carried out in plastic trays (0.60 m x 0.30 m x 0.03 m), containing substrate with a 1:1 (v/v) ratio of cow manure organic matter and soil, following a completely randomized block experimental design, with four replications. Plant height, number of leaves, root length, aerial and root dry mass were evaluated 18 days after emergence. With the application of EcoMic® biofertilizer at the seedling stage, rice plants colonized by Arbuscular Mycorrhizal Fungi (AMF) were transplanted, which have a greater development in terms of height, root length, aerial and root dry mass, compared to untreated plants. The genetic component has a marked effect on plant growth and development, even when plants are colonized by AMF. Based on these results, the EcoMic® biofertilizer application is suggested as a nutritional alternative in seedbeds for mechanized rice transplanting.

Key words: Arbuscular mycorrhizal fungi, colonization, *Glomus*, *Oryza sativa*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the main cereals used for human food in the world, with the largest world production concentrated in China and India [1]. According to estimates of the Organization for Economic Cooperation and Development (OCDE, Organización para la Cooperación y el Desarrollo Económico) and the Food and Agriculture Organization of the United Nations (FAO), for the next decade, it is projected that world rice production will reach 582 Mt in 2029; that is, an increase of 15 % and that Asia will contribute most of this increase, with an additional 61 Mt [2]. To achieve these goals, genetic improvement programs continue in this cereal.

In Cuba, the genetic breeding program is working to obtain new cultivars with excellent agronomic qualities and good industrial performance, resistant to main pests and diseases that cause economic damage to the crop, and at least tolerant to abiotic stress [3]. Among the procedures in the multiplication and reproduction of commercial cultivars, rice transplanting is applied in the case of original and basic seed [3,4]. Rice transplanting is one of the most laborious technological operations in this crop; an activity that is generally carried out manually by our farmers. Only in some reference farms and research institutions is rice transplanting carried out mechanized, due to the high cost of this technology and the technical requirements needed to achieve a stable production of this grain [5]. Mechanical rice transplanting is the process of transplanting seedlings grown in a seedbed, and then using a self-propelled transplanter. This activity reduces the cost of crop establishment, saves labor, ensures timely transplanting and achieves an optimal plant density that contributes to high productivity [6].

Transplanting, manually and mechanized, is preceded by a seedbed period, which can be carried out directly in the soil (traditional) and technified in carpet or plastic trays (7). In the case of this technified seedbed, in the technology transfer process, the fertilization activity is carried out with edaphic fertilizers (7) and does not include biological alternatives in order to find efficiency in EcoMic® bioproduct application, based on Arbuscular Mycorrhizal Fungi (AMF) in this crop. AMF provide hosts with minerals that are absorbed through hyphal networks in the soil and, in return, receive carbon sources such as sugars and lipids derived from plant photosynthates. This is considered an obligate symbiotic relationship, since AMF can complete their life cycle only through colonization of their host (8). Arbuscular mycorrhizal (AM) symbiosis, in most plant roots, enhances the uptake of water and nutrients from the soil, due to a vast mycelial network that can access beyond the root depletion zone in the rhizosphere (9). Inside the plant, they bring numerous benefits to plant physiology, the most obvious being the stimulation of plant growth and the improvement of mineral nutrition (10). Taking into account the above, the objective of the research was to evaluate EcoMic® biofertilizer application effect in a technified seedbed for the mechanized transplanting of seed rice.

MATERIALS AND METHODS

The research was carried out under conditions of technified seedbeds for mechanized transplanting of rice in seed production at the Scientific and Technological Base Unit "Los Palacios" (UCTB "Los Palacios"), Cuba, at 22°34'32.73" N and 83°14'11.95" W, belonging to the National Institute of Agricultural Sciences (INCA), for which two experiments were established, one in February 2020 and other in March of the same year. The experiments consisted of the EcoMic® biofertilizer application, based on AMF (fungal richness of 20 spores g soil^{-1}), by the seed coating method (11) at a dose of 6 % based on the seed mass of four commercial rice cultivars (INCA LP-7, Roana, Guillemar and José), which were compared to these same cultivars, but without the EcoMic® application.

The seedbed was planted in plastic trays (0.60 m x 0.30 m x 0.03 m), containing substrate with a 1:1 ratio (v/v), and organic matter from cattle manure and soil from the areas of the UCTB "Los Palacios". The trays were placed outdoors in an area established for the seedbeds, with irrigation and good drainage.

Prior to the sowing of four rice cultivars, the seed was classified by the density method, which consisted of submerging the rice seeds in irrigation water and eliminating all the seed that floated, in order to achieve maximum seed germination [7]. AMF inoculation was carried out through the EcoMic® biofertilizer, at 50 % of seed. A total of 150 g of seed per tray was sown at 1 cm depth. Four trays per treatment were used and the experiment was repeated at two points in time, following a completely randomized block experimental design.

The seedbed had a cycle in days, from sowing to the evaluation time of 18 days, at which time it was considered that the plants could be transplanted to the field [7]. The agrotechnical activities of seedbed were carried out according to the Agricultural Technology manual for the Mechanized Transplanting of Rice in Cuba [7], with the exception of irrigation water management. The seedbeds (trays) were maintained from the moment of sowing with daily passes of water by flooding, until 10 days after emergence (DAE), then a film of water of up to 5 cm was established and reestablished every 48 h. In the period from February to March the average temperature was 24.1 ºC, relative humidity was 76.58 % and rainfall was below 3 mm, according to data provided by the Meteorological Station "Paso Real de San Diego", # 317, in Los Palacios, which is located 5 km from the research area.

The trays were placed outdoors in an area established for seedbeds, with irrigation and good drainage.

Evaluations conducted

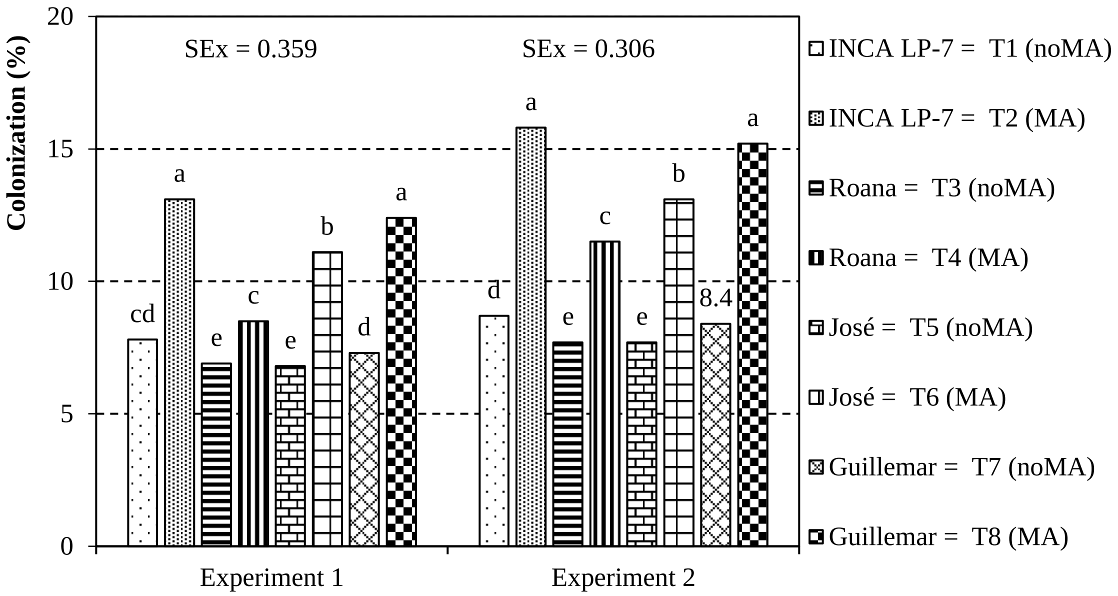
Ten plants per tray were taken to evaluate plant height, root length, aerial dry mass and root dry mass at the end of the seedling stage (18 DAE), as well as mycorrhizal colonization percentage. Plant height was measured from the surface of the substrate to the top of the longest leaf, projected in the direction of aerial growth [12]. Roots were washed with abundant water to remove the adhering substrate and root length was measured up to the end of their projection, in the direction of their growth. The number of leaves was determined by counting each plant and the aerial and root mass was determined separately and placed in an oven at 70 ºC until constant mass was reached.

All roots of 10 plants per tray were taken to form a pull, which were stained with Parker QuinK ink [13], later the intercept method was used [14], to quantify the mycorrhizal colonization. Four counts were made for each repetition.

The data obtained were analyzed by Simple Classification Analysis of Variance and when there was a significant difference, means were compared according to Duncan's Multiple Range Test ($p \leq 0.05$), for which the SPSS program on Windows, version 22, was used.

RESULTS AND DISCUSSION

EcoMic® biofertilizer application caused increases in mycorrhizal colonization percentages in all rice cultivars at 18 DAE, but differential behavior was observed depending on the cultivar (Figure 1). The highest mycorrhizal colonization values were observed in the cultivars INCA LP-7 and Guillemar.



Means with equal letters do not differ significantly (p≤0.05) according to Duncan's Multiple Range Test

Figure 1. Percentage of mycorrhizal colonization in rice plants inoculated with Arbuscular Mycorrhizal Fungi (AMF) and non-inoculated (no-AFM)

Even when mycorrhizal colonization has been observed in rice roots, it can be considered that the values of this indicator are low, if it is compared them with those reported in other crops grown under aerobic conditions [12,15]. The flood irrigation condition, as well as the presence of a water film, can condition a lack of oxygen in the soil, which could limit the germination of fungal spores. In addition, the signaling pathway between plant roots and AMF may be affected by the exudation of strigolactones in rice plant roots [16,17]. Reportedly, rice plants have to produce sufficient strigolactone exudates that stimulate colonization [18]. In this investigation, as there is a high concentration in the number of rice plants per trays (approximately 5,000 in 0.18 m^2) that grew from seed coating with EcoMic® biofertilizer, colonization of rice roots, both in biofertilizer-treated and untreated plants, is justified due to the possible increase in strigolactone exudates. In the specific case of treatments without EcoMic® biofertilizer application, the AMF presence in roots of these treatments responds to the fact that the substrate was not sterilized and, therefore, the resident AMF colonized roots of these rice cultivars.

When analyzing the behavior of the height of rice plants at 18 DAE, it was found that those inoculated with AMF showed the greatest magnitudes in all cultivars and in the two experiments, with respect to those not inoculated (Table 1). In general, the increase in plant height of AMF-inoculated plants compared to non-inoculated plants was between 8 and 15 %.

Table 1. Growth and development variables of rice plants inoculated with Arbuscular Mycorrhizal Fungi (AMF) and non-inoculated (non-AFM)

Treatments		**Experiment 1**					**Experiment 2**				
		HE	**N_Leaves**	**L_Root**	**ADM**	**RDM**	**HE**	**N_Leaves**	**L_Root**	**ADM**	**RDM**
INCA LP-7	noAMF	17.41 b	4.00 a	5.05 c	0.0140 cd	0.0050 bc	18.18 cd	4.10 a	6.82 bc	0.0158 b	0,0057 de
	AM	19.70 a	4.20 a	7.67 a	0.0165 a	0.0061 a	20.42 a	4.10 a	8.53 abc	0.0174 a	0,0067 ab
Roana	noAMF	16.29 b	4.00 a	5.09 c	0.0135 e	0.0041 d	17.51 d	4.00 a	6.35 cd	0.0141 cd	0,0057 e
	AM	18.94 a	4.00 a	5.46 c	0.0150 b	0.0058 a	20.29 ab	4.10 a	7.61 abc	0.0156 b	0,0063 bc
José	noAMF	16.76 b	3.90 a	5.21 c	0.0124 e	0.0043 d	18.78 bc	4.00 a	7.14 abc	0.0135 d	0,0055 e
	AM	18.58 a	4.10 a	6.47 ab	0.0148 bc	0.0057 ab	19.16 ab	4.10 a	9.07 ab	0.0154 b	0,0062 cd
Guillemar	noANF	17.13 b	3.90 a	5.23 c	0.0138 d	0.0045 cd	18.14 cd	4.10 a	6.23 d	0.0145 c	0,0057 de
	AM	19.28 a	4.20 a	6.08 b	0.0159 a	0.0058 a	19.74 a	4.20 a	8.63 a	0.0171 a	0,0070 a
SEx		0,402	0.174 NS	0.259	0.0004	0.00021	0.413	0.140 NS	0.698	0.0003	0.00024

Means with equal letters do not differ significantly ($p \leq 0.05$) according to Duncan's Multiple Range Test
Plant height (HE); Number of leaves (N_Leaves): Root length (L_Root): Aerial dry mass (ADM); Root dry mass (RDM)

Regarding the number of leaves per plant, no significant differences were recorded in the two experiments carried out between plants with AMF and non-AMF. However, differences in root length were found in experiment 1, with the exception of Roana cultivar, which showed no differences between plants with AMF and non-AMF. In the rest of cultivars, greater root length was observed in the inoculated treatments. In experiment 2, only differences between AMF and non-AMF plants were observed in cv. Guillemar. The accumulation of aerial and root dry mass showed contrasting behavior, caused by the AMF presence in roots and by the varietal response (Table 1).

The accumulation of aerial and root dry mass, in general, plants inoculated with AMF showed higher values than the non-inoculated control. It is important to note that the cultivars INCA LP-7 and Guillemar inoculated with AMF showed the highest accumulation of aerial dry mass in both experiments. In the case of *cv*. INCA LP-7, the increase in ADM with respect to cultivars without the EcoMic® biofertilizer application (INCA LP-7, Roana, José and Guillemar) represented 13.74, 27.02, 28.48 and 15.60 %, respectively. The *cv*. Guillemar with AMF increased by 17.92, 31.69, 33.21 and 9.73 %, respectively, with respect to the rest of cultivars (INCA LP-7, Roana, José and

Guillemar). However, at the level of root dry mass accumulation, in experiment 1, no differences were found among the cultivars inoculated with AMF, and in the second repetition the best results corresponded to cultivars INCA LP-7 and Guillemar, followed by Roana, but without differences with Guillemar (Table 1).

The increase that was appreciated in plant height, aerial and root dry mass, results correspond to those reported in other investigations (18), when rice plants were inoculated with different AMF strains under similar soil and crop conditions. In addition, the values of plant height and mat formation by root interlacing corresponded with the quality parameters of seedlings, ready for mechanized transplanting (7).

The results of this research showed that the flooding conditions affected colonization, so that at the time of transplanting at 18 DAE, uncolonized plants could be taken to the field. However, the above mentioned allowed explaining that the increase in the development of inoculated plants (height, number of leaves, length of roots, dry mass of aerial part and roots) in relation to non-inoculated plants, may be due, fundamentally to the formation of extraradical mycelium, which facilitates the absorption of water and nutrients by the plants, as previously reported (8,19). Although it may be associated to the exudation by the fungus of growth hormone and to a better nutritional status of the plants (20,21), if we take into account that the volume of roots in the trays is dense and the competition for nutrients and growth in a seedbed increases; therefore, AMF must increase its efficiency inside the root so that it can provide it with elaborated substances (8).

CONCLUSIONS

- With the application of EcoMic® biofertilizer in the seedling stage, rice plants colonized by Arbuscular Mycorrhizal Fungi can be transplanted. With percentages between 10 and 16 %, in addition to a greater development in terms of height, root length, aerial and root dry mass, compared to untreated plants.
- The genetic component has a marked effect on plant growth and development, even when plants are colonized by AMF.
- Based on these results, EcoMic® biofertilizer application is suggested as a nutritional alternative in seedbeds for mechanized rice transplanting.

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