ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

GROWTH, WATER RELATIONS AND EFFICIENCY IN NUTRIENTS UTILIZATION BY TOMATO PLANTS INOCULATED WITH A MYCORRHIZAL INOCULANT IN LIQUID SUPPORT

Crecimiento, relaciones hídricas y aprovechamiento nutricional en el tomate inoculado con un inoculante micorrízico en soporte líquido

José M. Dell'Amico[1]✉, Félix Fernández[2], Emilio Nicolás Nicolás[3] and María de J. Sánchez-Blanco[3]

ABSTRACT. The work was performed in order to know the effects of different doses of a mycorrhizal inoculant in liquid support in growth variables, water relations and efficiency in the use tomato plants nutrients. For its realization, tomato plants INCA 9 [1] were grown in pots and placed in a polycarbonate greenhouse. Three doses were studied (D1, D2 and D3) 75 (D1), 150 (D2) and 300 spores plant^{-1}, applied in seeding through injection by irrigation system and a non-inoculated control. At 25 and 40 days after germination (DDG) evaluation of fungal variables, growth on dry biomass, leaf water potential and its components, stomatic conductance the concentration of foliar nutrients and efficiency use of these. The results showed that with any of the applied doses important benefits are achieved by symbiosis with increases of fungal variables, the content of dry biomass, water relations and the best efficiency in the use of nutrients. However, in general only with the application of the D1 dose, these benefits were obtained, resulting in this work the most appropriate and economic dose.

Key words: tomato, soil fungi, growth, plant water relations, nutrition

RESUMEN. El trabajo se realizó con el objetivo de conocer los efectos de diferentes dosis de un inoculante micorrizógeno en soporte líquido en variables del crecimiento, las relaciones hídricas y la eficiencia en el aprovechamiento de los nutrientes de plantas de tomate. Para su realización, se cultivaron en macetas plantas de tomate del cultivar INCA 9 [1] colocadas en un invernadero de policarbonato. Se estudiaron tres dosis (D1, D2 y D3) 75 (D1), 150 (D2) y 300 esporas planta^{-1} (D3), aplicadas en la siembra mediante su inyección por el sistema de fertirriego y un tratamiento control, sin inocular. A los 25 y 40 días después de la germinación (DDG) se realizaron evaluaciones de variables fúngicas, de crecimiento en biomasa seca, del potencial hídrico foliar y sus componentes, la conductancia estomática y a los 40 DDG se realizó un análisis foliar para evaluar la concentración foliar de nutrientes y la eficiencia de utilización de estos. Los resultados mostraron, que con cualquiera de las dosis aplicadas se alcanzan beneficios importantes de la simbiosis, con incrementos en las variables fúngicas, el contenido de biomasa seca, las relaciones hídricas y la mejor eficiencia en la utilización de los nutrientes. Sin embargo, en general, solo con la aplicación de la dosis D1 se obtuvieron estos beneficios, resultando en este trabajo la dosis más adecuada y rentable.

Palabras clave: tomate, hongos del suelo, crecimiento, relaciones planta agua, nutrición

INTRODUCTION

The fundamental interest generated by the association of plants with arbuscular mycorrhizal fungi (AMF), is due to its universality among families of vascular plants, its apparent lack of specificity to inoculate already ample evidence of its influence on the growth of plants by greater incorporation of nutrients and improving their water relations (1, 2).

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

[2] Empresa SYMBORG, Campus de Espinardo 7 edif. CEEIM CP 30100, Murcia, España.

[3] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Murcia, España.

✉ amico@inca.edu.cu

In natural and semi-natural ecosystems, AMF are the most abundant group and functionally the most important on the ground and the dependence mycotrophic responsible for 90 % of terrestrial plants (3).

It is due to their beneficial effects on plants which are applied in agricultural practice as biofertilizers. In addition, their use significantly reduces environmental impact in agricultural areas (4, 5).

These products are generally produced in solid support and in Cuba; in the National Institute of Agricultural Sciences (INCA) from 2000, a liquid inoculant was obtained, LicoMic (6) allowing use through irrigation systems and diversify the ways of applying these fungi with more efficient and profitable adequate doses.

For the above, the following work was performed with the fundamental objective of knowing the effects of different doses of a mycorrhizal inoculant in liquid support in variables of growth, water relations and efficiency in the nutrients´ use of tomato plants.

MATERIALS AND METHODS

The work was performed at the experimental farm of the Center for Soil Science and Applied Biology Segura (CEBAS-CSIC), in Santomera-Murcia town, Spain. Seeds of Solanum *Lycopersicon* L. INCA variety 9 [1] of the determined growth, for the breeding program tomato INCA-MES were sown directly in containers (pots) of 5 L of capacity, containing a substrate compound for: soil-washed sand-vermiculite mixed in ratio 3: 2: 1 v/v. The characteristics of the soil used appear in Table I.

Table I. Characteristics of the soil used

Organic material	1,70 %
Total organic carbon	0,98 %
Total Nitrogen	0,132 %
Relation C/N	7,42
Total carbonates	12,14 %
Active limestone	3,98 %
Assimilable phosphorus	196,68 ppm
Chlorides	0,05 meq 100 g^{-1}
Sulfates	0,29 meq 100 g^{-1}
Iron assimilable	8,51 ppm
Copper assimilable	1,60 ppm
Manganeso asimilable	42,01 ppm

Treatments

Four treatments were studied: three doses (D1, D2 and D3) of a mycorrhizal inoculant liquid support based on *Glomus fasciculatun* currently *Glomus cubensis* (7), 75 (D1), 150 (D2) and 300 spores $plant^{-1}$ (D3), applied at planting. These doses were compared against a control treatment without inoculation. To each treatment corresponded 25 pots distributed in a completely randomized design and each one was placed in a greenhouse polycarbonate equipped with a refrigeration system, cooling type.

Inoculation was by the irrigation system, using a jet pump with flow rate of 30 L h^{-1}. For the application of different doses, valves were inserted into the irrigation lines that allowed wean each treatment (dose) and to make a correct inoculation.

Irrigation was carried out by a fertigation system and each container (pot) was placed an emitter (dropper) of 2 L h^{-1} and a delivery device for uniform water on the substrate surface distribution.

From planting to germination starting irrigation water was only at 0,5 L applied twice daily to each container to ensure good germination. After germinated plants, they received 0,40 L twice a week to 12 days after planting. From this date fertigation began, adding to a tank of 2000 L fertilizer the following:

$NH_4 NO_3$ (96 %) ——— 86 g
KNO_3 (99,8 %) ——— 1430 g
$Ca (NO_3)_2$ (91 %) ——— 828 g
H_3PO_4 (72 %) ——— 408 g
HNO_3 (54 %) ——— 352 g

At the beginning, the fertigation was applied at a rate of 0,40 L per container, three times a week and remained so until the early fruiting, when it began to water daily. Irrigation and fertigation always applied equally to all treatments.

The analysis results of the water used for irrigation of the experiment are presented below:

PH _____ 7.90
C.E. _____ 1,166 mmhos cm^{-1}
S.T.D. _____ 0, 830 g L^{-1}

These values show that the water used was of good quality.

Evaluations conducted

Fungal variables

- Radical Colonization: Trypan Blue method (8).
- Fungal percentage occupation (9).
- Mycorrhizal dependence (DM) based on the total dry mass and by the formula:

DM= Dry mass of plants with AMF-Dry mass of plants without AMFA (10)
Dry mass of plants with AMF

Evaluations were conducted in rootlets of three plants per treatment and at two samples, at 25 and 40 days germinated (DAG). For colonization and occupation percentage samples they were observed in a stereomicroscope and microscope Olympus.

Leaf water potential (Y_f) (Mpa) and its components. It was measured on five plants per treatment to 25 and 40 DAG. The first evaluation was carried out between 9:00 to 10:00 a.m. and the second between 5:00 to 6:00 a.m. (before dawn) and for this purpose a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, C A) it was used. For the current osmotic potential foliar (Ψact. Foliar) immediately after evaluating Ψ_f leaves were covered with foil and frozen in liquid nitrogen and for determining the osmotic potential saturated leaf (ΨOact $_{foliar}$) surrounding leaves were taken at selected for measuring Y_f and placed in hydration chambers with distilled water, darkness and between 6 and 8 °C for 24 hours. Immediately after they were wrapped with aluminum foil for freezing in liquid nitrogen and stored in a freezer at -80 °C.

Subsequently they were thawed at room temperature, placed in Ependorf with small holes at the base, these were placed in another, similar but unperforated and cell juice was extracted by centrifuging for three minutes at 3 000 rpm. From aliquots of 100 ul, the current osmotic potential (ΨOact.$_{foliar}$) and saturated osmotic potential leaf (leaf ΨOsat$_{foliar}$) was determined with a vapor pressure osmometer Wescor of 5500.

Pressure potential (Ψp) was calculated from the difference between the leaf water potential and osmotic potential current leaf, using the following equation:

$$Y\ p = \Psi f - \Psi O\ act._{foliar} \quad (Mpa).$$

Stomatal conductance (gs). These evaluations were conducted in three plants per treatment at 25 and 40 DAG. In all cases, assessments began at 10:30 a.m, using a porometer of diffusion *steady state* (LIQUOR-1600, LIQUOR, UK). For assessments for the leaf water potential and stomatal conductance, leaves of the upper third of the plants and well exposed to the sun were taken.

Dry mass of root and aerial part. These variables were evaluated in two moments of the vegetative cycle of plants, at 25 and 40 DAG. To do this, 10 plants were taken for treatment and were separated into root and aerial part and dried in a forced system oven at 75 °C to constant weight.

Mineral analysis. At 40 DAG a foliar analysis in order to know the effects of treatments on concentrations (%) of Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) was performed. To do this, five samples per treatment, consisting of 15 leaves fully developed and taken at random were taken.

Mineral determinations were performed in a specialized laboratory to this type of analysis. The percentages of N and P were determined by the Nessler's reagent method and spectrometric and concentrations of K, Ca and Mg using a Shimadzu Atomic Absorption Spectrophotometer AA-680 (Shimadzu Co. Ltd. Kyoto, Jp.). The efficiency of utilization of nutrients was defined as the amount of biomass produced per unit of nutrient in tissues and was calculated by the relationship:

Biomass production (g) (10)
Nutrient concentration (%)

The data on the percentage of mycorrhizal colonization were transformed by the 2arcsen$\sqrt{x}$ function. This variable, like other indicators evaluated was subjected to an analysis of variance of simple classification (ANOVA). For processing data and comparison of means, the SPSS 10.0 statistical software for *Windows* was used and means with significant differences were compared according to the multiple range test of Duncan for p≤0,05.

RESULTS AND DISCUSSION

When analyzing the behavior of mycorrhizal colonization (Table II), it was found that both 25 as at 40 DAG no significant differences were found in plants inoculated with different doses studied and the highest percentages of colonization were presented to 40 DAG, corresponding to the stage of fruiting tomato and inoculated plants. It should be noted that control treatment plants presented at both times, minimum values infection, whereas those for the inoculated treatments showed relatively high values compared to that reported by other researchers working on this crop (11).

The fact that there are no significant differences in the percentages of colonization among the doses studied this inoculant in liquid formulation, confirmed the effectiveness of inoculation with this product, behavior has been noted before.[A]

[A] Fernández, F.; Dell'Amico, J.; Pérez, Y.; Morte, A.; Honrubia, M. y Providencia, I. ''Viabilidad y capacidad de colonización de hongos micorrízicos arbusculares en medio líquido (LicoMic)'', eds. Frías-Hernández, J.T., Olalde-Portugal, V., y Ferrera-Cerrato, R., *Avances en el conocimiento de la biología de las Micorrízas*, edit. Universidad de Guanajuato, México, 2004, pp. 237-251.

Table II. Percentage of colonization, fungal occupation and mycorrhizal dependence of tomato plants Inoculated with different doses of a mycorrhizal inoculant in liquid formulation based on *G. cubensis*

Treatment	Colonization %	Fungal occupation %	Mycorrhizal dependence (Based on total dry mass) %
25 DDG			
Control	11,13 b	0,14 c	0
D1	63,37 a	3,37 b	54,37 a
D2	49,27 a	6,65 a	49,50 a
D3	54,73 a	1,91 b	39,75 a
E. est. X	2,1003 *	1,5011 *	5,8729 *
40 DDG			
Treatment			
Control	20,79 b	1,53 b	0
D1	83,14 a	17,49 a	37,10 ab
D2	75,26 a	13,73 a	49,01 a
D3	76,58 a	10,70 a	31,99 b
E. est. X	5,2112 *	3,7004 *	4,5514 *

* Statistically significant difference

Moreover, the percentages of fungal occupation, which represent the intensity of mycorrhizal colonization, increased significantly with the development of the crop. At 25 DAG, values of this variable were significantly higher in plants inoculated with dose (D2) and 40 DAG no statistically significant differences between different doses were observed. The fact of being a fungal occupation at 40 DAG warrants further symbiotic efficiency, more fungal structures exist inside root cells, and therefore a higher fungus-host exchange, regardless of the dose used.

As mycorrhizal dependence (DM), at 25 DAG a similar behavior to the colonization percentage was found and at 40 DAG, most mycorrhizal dependency corresponded to plants inoculated with D2, followed by plants of D1 and D3 treatments, respectively. It should be noted that at the 40 DAG, DM decreased in plants inoculated with D1 and D3, with respect to 25 DAG, while values in inoculated with D2 remained similar.

It is noteworthy that in this variable no statistically significant differences among plants inoculated with D1 and D2 in the evaluated moments, suggesting that symbiosis was effective regardless of applied spore number and inoculated plants reached growth of total biomass between 40 to 54 % higher than the uninoculated ones to 25 DAG.

In this sense, in the olive cultivation (*Olea europaea*) DM values between 39 and 62 %, depending on the *Glomus* strain used (10) were found. In the species *Oyedaea verbesinoides* (12) inoculating with strain *Glomus manihotis* have reported high values of DM.

Other researchers point out that the woody species present a mycorrhizal dependence greater than herbaceous, which apparently is associated with the lack of absorbent hairs. Moreover, there are evidences that plants with branched roots are more dependent on mycorrhizal associations that non-branched. Other studies also suggest that the fibrous roots of tropical species are colonized rapidly with greater growth response as a result of the symbiosis (13).

In Table III, Ψ_f values and their ΨO act.$_{foliar}$ and Ψp components are presented as well as the ΨOsat.$_{foliar}$ where it was found that the 25 plants inoculated with DAG dose D1 showed the highest values of Ψf with significant differences with other treatments, similarly occurred with Ψ p values, although in this case there were no significant differences from the other doses used and other control plants. As for the ΨO act.$_{foliar}$, this variable was similar in all treatments and the ΨOsat.$_{foliar}$ the most negative values corresponded with the inoculated plants with D1 and D3 doses, respectively, with no statistically significant differences among them are appreciated.

At 40 DAG were no significant differences between the variables and their component Ψf ΨO act.$_{foliar}$ leaf from plants of the treatments under study, although less negative values corresponded to treatment plants D1 and D3, with very similar values. As for the Ψp, no differences between plants inoculated with the three doses applied were found, but these with control treatment.

Table III. Behavior of leaf water potential (Ψ_f) and its osmotic components (ΨOact. $_{foliar}$) and pressure (Ψp) and saturated osmotic potential (ΨOsat $_{foliar}$) in young tomato plants inoculated with three doses of *G. cubensis* in liquid formulation

	Leaf water potential and its components (MPa)			
Treatment	**Leaf water potential**	**Osmotic potential**	**Pressure potential**	**Saturated osmotic potential**
25 DDG (9:00 a.m.)				
Control	- 0,47 c	- 0,91	0,44 b	- 0,66 a
D1	- 0,26 a	- 0,85	0,59 a	- 0,74 ab
D2	- 0,36 b	- 0,89	0,53 a	- 0,69 a
D3	-0,37 b	- 0,88	0,51 ab	- 0,81 b
E. est. X	0,0223	0,0098	0,0186	0,022
	*	n.s.	*	*
40 DDG (6:00 a.m.)				
Control	-0,37	- 0,80	0,43 b	- 0,90 c
D1	-0,31	-0,80	0,49 ab	-0,84 bc
D2	-0,34	-0,84	0,50 ab	-0,78 ab
D3	-0,32	-0,84	0,52 a	- 0,70 a
E. est. X	0,0102	0,0099	0,0186	0,0241
	n.s.	n.s.	*	*

* Statistically significant difference

In the case of ΨOsat.$_{foliar}$ even when plants of the control treatment had the lowest values, followed by inoculated with D1, not the occurrence of the process of osmotic adjustment was evident, given the values of Ψp reached by plants of these treatments.

In general, mycorrhizal inoculation with any of the doses used favored water relations of plants at both time points assessed, but based on the values of Ψf no symptoms of water deficiency demonstrated in plants of any of the treatments, indicating that the irrigation was effective and uniformly applied to all plants.

The benefits of this mutualistic symbiosis in water relations of different tomato varieties have been reported previously with different strains, doses, types and forms of inoculant inoculation (14, 15).

These results are also consistent with those report that the extensive network of hyphae extramatrical mycelium produced by mycorrhizal symbiosis acts as an extension of the root in the ground (16), so that the plant obtains additional availability of nutrient absorption, mainly N, P and soil water (17). Moreover others point out that the HMA prevent the formation of large spaces between the roots and the soil, which facilitates the continuity of water through the root-soil interface (18).

In addition to the beneficial effects of arbuscular mycorrhizal fungi in plant physiology, they have been attributed benefits in other ecological processes, including their contribution to soil structure (19) which is of vital importance for the sustainability of agro-ecosystems. Also, it has be said that they have a marked influence on the formation of soil aggregates through biochemical, biophysical and biological processes, which include mechanical actions of the hyphae, excretion of glycoproteins and other extracellular compounds and interactions with soil biota (1). These aspects, undoubtedly, promote soil water relations and thus increase the availability of water for plants.

In Table IV the results of evaluations of stomatal conductance (gs) plants are shown.

At 25 DAG highest values of this variable corresponded to plants inoculated with D1, showing statistically significant differences with plants of the three remaining treatments. Moreover, the lowest values occurred in control treatment plants, while at the 40 DAG showed no significant differences in the treatments plants.

Table IV. Stomatal conductance values of tomato plants inoculated with different doses of a mycorrhizal inoculant in liquid formulation

Treatments	Stomatal conductance ($mmolH_2O\ m^{-2}\ s^{-1}$)
25 DDG	
Control	152 c
D1	180 a
D2	166 b
D3	168 b
E. est. X	4,0685 *
40 DDG	
Control	173
D1	135
D2	158
D3	175
E. est. X	8,3005 n.s.

* Statistically significant difference

These results indicate that both the gs, as in Ψf, the beneficial effects of inoculation were less obvious in as the crop (40 DAG) was developed, even in the case of gs values of this variable in plants they inoculated with D1 and D2 were the lowest. Also, it keeps in mind that these variables depend largely on the environmental conditions prevailing at the time of the measure and also respond to many disturbances linked to hydraulic continuous soil-plant-atmosphere.

The effect of inoculation treatments in dry biomass accumulation of plants are presented in Table V, where it was found that both 25, as 40 DAG existed a marked beneficial effect of inoculation with AMF in the dry root, aerial and total mass standing out in this sense generally plants inoculated with D1 and D2 doses. However, in the dry mass ratio of root/shoot only statistically significant differences at 40 DAG for plants inoculated with D2 were presented, while corresponding to the control treatment showed smaller values.

It should be noted that there is a large body of evidence about the benefits of mycorrhization in plant growth, tomato (20), tobacco *Nicotiana tabacum* (21), pepper *Capsicun annum* (22) in rice *Oryza sativa* (16, 23, 24) in hard wheat *Triticum durum* (25) in sorghum *Sorghum vulgare* (26), among others, mainly due to a greater absorption of nutrients, mainly phosphorus (27). Furthermore, it has been shown that these fungi naturally associated with other microorganisms also contribute positively to the growth of plants.

Moreover, in the tomato and wheat cultivation, it has been found that mycorrhizal inoculation in liquid formulation was more effective than in solid support (EcoMic) mainly due to some properties conferred the liquid medium to spores, favoring the germination and infectivity of these.

Table V. Effect of different doses of liquid mycorrhizal inoculant on tomato growth variables

	Plant Growth Variables			
Treatment	Dry root mass (g)	Aerial dry mass (g)	Dry mass of total (g)	Root / shoot dry mass ratio
25 DDG				
Control	0,050 b	0,200 c	0,250 c	0,26
D1	0,106 a	0,443 a	0,549 a	0,24
D2	0,118 a	0,363 ab	0,481 ab	0,33
D3	0,103 a	0,302 b	0,405 b	0,35
E. est. X	0,0076 *	0,0232 *	0,0288 *	0,0178 n.s.
40 DDG				
Control	0,452 b	2,473 b	2,925 c	0,19 b
D1	1,308 b	3,320 a	4,629 b	0,40 b
D2	2,413 a	3,676 a	6,088 a	0,67 a
D3	0,729 b	3,331 a	4,059 bc	0,22 b
E. est. X	0,2114 *	0,1571 *	0,3120 *	0,0577 *

* Statistically significant difference

In analyzing the results in terms of foliar nutrient concentration (Table VI A) it was found which no significant differences among the plants of different treatments, it is presented in conjunction with foliar concentrations of N, Ca and Mg and only these differences were presented in the concentrations of P and K, without strong effect of inoculation treatments are appreciated because in the case of P, as well as other nutrients, generally higher concentrations corresponded to plant control treatment and inoculated with D2 and in the specific case of K, the highest concentration corresponded to plants inoculated with D2 and D3 and the lowest values were the ones inoculated with D1. Moreover, it was observed that the lowest concentration values were presented in the P and Mg. These results apparently are associated with the greatest growth increased demand for nutrients, especially P and this caused a decrease in nutrient concentrations in the biomass of the inoculated plants. This behavior was observed previously by researchers working with different grass species (28, 29).

However, considerably mycorrhizal inoculation favored the efficiency use of nutrients (Table VI B) and in all cases the efficiency was higher in plants of inoculation treatments, without statistically significant differences among them.

It is noteworthy that the largest magnitude of utilization efficiency was presented in the P and Mg, contrary to what was observed in concentration and the lowest corresponded to N and Ca.

These results indicate that plants inoculated with either studied dose used more efficiently soil nutrients in the production of biomass than the control treatment. Similar results were found when we worked with positions of tobacco in technified seedbeds (21). Moreover, (Roberts 2006) cited by (Stewart 2007) suggested rightly that improve the efficiency of nutrient use is a major challenge facing agriculture in general, there are tools to achieve this goal, with the use of biofertilizers such an element of paramount importance (30). However, it should avoid these improvements are made to the detriment of producers or the environment, ie, avoid reduced yields and reduce the use of chemical fertilizers.

CONCLUSIONES

Due to the results achieved in each of the variables analyzed and the beneficial effect produced on them by the use of doses of LicoMic, it is clear recommend the application of D1 dose as adequate and economically profitable, regardless of continuing studies in the use of lower doses and in other aspects of nutrient absorption.

Table VI. Foliar concentration of N, P, K, Ca and Mg (A) and efficiency in nutrient utilization (B) of tomato plants inoculated with different doses of a mycorrhizal inoculant in liquid formulation

Treatments	N	P	K	Ca	Mg
The concentration (%)					
Control	5,20	0,48 a	2,93 b	4,67	0,92
D1	4,97	0,45 b	2,79 c	4,40	0,87
D2	5,07	0,48 a	2,97 ab	4,49	0,93
D3	4,97	0,42 b	3,05 a	4,63	0,92
Est. X	0,0502 n.s.	0,0078 *	0,0288 *	0,0750 n.s.	0,0164 n. s.
B (Efficiency of use)	Concentration (%)				
Control	0,48 b	5,15 b	0,84 b	0,53 b	2,70 b
D1	0,67 a	7,47 a	1,19 a	0,76 a	3,83 a
D2	0,73 a	7,65 a	1,24 a	0,83 a	3,99 a
D3	0,67 a	7,88 a	1,09 a	0,72 a	3,61 a
Est. X	0,0122 *	0,3599 *	0,0496 *	0,0354 *	0,1659 *

* Statistically significant difference

BIBLIOGRAPHY

1. Harris-Valle, C.; Esqueda, M.; Valenzuela-Soto, E.M. y Castellanos, A.E. ''Tolerancia al estrés hídrico en la interacción planta-hongo micorrízico arbuscular: metabolismo energético y fisiología'', *Revista Fitotecnia Mexicana*, vol. 32, no. 4, 2009, pp. 265–271, ISSN 0187-7380.
2. Pérez-Moreno, J. y Negreros-Castillo, P. ''Los hongos micorrízicos arbusculares y su implicación en la producción y manejo de especies neotropicales forestales, con énfasis en meliáceas'', *Interciencia*, vol. 36, no. 8, 2011, pp. 564–569, ISSN 0378-1844.
3. Smith, S.E. y Read, D.J. *Mycorrhizal Symbiosis*, edit. Academic Press, 25 de octubre de 1996, p. 621, ISBN 978-0-08-053719-1.
4. Miransari, M. ''Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress'', *Plant Biology*, vol. 12, no. 4, 1 de julio de 2010, pp. 563-569, ISSN 1438-8677, DOI 10.1111/j.1438-8677.2009.00308.x.
5. Pérez, A.; Rojas, S.J. y Montes, V.D. ''Hongos formadores de micorrizas arbusculares: una alternativa biológica para la sostenibilidad de los agroecosistemas de praderas en el Caribe colombiano'', *Revista Colombiana de Ciencia Animal*, vol. 3, no. 2, 2011, pp. 366-385, ISSN 2027-4297.
6. Fernández, F.; Dell'Amico, J.M. y Pérez, Y. *Inoculante micorrizógeno líquido*, no. WO 2006/060968 A1, pp. 1-15.
7. Rodríguez, Y.; Dalpé, Y.; Séguin, S.; Fernández, K.; Fernández, F. y Rivera, R.A. ''*Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba'', *Mycotaxon*, vol. 118, no. 1, 5 de enero de 2012, pp. 337-347, DOI 10.5248/118.337.
8. Phillips, J.M. y Hayman, D.S. ''Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection'', *Transactions of the British Mycological Society*, vol. 55, no. 1, agosto de 1970, pp. 158-IN18, ISSN 0007-1536, DOI 10.1016/S0007-1536(70)80110-3.
9. Giovannetti, M. y Mosse, B. ''An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots'', *New Phytologist*, vol. 84, no. 3, 1 de marzo de 1980, pp. 489-500, ISSN 1469-8137, DOI 10.1111/j.1469-8137.1980.tb04556.x.
10. Porras-Soriano, A.; Soriano-Martín, M.L.; Porras-Piedra, A. y Azcón, R. ''Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions'', *Journal of Plant Physiology*, vol. 166, no. 13, 1 de septiembre de 2009, pp. 1350-1359, ISSN 0176-1617, DOI 10.1016/j.jplph.2009.02.010.
11. Fundora, L.R.; Mena, A.; Rodríguez, Y.; González, P.J.; Rodríguez, P. y González-Peña, D. ''Estabilidad de la eficiencia de la cepa *Glomus mosseae* en la respuesta del tomate a condiciones de estrés hídrico fuera de su periodo óptimo'', *Cultivos Tropicales*, vol. 29, no. 4, diciembre de 2008, pp. 47-53, ISSN 0258-5936.
12. Flores, C. y Cuenca, G. ''Crecimiento y dependencia micorrízica de la especie pionera y polenectarífera *Oyeda verbesinoides* (tara amarilla), asteraceae'', *Interciencia*, vol. 29, no. 11, noviembre de 2004, pp. 632-637, ISSN 0378-1844.
13. Zangaro, W.; Nishidate, F.R.; Camargo, F.R.S.; Romagnoli, G.G. y Vandressen, J. ''Relationships among arbuscular mycorrhizas, root morphology and seedling growth of tropical native woody species in southern Brazil'', *Journal of Tropical Ecology*, vol. 21, no. 05, septiembre de 2005, pp. 529–540, ISSN 1469-7831, DOI 10.1017/S0266467405002555.
14. Dell'Amico, J.; Rodríguez, P.; Torrecillas, A.; Morte, A. y Sánchez-Blanco, M.J. ''Water and growth parameter responses of tomato plant associated with arbuscular mycorrhizae during drought and recovery'', *Journal of Agricultural Science*, vol. 138, 2002, pp. 387-393, ISSN 0021-8596.
15. Dell'Amico, J.M.; Fernández, F.; Nicolás, E.; López, L.F. y Sánchez-Blanco, M.J. ''Respuesta fisiológica del tomate a la aplicación de dos inoculantes a base de *Glomus* sp1 (*INCAM 4*) por dos vías de inoculación diferentes'', *Cultivos Tropicales*, vol. 28, no. 2, 2012, pp. 51–58, ISSN 0258-5936.
16. Mena Echevarría, A.; Fernández Suárez, K.; Jerez Mompie, E.; Olalde Portugal, V. y Serrato, R. ''Influencia de la inoculación con *Glomus Hoi-Like* y un conglomerado de especies de HMA en el crecimiento de plantas de sorgo sometidas o no a estrés hídrico'', *Cultivos Tropicales*, vol. 32, no. 1, marzo de 2011, pp. 16-27, ISSN 0258-5936.
17. Ruiz Sánchez, M.; Ruiz-Lozano, J.M. y Muñóz Hernández, Y. ''Las acuaporinas, su relación con los hongos micorrízicos arbusculares y el transporte de agua en las plantas'', *Cultivos Tropicales*, vol. 32, no. 3, septiembre de 2011, pp. 18-26, ISSN 0258-5936.
18. Augé, R.M. ''Arbuscular mycorrhizae and soil/plant water relations'', *Canadian Journal of Soil Science*, vol. 84, no. 4, 1 de noviembre de 2004, pp. 373-381, ISSN 0008-4271, DOI 10.4141/S04-002.
19. Rillig, M.C. y Mummey, D.L. ''Mycorrhizas and soil structure'', *New Phytologist*, vol. 171, no. 1, 1 de julio de 2006, pp. 41-53, ISSN 1469-8137, DOI 10.1111/j.1469-8137.2006.01750.x.
20. Mujica Pérez, Y. y Batlle Sales, J. ''Funcionamiento de la inoculación líquida con hongos micorrízicos arbusculares (HMA) en plantas de tomate (*Solanum lycopersicum* L.)'', *Cultivos Tropicales*, vol. 34, no. 4, diciembre de 2013, pp. 5-8, ISSN 0258-5936.
21. Cruz Hernández, Y.; García Rubido, M.; Hernández Martínez, J.M. y León González, Y. ''Influencia de las micorrizas arbusculares en combinación con diferentes dosis de fertilizante mineral en algunas características morfológicas de las plántulas de tabaco'', *Cultivos Tropicales*, vol. 33, no. 3, septiembre de 2012, pp. 23-26, ISSN 0258-5936.
22. Montero, L.; Duarte, C.; Cun, R. y Cabrera, J.A. ''Efectividad de biofertilizantes micorrízicos en el rendimiento del pimiento (*Capsicum annuum* L. var. Verano 1) cultivado EN diferentes condiciones de humedad del sustrato'', *Cultivos Tropicales*, vol. 31, no. 3, septiembre de 2010, pp. 00-00, ISSN 0258-5936.
23. Fernández, F.; Dell'Amico, J.M.; Angoa, M.V. y de la Providencia, I.E. ''Use of a liquid inoculum of the arbuscular mycorrhizal fungi Glomus hoi in rice plants cultivated in a saline Gleysol: A new alternative to inoculate'', *Journal of Plant Breeding and Crop Science*, vol. 3, no. 2, 2011, pp. 24–33, ISSN 2006-9758.

24. Ruiz Sánchez, M.; Polón Pérez, R.; Vázquez Del Llano, B.; Muñoz Hernández, Y.; Cuéllar Olivero, N. y Ruiz-Lozano, J.M. ''La simbiosis micorrizíca arbuscular en plantas de arroz (*Oryza sativa* L.) sometidas a estrés hídrico: Parte I. Mejora la respuesta fisiológica'', *Cultivos Tropicales*, vol. 33, no. 4, diciembre de 2012, pp. 47-52, ISSN 0258-5936.
25. Ruiz-Sánchez, M.; Aroca, R.; Muñoz, Y.; Polón, R. y Ruiz-Lozano, J.M. ''The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress'', *Journal of Plant Physiology*, vol. 167, no. 11, 15 de julio de 2010, pp. 862-869, ISSN 0176-1617, DOI 10.1016/j.jplph.2010.01.018.
26. Plana, R.; González, P.J.; Fernández, F.; Calderón, A.; Marrero, Y. y DellAmico, J.M. ''Efecto de dos inoculantes micorrízicos arbusculares (base líquida y sólida) en el cultivo del trigo duro (*Triticum durum*)'', *Cultivos Tropicales*, vol. 29, no. 4, diciembre de 2008, pp. 35-40, ISSN 0258-5936.
27. Andrade, S. a. L.; Mazzafera, P.; Schiavinato, M.A. y Silveira, A.P.D. ''Arbuscular mycorrhizal association in coffee'', *The Journal of Agricultural Science*, vol. 147, no. 02, abril de 2009, pp. 105–115, ISSN 1469-5146, DOI 10.1017/S0021859608008344.
28. Johnson, N.C.; Rowland, D.L.; Corkidi, L. y Allen, E.B. ''Plant winners and losers during grassland n-eutrophication differ in biomass allocation and mycorrhizas'', *Ecology*, vol. 89, no. 10, 1 de octubre de 2008, pp. 2868-2878, ISSN 0012-9658, DOI 10.1890/07-1394.1.
29. Costa, K.A. de P.; Faquin, V.; Oliveira, I.P. de.; Severiano, E. da C.; Simon, G.A. y Carrijo, M.S. ''Extração de nutrientes pela fitomassa do capim-marandu sob doses e fontes de nitrogênio'', *Revista Brasileira de Saúde e Produção Animal*, vol. 10, no. 4, 16 de diciembre de 2009, ISSN 1519-9940, [Consultado: 19 de junio de 2015], Disponible en: <http://www.rbspa.ufba.br/index.php/rbspa/article/view/1410>.
30. Stewart, W.M. ''Consideraciones en el uso eficiente de nutrientes'', *Informaciones Agronómicas*, vol. 67, 2007, pp. 1–7, ISSN 2222-0178.

Received: October 31st, 2014
Accepted: January 14th, 2015