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Short communication
EVALUATION OF *Glomus cubense* INFECTIVITY IN LIQUID FORMULATION SUBJECTED TO DIFFERENT HYDROSTATIC PRESSURES

Comunicación corta
Evaluación de la infectividad de *Glomus cubense* en formulación líquida sometida a diferentes presiones hidrostáticas

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ABSTRACT. In order to confirm *Glomus cubense* infectivity in liquid formulation exposed to different hydrostatic pressures, an experiment under controlled conditions was conducted. AMF spores were exposed to different pressures (Pressure I: 0,15, Pressure II: 0,3 and Pressure III: 0,5 MPa, respectively) for one minute before applying the inoculant into seeds of sorghum and corn. Plants grew for 30 days and fungal indicators and variables related to their growth were evaluated. The effect of different pressures didn't affect the fungal structures morphologically. A positive response to fungal inoculation was found in sorghum and corn plants, as there were significant differences in relation to control plant. These results show that it is possible to include the liquid inoculant through fertigation systems.

Key words: mycorrhiza, spores, morphology, pressure

RESUMEN. Con el objetivo de comprobar la infectividad de *Glomus cubense* en formulación líquida expuesta a diferentes presiones hidrostáticas, se condujo un experimento en condiciones controladas. Las esporas de HMA se expusieron a diferentes presiones (Presión I: 0,15; Presión II: 0,3 y Presión III: 0,5 MPa, respectivamente), durante un minuto antes de aplicar el inoculante en semillas de sorgo y maíz. Las plantas crecieron durante 30 días y se evaluaron indicadores fúngicos y variables relacionadas con su crecimiento. El efecto de las diferentes presiones no afectó morfológicamente a las estructuras fúngicas. Se encontró una respuesta positiva a la inoculación fúngica en las plantas de sorgo y maíz, al existir diferencias significativas con relación a las plantas no inoculadas. Estos resultados demuestran que es posible la inclusión del inoculante líquido, mediante los sistemas de fertirriego.

Palabras clave: micorrizas, esporas, morfología, presión

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) represent a group of edaphic microorganisms that, due to their direct effects on mineral nutrition, especially phosphorus and nitrogen (1,2); the induction of tolerance to biotic (pathogens) and abiotic (drought and salinity) stress conditions (3,4); their participation in the phytoremediation processes (5,6) and their contribution in the stability of the soil aggregates (7), are used in the production of inoculants.

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Due to the condition of obligate symbionts, AMFs require a host to complete their life cycle (8), but this has not been a factor limiting their multiplication (9) and they are currently formulated as commercial products (solids, granulates and liquids), but practical experiences have shown that the application of each inoculant depends on the type of crop and the conditions for its management; being one of the immediate challenges, the obtaining of products that adjust to any agricultural system (10). The Cuban experience with the management of mycorrhizal symbiosis, using solid carriers, has provided valuable results during more than 20 years of sustained studies with these symbionts, in which the following principles

have been established: the inoculation of efficient species of AMF and the effect of the type of soil in the selection of said strains, in correspondence with the nutrient balance (11).

On the other hand, the agricultural system in Cuba is going through a process of improvement in its production models and, recently, the joint action of decisive actors, scientific centers that promote their research towards this sector and the implementation of collaborative projects, strengthen the fertigation practice, as a technology that guarantees the efficient and rational use of natural resources (12). In the 2000s, a liquid inoculant based on said fungi was obtained in order to guarantee its application through fertigation, but it is not known if the fungal structures retain their integrity once they are exposed to the hydrostatic pressures of these systems.

For this reason, the objective of this study was to evaluate the effect of the inoculation of Glomus cubense in liquid formulation subjected to different hydrostatic pressures in plants of sorghum (*Sorghum bicolor* L.) and corn (*Zea mays* L.).

MATERIALS AND METHODS

General Experimental Conditions

The experiment was conducted under controlled conditions in the Physiology and Plant Biochemistry Laboratory of the National Institute of Agricultural Sciences (INCA), San José de la Lajas, Mayabeque province, with an average temperature that ranged between ± 18-25 °C, humidity relative of 75 % and the photoperiod was adjusted to 16 light hours/8 dark hours for 30 days (July and August) of 2016.

The clay was autoclaved at 121 °C for two hours, in continuous cycles of three days and classified as humic carbonate Gleysol (13) and some of its chemical characteristics as well as the content of resident AMF spores, are shown in the Table 1.

Chemical determinations: pH, potentiometry; organic matter (MO), Walkley Black (14); phosphorus (P2O5), extraction with 0.025 M H_2SO_4 and determination by spectrometer; interchangeable cations, Ca^{2+} Mg^{2+} (extraction with NH4Ac 1 mol L^{-1} at pH 7 and determination by complexometry), Na+and K+ (extraction with boiling HNO_3 and determination by flame photometry); spores of HMA, Gerdemann and Nicholson (15).

Obtaining the fungal material for the liquid inoculant

The INCAM-4 strain of *Glomus cubense* (Y. Rodr. Dalpé) (16), from the collection of strains of the National Institute of Agricultural Sciences (INCA) of Cuba, was reproduced in a sterile clay with sorghum plants (*Sorghum bicolor* L. Moench cv. 'CIAP 1322') and 9 0 days after sowing, the aerial part was eliminated and the substrate with mycorrhizal roots was used as a source of inoculant. The material was manually homogenized, dried at room temperature and stored for 15 weeks at 4 °C.

For the isolation of the spores 50 g of the homogenized material were taken and a wet sieve (15) was made of the paste obtained by mixing the solid with water, between two sieves (40 and 400 µm of light) with the addition of water to facilitate the process. The remaining residue was collected in the 40 µm sieve, with a spatula was passed to a centrifuge tube in which it was mixed with sucrose solution (720 g of sucrose and 20 g of Tween 80 L^{-1}) and centrifuged 2000 rpm for five minutes. Subsequently, the liquid fraction was decanted with the fungal propagules that were deposited in 1.5 mL Eppendorf tubes, with 300 µL of Ringer solution to preserve them until disinfection. One liter of the Ringer solution contained 7.5 g NaCl, 0.75 g KCl, 0.1 g $CaCl_2$ and 0.1 g $NaHCO_3$.

The fungal propagules were contacted with a 2 % Chloramine T solution and two drops of Tween 20 for 10 minutes. Subsequently, they were washed three times with sterile distilled water and placed in an antibiotic solution containing streptomycin sulfate (0.02 %) and gentamicin sulfate (0.01 %) for equal time (17). Finally, they were washed with sterile distilled water and stored in an osmoprotective solution.

The spores contained in said osmoprotective solution were exposed for one minute at three different pressures (0.15, 0.3 and 0.5 MPa, respectively) in a Scholander pressure chamber, Soil Moisture, Model P80 L08.

Table 1. Chemical characteristics and content of resident AMF spores of the clay used in the experiment

pH	MO	P_2O_5	Ca^{2+}	Mg^{2+}	K+	Na+	CIB	Esp. HMA
	(g kg^{-1})	(mg 100 g^{-1})		(cmolc kg^{-1})				(g $soil^{-1}$)
7,1	26,4	14,8	12,5	3,7	0,48	0,10	16,78	2

MO: Organic matter; CIB: Base exchange capacity, Esp. AMF: number of AMF spores per gram of dry soil

Posteriormente se observaron en el microscopio de disección (Carl Zeiss, Stemi 2000-C/50x) para evaluar si se produjo alguna modificación en su estructura (ruptura) y finalmente se pipeteó 1 mL del inoculante con una concentración de 20 esporas por mL. La aplicación de la formulación líquida se realizó en el momento se la siembra.

Vegetal material

Sorghum seeds (*Sorghum bicolor* L. Moench 'CIAP 1322') and maize (*Zea mays* L 'VSF-6') were used, with 96 and 94 % germination respectively, were disinfected with a commercial solution of sodium hypochlorite (10 %) for ten minutes and were planted in 250 cm^3 pots containing sterile clay, at a rate of three seeds. Seven days after the emergence of the plants, thinning was carried out leaving one plant per pot. The irrigation was applied manually, in correspondence with the water needs of the plants and ten days after sowing, 20 mL of Long Ashton nutrient solution was applied per pot (modified with 22 µg mL^{-1} of phosphorus in the inoculated treatments) (18).

Experimental design, evaluations and statistical analysis

The treatments under study consisted of the three pressures evaluated (Pressure I: 0.15, Pressure II: 0.3 and Pressure III: 0.5 MPa, respectively), in addition to a control without inoculation and were arranged in a completely randomized design with ten pots per treatment (n = 10).

The determinations were made 30 days after sowing and the following indicators were evaluated:
Fungal indicators: 250 mg of secondary roots were taken for the determination of the variables related to mycorrhizal functioning, washed carefully with abundant water, dried in an oven at 70 ºC until constant mass was obtained and clarified and stained (19). The intercept method was used to determine the frequency of mycorrhizal colonization and the intensity of colonization or visual density (DV) (20). The number of spores was determined from 50 g of dry soil, according to the extraction method based on sieving and wet decanting (15). The spores were collected on a mesh of 40 µm opening, separated by centrifugation with sucrose and Tween 80 and quantified in the dissection microscope (Carl Zeiss, Stemi 2000-C / 50x).

Plant growth indicators: the aerial and root dry masses were determined by drying the samples at 70 °C in the oven, to constant mass and weighing them on a digital technical scale (Acom JW-1, accuracy level 0.001). The root dry matter/aerial dry mass ratio was determined from the equation:

R = Dry Root Mass/Dry Air Mass

The data were processed statistically using a Simple Classification Analysis of Variance (ANOVA). The comparison of the means was performed, according to the Tukey test ($p \leq 0.05$), when significant differences were found between the treatments. All the statistical analyzes were executed with the statistical package IBM SPSS version 19.0 (21).

RESULTS AND DISCUSSION

Table 2 describes the effect of the inoculation on the different variables studied for both crops and it was possible to verify the existence of significant differences, with respect to the control plants. It was observed that the inoculation of the AMF increased the air and root dry matter, an effect that was reaffirmed with the dry root mass / dry mass aerial part ratio.

With regard to fungal indicators, significant differences were found in relation to non-inoculated plants and this response may indicate that the treatment of pressures, prior to inoculation, did not limit the efficiency of the symbiont, since during observation of fungal propagules, before and after their exposure to said treatment, it was found that they retained their initial structure, without finding visible damage to their external walls.

The direct effect of AMF on the growth and development of plants has been one of the most in-depth aspects of the research carried out with these symbionts and this response is due to factors related to the diameter of the HMA hyphae, which they allow to increase the volume of absorption of the root system and, therefore, to increase the efficiency in the intake of water and nutrients of low mobility (22-24).

Although it has been pointed out that the induction of modifications in cellular structures by the action of hydrostatic pressures is related to the intensity of the pressure treatment imposed and its duration (25), the results of this work did not reveal such affectation before the levels of pressure and time evaluated.

Table 2. Behavior of the indicators evaluated in sorghum and corn plants

Treatments	Dry mass Aerial part (g)	Dry mass Root (g)	Ratio MSRoot/ MS Air Part	Frequency (%)	Intensity (%)	AMF spores (g soil^{-1})
Sorghum						
Control	0,12 b	0,03 b	0,24 b	7,33 b	0,07 b	0 b
Pressure (I)	0,38 a	0,18 a	0,47 a	30,67 a	0,57 a	0,14 a
Pressure (II)	0,38 a	0,18 a	0,47 a	27,67 a	0,52 a	0,14 a
Pressure (III)	0,38 a	0,19 a	0,50 a	29,67 a	0,58 a	0,14 a
Es_x	0,01	0,01	0,02	0,01	0,03	0,01
Corn						
Control	0,56 b	0,06 b	0,11 b	8,33 b	0,08 b	0 b
Pressure (I)	1,10 a	0,22 a	0,20 a	27,67 a	0,50 a	0,14 a
Pressure (II)	1,09 a	0,21 a	0,19 a	29,67 a	0,57 a	0,136 a
Pressure (III)	1,08 a	0,21 a	0,19 a	28,67 a	0,51 a	0,132 a
Es_x	0,02	0,01	0,01	0,02	0,03	0,01

In the literature there are not enough studies related to the resistance of fungal propagules at different hydrostatic pressures, and although some investigations have referred to the ranges of susceptibility and resistance of each microbial group at pressure levels evaluated in some Gram-bacteria. Positive and fungi (26), the results of this study allow to reveal that the behavior of *Glomus cubense* spores before the hydrostatic pressures studied, indicate that it is possible to include them through fertigation systems with a pressure of up to 0.5 MPa, without affect the infectivity of the fungus.

CONCLUSION

With the results of this investigation it was demonstrated that the effect of the three hydrostatic pressures did not affect the infectivity of the symbiont, so that *Glomus cubense* can be applied through fertigation.

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