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RELATIONSHIP BETWEEN *Canavalia ensiformis* RESPONSE TO MYCORRHIZAL INOCULATION AND SOME CHEMICAL PROPERTIES OF SOIL

Relación entre la respuesta de *Canavalia ensiformis* a la inoculación micorrízica y algunas propiedades químicas del suelo

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ABSTRACT. In Cuba, it have been carried out investigations related with the inoculation of species of arbuscular mycorrhizal fungis (AMF) in diverse crops and soil types, being that, for a same soil type, the species that bigger stimulation of the growth of the host plant is always the same, what has allowed infering that a high specificity efficient species of AMF exists for soil type. This work was carried out to evaluate if some properties of the soil have relationship to the response of *Canavalia ensiformis* to the AMF inoculation, cultivated in three types of soils. They were used the results of experiments conduced in Nitisol, Cambisol and Plintosol soils. The canavalia was inoculated with the species *Glomus cubense*, *Rhizophagus intraradices* and *Funneliformis mosseae*. In all the cases was used at complete randomized blocks design. The results of the behavior of the canavalia inoculated with the most efficient species and the chemical properties of the soil were processed by means of a correlation analysis through the coefficient of Pearson. Also, it was carried out an analysis of main components for all the valued indicators. It is demonstrated that the soil type, their nutrients content and the pH have a high relationship with the answer type found in canavalia, being therefore, the efficient specie that was different in each case, in correspondence with the chemical properties of the soil in each analyzed experimental situation.

Key words: green manures, nutrient content (soil), organic matter, arbuscular mycorrhizae, soil pH

RESUMEN. En Cuba se han realizado investigaciones relacionadas con la inoculación de especies de hongos micorrízicos arbusculares (HMA) en diversos cultivos y tipos de suelo; encontrándose que, para un mismo tipo de suelo, la especie de mayor estimulación del crecimiento de la planta hospedante siempre es la misma, lo que ha permitido inferir que existe una alta especificidad en la especie eficiente de HMA por tipo de suelo. Este trabajo se realizó para evaluar si algunas propiedades del suelo tienen relación con la respuesta a la inoculación micorrízica de *Canavalia ensiformis*, cultivada en tres tipos de suelos. Se emplearon los resultados de experimentos conducidos en suelos Ferralítico Rojo Lixiviado, Gley Nodular Ferruginoso y Pardo mullido carbonatado. La canavalia fue inoculada con las especies *Glomus cubense*, *Rhizophagus intraradices* y *Funneliformis mosseae*. En todos los casos se empleó un diseño de bloques al azar con cuatro réplicas. Los resultados del comportamiento de la canavalia inoculada con la especie más eficiente y las propiedades químicas del suelo se procesaron mediante un análisis de correlación a través del coeficiente de Pearson. Asimismo, se realizó un análisis de componentes principales para todos los indicadores evaluados. Se demuestra que el tipo de suelo, su disponibilidad de nutrientes y el pH tienen una alta relación con el tipo de respuesta encontrada en el cultivo de la canavalia ante la inoculación micorrízica, estando por lo tanto, la cepa eficiente, que fue diferente en cada caso, en correspondencia con las propiedades químicas del suelo en cada situación experimental analizada.

Palabas clave: abonos verdes, contenido de nutrientes (suelo), materia orgánica, micorrizas arbusculares, pH del suelo

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INTRODUCTION

Legumes used as green manures/cover crops have a positive impact on the nutrient cycle in agricultural systems in three different ways: recycling of nutrients in the soil, increasing the availability of nutrients to be used by crops and fixing the atmospheric N_2, making it available for the main crop (1).

Inherent to the use of green fertilizers is the association of these with the native populations of arbuscular mycorrhizal fungi (AMF) and thus cause qualitative and quantitative changes in the population of these soil fungi and in the colonization of subsequent crops (2), although in general this mycorrhization does not become totally effective and the crops respond to the inoculation with efficient species of AMF (3).

In Cuba, several studies have been carried out to confirm that the type of soil defines which AMF species are efficient for a given edaphoclimatic condition (high soil-species specificity of AMF), although the effectiveness achieved by the inoculation depends on the management given to the plant and the soil. Thus, *Rhizoglomus intraradices* (strain INCAM 11) is reported to be the most efficient in high fertility clay soils, *Funneliformis mosseae* (strain INCAM 2) favors colonization in acid soils of low fertility and the species *Glomus cubense* (strain INCAM 4) is more effective on medium to high fertility soils (4).

In correspondence with this result, in studies of selection of AMF species by soil types for the cultivation of Canavalia ensiformis used as green manure made in Cuba it has been found that the most efficient species of AMF depended on the type of soil (5-7); however, no analysis has been done to relate the inoculated AMF species with better response in this plant with some properties present in the soils under study.

According to the principles of ecology, the success of the mycorrhizal symbiosis depends not only on the genotypes of plants and fungi, but also on the environmental conditions (8) and although the influence of the soil on the AMF genotypes is still poorly understood, some authors recognize that the soil imposes a strong selection pressure on AMF (9,10).

There is much evidence that supports the hypothesis that soil properties exert a great influence on AMF (11). For example, the pH and the contents of mineral phosphates present in the soil influenced the diversity and abundance of AMF spores (12). In addition, the diversity and composition of the AMF community depended strongly on the type of soil and the intensity of land use (13).

This work was carried out to evaluate if some soil properties are related to the response to mycorrhizal inoculation of Canavalia ensiformis, cultivated in three soil types.

MATERIALS AND METHODS

A series of experiments were carried out in three soil types: Carbonated Fluffy Brown, from the Mountain Agroforestry School; Ferralitic Red Leachate, from the National Institute of Agricultural Sciences and Ferruginous Gley Nodular, from the Cascajal Pastures and Forages Station, located in Guantánamo provinces, Mayabeque and Villa Clara, respectively.

Table I presents the evaluations made to the soils of all the experiments and the method of chemical analysis used in each case.

Table I. Assessments made to the soil and methodology of used chemical analysis

Evaluation	Method (14)
pH (H_2O)	potentiometric, with soil relation: solution of 1:2,5
Organic matter of soil (%)	Walkley and Black
P assimilable (mg kg^{-1})	extraction with H_2SO_4 0,1N with ratio soil:solution 1:2,5
Exchangeable cations($cmol_c$ kg^{-1})	extraction with NH_4Ac 1 Mol l^{-1} to pH 7 complexometry (Ca and Mg) flame photometry (Na and K)

20 soil samples were taken per experiment, in simple random sampling form (15), at a depth of 0-20 cm (arable layer).

In all the experiments, the response of Canavalia ensiformis to inoculation with different AMF species was studied. The commercial product EcoMic® based on the species was used as mycorrhizal inoculum: *Glomus cubense*, INCAM 4 strain (Y. Rodr. & Dalpé) (16), *Funneliformis mosseae*, INCAM 2 strain (Nicol. & Gerd.) Walker & Schüßler (17) and *Rhizoglomus intraradices*, INCAM 11 strain (Schenck & Smith) (18), with a guaranteed minimum quality of 20 g^{-1} spores of inoculant, a nontoxic and pathogen-free product, produced by INCA (19). The product contained each strain separately.

A randomized block design and four replications were used in each experiment; the treatments under study were the three species of AMF and a treatment without inoculation.

The inoculation was carried out by the method of seed coating, using a dose of inoculant corresponding to 10 % of the weight of the seed used in each treatment (19). The canavalia was planted with a planting distance of 0,90 x 0,30 m and allowed to grow until 60 days after germination (20).

At that time, samples were taken to determine the dry mass and the variables of mycorrhizal functioning such as: the colonization percentage, the percentage of the visual density and the number of spores in 50 g of dry soil.

The spore count was carried out according to the sieving method and wet decanting of the fungus propagules (21). For the determination of mycorrhizal colonization, the roots of the collected plants were taken to a depth of 15 cm, washed with running water and air dried. The finest rootlets were taken and crumbled. For the determinations, approximately 200 mg of rootlets that were dried at 70 °C were weighed to be stained with Trypan blue. The evaluation was made by the intercepts method. The determination of the percentage of visual density (% DV) was carried out evaluating the fungal occupation of each intercept and a level was assigned to it and subsequently, the intensity of the fungal occupation within the root was evaluated (21).

To determine the dry leaf mass (t ha^{-1}), the aerial organs of the plants (leaves and stems) were taken. The total fresh mass of each organ was weighed separately on a Sartorius scale, from there, a 100 g fraction of fresh mass was taken and dried in the oven at 70 °C until reaching constant mass values, subsequently it was estimated the dry mass, expressed in t ha^{-1}.

The data related to the behavior of the canavalia inoculated with the most efficient species and the type of soil were processed through a correlation analysis using the Pearson coefficient. Likewise, an analysis of the main components was carried out for all the indicators evaluated. All this through the statistical package Statgraphics Centurion XV (22).

RESULTS AND DISCUSSION

The range of values of the chemical properties analyzed was typical for these types of soils and they are presented in Table II. The pH ranged from very acidic for the Gley Nodular Ferruginous soil to slightly acidic for the Carbonated Fluffy Brown and slightly alkaline for the leached Red Ferralitic, in correspondence with its source material.

The contents of organic matter oscillated from low to medium, because they are soils under permanent agricultural exploitation. The exchangeable potassium contents ranged from very low to low in the Gley Nodular Ferruginous soil and in the other soils it was high. Phosphorus was very low in the Gley soil and high in the other two types of soil. As for the sodium content was very low in the three soils, the magnesium was low in the Gley soil and in the Ferralitic and high in Brown soil. Finally, calcium was very low in Gley, low in Ferralitic and very high in Brown (23).

The range of values for the Base Exchange Capacity (CIB) is typical for each type of soil, very low for the Ferruginous Nodular Gley, medium for the Ferralitic Red Leached and high for the Carbonated Fluffy Brown.

Table III provides a summary of the most efficient AMF species by soil type in each experiment (5-7) and the values reached in the dry mass and the fungal functioning variables in the canavalia.

In all cases, the canavalia presented dry mass values, superior to 5 t ha^{-1} which is the minimum value of dry mass that a green manure must provide to be considered promising in tropical conditions (20).

Table II. Main chemical properties of the soils were the experiments were carried out (0-20 cm of depth)

Exp	Type of soil	pH (H_2O)	% MO	P (mg kg^{-1})	Na	K	Ca	Mg (cmol kg^{-1})	CIB
1	Carbonated Mollic Brown	6,9	3,04	216,0	0,56	0,61	40,0	12,5	53,67
2	Carbonated Mollic Brown	6,8	2,83	177,0	0,50	0,51	35,0	19,0	55,01
3	Ferralitic Red Leachate	7,05	3,50	190,5	0,17	0,46	13,15	2,2	15,98
4	Ferralitic Red Leachate	7,36	3,79	122,6	0,21	1,15	16,84	2,66	20,86
5	Ferralitic Red Leachate	7,31	3,93	292,0	0,20	0,40	13,94	4,83	19,37
6	Gley Nodular Ferruginous	4,2	2,15	0,9	0,03	0,2	3,7	1,6	5,53
7	Gley Nodular Ferruginous	4,7	2,45	1,4	0,08	0,1	4,2	1,8	6,18
8	Gley Nodular Ferruginous	5,2	2,75	1,9	0,13	0,2	4,7	2	7,03

Exp: experiment. MO: organic matter of soil

Tabla III. Especie eficiente de HMA para *Canavalia ensiformis*, seleccionada en cada tipo de suelo y comportamiento agronómico de las variables evaluadas en la leguminosa

Exp	Type of soil	Specie of AMF	MS	Nesp	Col	DV
1	Carbonated Mollic Brown	*Rhizophagus intraradices*	7,12	1227	81,73	9,47
2	Carbonated Mollic Brown	*Rhizophagus intraradices*	6,2	698,64	77,67	9,26
3	Ferralitic Red Leachate	*Glomus cubense*	9,54	232	63,67	3,83
4	Ferralitic Red Leachate	*Glomus cubense*	9,89	258,5	75,75	4,92
5	Ferralitic Red Leachate	*Glomus cubense*	14,94	563,67	70,87	5,27
6	Gley Nodular Ferruginous	*Funneliformis mosseae*	10,07	1054	52	2,76
7	Gley Nodular Ferruginous	*Funneliformis mosseae*	10,19	1006	47	2,49
8	Gley Nodular Ferruginous	*Funneliformis mosseae*	10,03	914	49	2,6

Exp: experiment. MS: dry mass (t ha^{-1}). Nesp: number os spores of AMF in 50 g of dry soil
Col: percentage of mycorrhizal colonization. DV: percentage of visual density

Regarding the fungal functioning variables, the strong capacity of this legume to establish the mycorrhizal symbiosis and multiply the population of this fungus in the soil, key aspect for the introduction and multiplication of efficient AMF species in crop sequences (3).

In Argentina it has been reported that the greater efficiency of the symbiosis is in correspondence with the soil type and its properties. The authors reached this conclusion when evaluating 36 soils with different agronomic management, which differed in the behavior of the fungal functioning variables, always using the same trap culture and attributed the differences found to the variation of the chemical properties of the soils in each one of the collection sites (24).

Based on this, in Cuba, when analyzing the results of more than 130 trials of mycorrhizal inoculation under field conditions, it was concluded that the properties of the soil influence the most effective functioning of the species that are inoculated and this appearance is the key to success in mycorrhizal inoculation under production conditions (11).

Table IV shows the results of the correlation analysis, in which it can be shown that the percentage of mycorrhizal colonization showed a positive and significant correlation with all the chemical characteristics of the soil evaluated, except for the content of organic matter. The percentage of the visual density presented this type of correlation with the sodium and magnesium contents.

A different result was obtained for the number of spores, which only showed significant and inverse correlation with soil organic matter. The dry mass of the canavalia did not present significant correlations with any of the soil properties evaluated.

This result may indicate that effective fungal functioning had a close relationship with some soil properties. In this regard, it has been reported that in the presence of high nutrient availability, the response of plants to mycorrhizal inoculation is limited, although this response will depend on many factors, including the possible availability of an element in the presence high or low amount of another chemical element that can affect the absorption of nutrients by the plant (25).

In the case of the dry mass it is possible that the poor correlation of the variable with the properties of the soil is an effect of the known hardiness of this plant, capable of growing, both in conditions of low fertility and water availability and in optimal conditions of soil and climate or perhaps other factors and even due to errors in the handling of the samples (26).

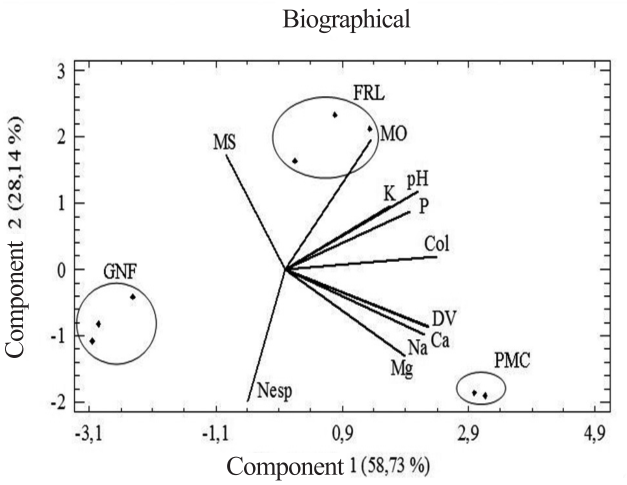
The analysis of the main components for the chemical characteristics of the soils with the variables of fungal functioning and dry mass of the canavalia (Figure) allowed us to separate the experiments according to the soil type. With the analysis, two components were formed, which allowed explaining the 86,87 % of the total variability, being 58,73 % of the variance explained by the main component 1 (horizontal axis).

The variables that contributed most to the formation of the first component were the percentage of mycorrhizal colonization, the percentage of visual density, pH of the soil and the contents of P, Na, Ca and Mg. For the second component, the variables with the greatest contribution were the dry mass of the canavalia, the number of AMF spores present in the rhizosphere and the percentage of organic matter in the soil.

Table IV. Pearson's linear correlation coefficient between the chemical characteristics of the soils and the variables evaluated in *Canavalia ensiformis*

	pH	MO	P	Na	K	Ca	Mg
MS	0,0084	0,4119	0,1469	-0,6112	-0,1834	-0,6037	-0,6281
Nesp	-0,6261	-0,7464*	-0,3856	0,1498	-0,5507	0,0919	0,1906
Col	0,8587*	0,5985	0,8109*	0,8341*	0,7541*	0,8844*	0,6919
DV	0,6121	0,2297	0,6595	0,9795*	0,4434	0,9885*	0,9267*

* Correlations significantly different from zero, with a confidence level of 95.0%; MS: dry mass; Nesp: number of spores of AMF in 50 g of dry soil; Col: percentage of mycorrhizal colonization; DV: percentage of visual density; MO: soil organic matter



MS: dry mass; Nesp: number of spores of AMF in 50 g of dry soil; Col: percentage of mycorrhizal colonization; DV: percentage of visual density; MO: soil organic matter; GNF: Gley Nodular Ferruginous soil; FRL: Red Ferralitic soil leachete; PMC: Brown Mollic Carbonated Soil

Figure. Main Component Analysis Results

Likewise, a close relationship was found between the leached Ferralitic Red soil and the Carbonated Fluffy Brown and all the variables analyzed except the number of spores and the dry mass, respectively. In contrast to this, the Gley Nodular Ferruginous soil showed only a close relationship precisely with the variables dry mass and number of spores, having little relationship with the rest of the evaluated variables. Regarding this last result, it has been pointed out about the greatest benefits for the host plant by establishing an effective mycorrhizal symbiosis in low fertility soils (4).

It is valid to highlight in the Figure how the soil pH has a close relationship with the variables related to the availability of nutrients, which are grouped in the quadrant opposite the Gley Nodular Ferruginous soil, this result may indicate that pH is the main factor that will determine the availability degree of nutrients by type of soil, which is very low precisely in acid soil. In this type of soil, an increase in the dry mass of the *Canavalia ensiformis* inoculated with *Funneliformis mosseae* has been reported with respect to the control without inoculation, superior to the increase found when this plant is inoculated with *Glomus cubense* in Leached Red Ferralitic soil, with higher capacity of cation exchange (10).

This may be because, as the pH approaches neutrality and increases soil fertility, the mycorrhizal dependence of the plants becomes weaker, because soil conditions are more favorable for optimal nutrition in the absence of symbiosis (27).

Possibly this aspect is what justifies the fact of the close relationship between the dry mass according to the type of soil, because it was precisely in the soil with lower fertility, where the greatest increase in the yields of the green manure was observed compared to the inoculation.

These results corroborate that the soil type and its fertility is the fundamental criterion to define which or which are the species and AMF efficient strains for a given soil-climatic condition, regardless of the type of existing crop. The explanation of this process means that the efficient strain for a given soil condition, establishes an effective symbiosis with any culture dependent on mycorrhization that is established in that soil.

CONCLUSION

It is demonstrated that the type of soil and its availability of nutrients have a high relation with the type of response to the mycorrhizal inoculation found in the cultivation of the canavalia, being, therefore, the efficient strain, which was different in each case, in correspondence with the availability of existing nutrients in each experimental situation analyzed.

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