THE USE OF NUCLEAR AND RELATED TECHNIQUES FOR EVALUATING THE AGRONOMIC EFFECTIVENESS OF PHOSPHATE FERTILIZERS, IN PARTICULAR ROCK PHOSPHATE, IN VENEZUELA: II. MONITORING MYCORRHIZAS AND PHOSPHATE SOLUBILIZING MICROORGANISMS

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Abstract. The objectives of the study were to quantify and isolate P solubilizing microorganisms (fungus and bacteria) from corn, sorghum and beans rhizosphere from El Sombrero soil, located in Guarico state, a very important agricultural area in Venezuela. Rhizospheric soil samples were taken from the crops in the field and taken to the laboratory to conduct a serial dilution procedure in specific medium culture to obtain pure cultures and isolate microorganisms according to their function. The spores of arbuscular mycorrhiza (AM) from the soils were reproduced using trap pots in the greenhouse and after 4-6 months a dilute soil sample was wet-sieved and decanted for isolation of AM spores which were used for classification and for obtaining native pure cultures. Finally, the infective potential of AM was determined by setting pots with test crops and determining the AM colonization and efficiency to produce potential infection in the root system. There were no differences in the total microflora in both crop rhizospheres but there was a tendency of higher values in the corn rhizosphere due to the root exudates. Two solubilizing fungi identified were *Aspergillus terreus* and *Aspergillus niger*.

1. INTRODUCTION

Several approaches have been proposed to achieve a sustainable P supply in agro ecosystems and one possibility is the use of rock phosphate and the exploitation of soil microbial activities to solubilize the rock phosphate [1-5]. Recent interest on the topic is based on the demonstrated activities of mycorrhizal fungi, particularly those forming arbuscular mycorrhiza. In such symbiotic association the fungus colonizes biotrophically the root system. The symbiotic fungi also develop an external mycelium, which colonize extensively the soil surrounding the roots. Thereby, the mycorrhizal symbiosis, by linking the biotic and geochemical portions of the ecosystem, can contribute to nutrient capture and supply. The fungi can also improve soil structure and stimulate other beneficial members of soil microbiota. Also some phosphate-solubilizing bacteria, able to solubilize rock phosphate in vitro, have been characterized as rhizobacteria, because of their "aggressiveness" in colonizing the root region of plants, where they can change phosphate bioavailability. Some rhizobacteria can even improve mycorrhiza formation. By manipulating such micro organisms the plant can be provided with an active myco-rhizosphere, which could be specifically tailored to try to improve the use of less available sources, like rock phosphate [6].

Selected micro organisms of the rhizosphere may be used as inoculants to enhance plant growth. These include rhizobacteria, which participate in many ecosystems, and symbiotic microorganisms such as mycorrhizal fungi, which develop an external mycelium acting as a bridge connecting the root with the surrounding soil microhabitats. Particularly, arbuscular mycorrhizal symbiotic associations play a direct role in making available to plants sparingly soil P forms [7-9].

The objective of this paper was to quantify and isolate P solubilizing microorganisms (fungus and bacteria) from corn and bean rhizosphere from El Sombrero soil, located in Guarico state, a very important agricultural area in Venezuela.

2. MATERIALS AND METHODS

In field experiments, where corn and beans were grown in El Sombrero soil, Guarico state, P solubilizing microorganisms (fungus and bacteria) were quantified and isolated. Table I shows that the main properties of the soil. The soil was acid, loam to silty loam, low organic matter content, high in available P, high to medium in available K, low in calcium, high to medium in available magnesium and low in available sodium. The methodology will be described according to the objectives of the paper.

2.1. Reproduction of Arbuscular Mycorrhizal (AM) spores from the native soil

A soil sample of 3 kg from the field was passed through a 0.4 cm sieve and placed in the trap pots in the greenhouse. One seed of corn or black beans were planted in each pot and they growth for about 4 to 6 months. Five replications of each pot were established. Then, a root sample is taken to evaluate the presence of the AM fungus. Also a soil sample is taken for the extraction of the fungus spores and to proceed to their reproduction. Diluted soil samples from the rhizosphere (1:20 soil - water ratio) were wet-sieved (250, 100 and 60 µm mesh sieves) and decanted for isolation of AM spores and were classified for native pure cultures. Figure 1 shows a diagram of the procedure.

2.2. Determining the infective potential of Arbuscular Mycorrhiza (AM)

The infective potential of AM spores obtained in the first objective, was determined in 3 kg soil-pots in the greenhouse by planting sorghum (*Sorghum vulgare)* and common beans *(Phaseolus vulgaris)* as test crops. Fifteen days after planting, 20 ml of the native pure culture of the isolated spores (objective a) in a concentration of 20 spores per g of soil together with mycelium and mycorrhizal root fragments are applied to each pot.

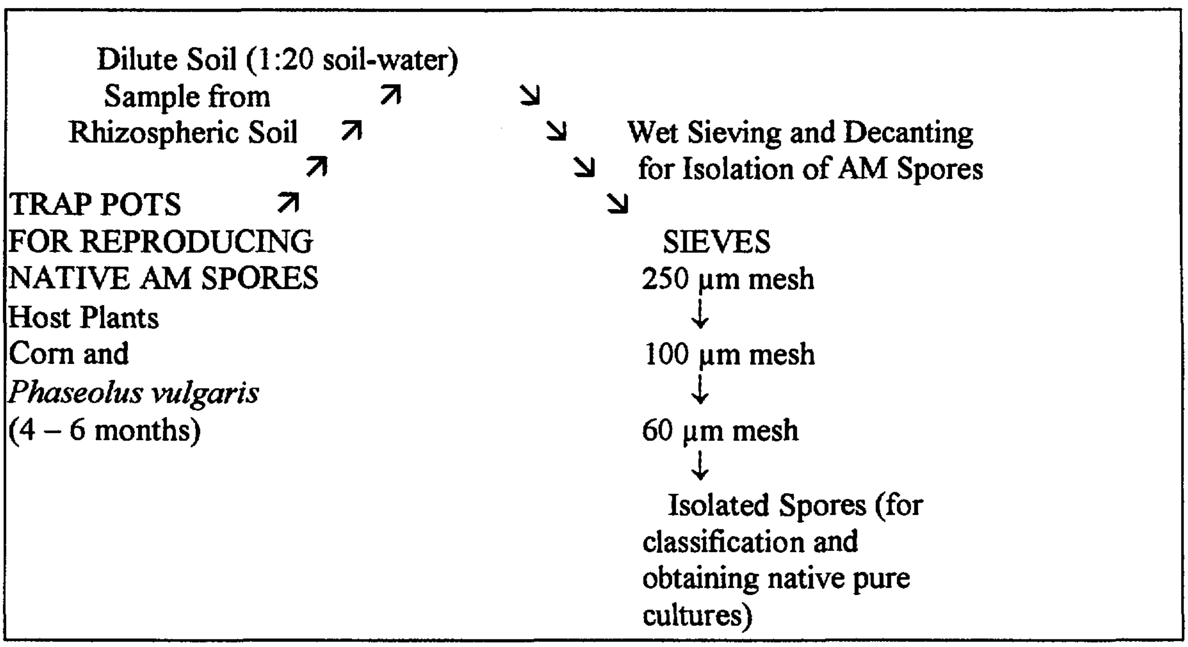

Fig. 1. Reproduction of Arbuscular Mycorrhizal (AM) Spores from Native Soil (Experimental plot in El Sombrero, Venezuela).

The plants were grown in a greenhouse under a day/night cycle of 12/12 h and 25°C and 50 % relative humidity and the pots were weighed and watered to 80% of field capacity daily, following the procedure described by Toro et al. [7]. After 6 weeks, the root system is observed to determine the % AM colonization and number of entry points. Using the most probable number test described by Toro et al. [7] the AM colonization was determined and the efficiency to produce potential infection in the root system. Figure 2 shows a diagram of the process.

2.3. Monitoring soil microorganisms of an agroecosystem and their manipulation for agricultural purposes

In order to identify P solubilizing fungi and bacteria that may have potential to solubilize rock phosphate applied to the soils, rhizospheric soil samples were taken from El Sombrero soil from corn and beans roots. A procedure similar to the one explained in Figure 1 was followed (seriate dilutions) until a pure culture is obtained. Threafter, the isolation of microorganisms according to their function is made and the identification of P solubilizing fungus and bacteria is done following the procedure described by Vassileva et al. [8]. Figure 3 shows a diagram of the procedure.

The total microflora in both rhizospheres was quantified. In addition, fungus and bacteria that solubilize phosphate were isolated and quantified using dicalcium phosphate and Riecito rock phosphate.

3. RESULTS AND DISCUSSION

3.1. Reproduction of Arbuscular Mycorrhizal (AM) spores from the native soil

The spores of fungus that formed AM were reproduced in each one of the soil samples where corn and bean were grown and after six months the quantity and variety of spores in each rhizosphere were measured as shown in Table II.

The corn rhizosphere had a higher quantity and variety of spores than the bean rhizosphere. Corn is known to have a rich-exudate rhizosphere, which could be the reason for the higher results of AM spores observed.

3.2. Determining the infective potential of Arbuscular Mycorrhiza (AM)

Even though no significant differences in the infective potential of AM were found in both crops (Table III), there was a trend for higher values in the bean. This could be explained by the higher affinity of AM for leguminous crops. They are more susceptible to colonization probably due to the particular chemical composition of their root exudates. The results indicate that for every 100 g of soil there is a potential of 2.58 and 3.40 infective spores that could colonize the sorghum and bean crops that are planted in them.

3.3. Monitoring soil microorganisms of an agrosystem and their manipulation for agricultural purposes

The last microbiological experiment isolated and quantified P solubilizing microorganisms from rhizospheres of corn and bean crops grown in El Sombrero soil. The values of total microflora, fungi and bacteria, which solubilize dicalcium phosphate and Riecito rock phosphate are shown in Table IV.

The values did not show significant differences for the total microflora for both rhizospheres but thre is trend for higher values of P solubilizing fungus and bacteria in the corn rhizosphere. The fungi *Aspergillus terreus* and *Aspergillus niger* were identified. They are known for their solubilizing capacity of sparingly P sources. In future studies, these microorganisms will be inoculated in greenhouse and field experiments to evaluate their effectiveness in solubilizing natural and modified rock phosphate using isotopic techniques (^{32}P). The effectiveness of this practice in soil is unknown because of the difficulties related to the translocation of phosphate ions to the root surface, if there is any solubilization of the riock phosphate product. The microbially-solubilised phosphate would be taken up by a mycorrhizal system, thereby developing synergistic microbial interactions [1].

* Natural soil samples from the field is used and passed through a 1 cm sieve

* 3 kg soil – pots are set.

* Setting pots with a test crop: *Sorghum vulgare* and *Phaseolus vulgaris*

* 20 ml of native pure culture of isolated spores (20 spores/g of soil) + mycelium + roots fragments is applied at 15 days after planting.

* After six weeks the root system is observed to determine % AM colonization and/or number of entry points (Most Probable Number Test)

* The infective potential of AM propagules is determined and the efficiency to produce a potential infection in the root system

Fig. 2. Determining the Infective Potential of Arbuscular Mycorrhizal (AM) in an agroecosystem.

MONITORED MICROORGANISMS :

* P- solubilizing Bacteria ↘ RIECITO ROCK PHOSPHATE
* P - solubilizing Fungi ↗ SOLUBILIZERS

* Rhizobium and / or Bradyrhizobium

* Celulolitic, Free Living N_2 Fixers and Others

RHIZOSPHERIC
SOIL SAMPLES
From corn and beans
↘
↘ Seriate dilutions ↗
↘ ↗
↘ ↗
SPECIFIC MEDIUM
CULTURES

OBTAINING
PURE CULTURE
↓
↓
↓
Isolation of
Microorganisms
According to
their Function

Fig. 3. Monitoring soil microorganisms in an agroecosystem for their manipulation with agricultural purposes.

TABLE I. MAIN PROPERTIES OF EL SOMBRERO SOIL WHERE CORN AND BEANS WERE PLANTED AND SPORES OF ARBUSCULAR MYCORRHIZA (AM) WERE OBTAINED

Crop	%clay	%silt	%sand	Texture	pH (1 :1)	Elect. Cond. S/m	OM %	Avail. P mg/kg	Avail. K mg/kg	Avail. Ca mg/kg	Avail. Mg mg/kg	Avail. Na mg/kg
Bean	20.4	46.8	32.8	Loam	4.62	0.120	2.31	53	101	156	114	43
Corn	16.4	50.8	32.8	Silty loam	4.74	0.090	1.85	85	50	215	74	19

TABLE II. QUANTITY AND VARIETY OF SPORES FROM FUNGUS THAT WILL FORM ARBUSCULAR MYCORRHIZA FROM THE CORN AND BEAN RHIZOSPHERE

	Corn Rhizosphere	Bean Rhizosphere
Spores quantity (# spores/80 g of dry soil)	60 a[1]	25 b
Diversity (types of spore)	6 a	3 b

[1] Different letters within the same line show significant differences according to Duncan Multiple Range Test (P = 0.05).

TABLE III. NUMBER OF INFECTIVE SPORES OF ARBUSCULAR MICORRHIZA PRESENT IN EL SOBRERO SOIL DURING THE RAINY SEASON OF 1997 IN SORGHUM AND BLACK BEANS

	Sorghum	Black bean
No of infective spores /100 g of soil	2.58[1]	3.40[1]

[1]The mean values are not statistically different according to the Duncan Multiple Range Test

TABLE IV. QUANTIFICATION OF TOTAL MICROFLORA, P SOLUBILIZING FUNGUS AND BACTERIA OF DICALCIUM PHOSPHATE AND RIECITO ROCK PHOSPHATE IN SOIL RHIZOSPHERES OF CORN AND BEAN IN EL SOMBRERO SOIL, GUARICO STATE IN THE RAINY SEASON OF 1997

	Corn Rhizosphere (cfu[1]/gr of soil)	Bean Rhizosphere (cfu/g of soil)
Total Microflora	1.7×10^7	2×10^7
Dicalcium phosphate fungus	1.7×10^4	-
Riecito rock phosphate fungus	4×10^5	5.6×10^4
Dicalcium phosphate (bacteria)	7.2×10^5	1.7×10^4
Riecito rock phosphate (bacteria)	1.1×10^5	4.5×10^4

[1] cfu: colony forming units

4. CONCLUSIONS

The isolation of P solubilizing microorganisms from rhizosphere of corn, sorghum and bean crops from El Sombrero soil, quantification of the total microflora, and isolation and quantification of fungus and bacteria that solubilize phosphate using dicalcium phosphate and Riecito rock phosphate showed no significant differences in the total microflora for both rhizospheres and a trend for higher values of P solubilizing fungus and bacteria in the corn rhizosphere. The fungi *Aspergillus terreus* and *Aspergillus niger* were identified. They are known for their solubilizing capacity of insoluble forms of P. In future studies, these microorganisms will be inoculated in greenhouse and field experiments to evaluate their effectiveness in solubilizing natural and modified rock phosphate using (^{32}P) isotopic techniques.

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