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Agronomical and Environmental Factors Influence Root Colonization, Sporulation and Diversity of Arbuscular Mycorrhizal Fungi at a Specific Phenological Stage of Banana Trees

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Abstract: The present research studied the influence of agronomical practices and seasonal changes on intraradical colonization and spore production patterns of Arbuscular Mycorrhizal Fungi (AMF) of Grand Nain banana clone plantations with contrasting management. It was studied if root colonization, spore production and spore diversity were determined by management and environmental changes. Roots and rhizosphere soil samples from Grand Nain banana plants at the fruiting phenological state were collected, for two years, at the beginning of the rainy season, the rainy season and during the dry season. Lower root colonization percentages, spore numbers and spore diversity indexes were found in the plantation that used an excess of Agrochemicals (AC), independent from the season. Root colonization was higher in the dry season and, to a lower extent, at the beginning of the rainy season than at the rainy season. The number of spores remained constant in the plantation AC while, in the low input management culture plantation (LI), an increment was observed when passing from the rainy season to the beginning of the following rainy season. Both plantations presented the following four dominant AMF spore types: Glomus (GC-1Ban), Glomus (GC-2Ban), Acaulospora (AH-1Ban) and Sclerocystis (SN-1Ban), where Glomus GC-1Ban was the most abundant type. The independent effect of agronomical management and environmental factors on root colonization, spore production and diversity was thus confirmed in plants sampled at the same phenological stage. This is further evidence suggesting multiple external control factors on the development and propagation of AMF, which may eventually affect the functioning of the symbiosis in field conditions.

Key words: Agrochemicals, banana, management, mycorrhiza, phenology

INTRODUCTION

The increasing interest on environmental protection and safety crop consumption without chemical residues has required the development of new approaches and alternative management and technologies.

The use of symbiotic microorganisms that increase plant nutrient uptake and promote plant growth is one of the most interesting alternatives. It has been demonstrated that through the association with Arbuscular Mycorrhizal Fungi (AMF), many plants have access to larger quantities of water and minerals from the soil[1,2]. As a result of an increased nutrient uptake, a mycorrhizal plant has often better growth and increases its resistance according to environmental stress and to root pathogen infection than a non-mycorrhizal plant[3].

Since most plants are colonized by AMF, the use of this symbiosis for agriculture benefit can be expected. However, it requires the selection of the best plant-fungus combination for the system.

Several studies[4,5] have been conducted to determine the dynamics of mycorhizal colonization, as well as spore production from cultivated plants in the field. Studies of grains and cereals, with a short life-cycle of four to six months, have been used as models and in general they have a common pattern of agronomic management. In those studies, a strong relationship has been found between the plant phenological state and the degree of mycorrhizal colonization. When these types of plants are at the beginning of flowering and fructification period, the roots show a high degree (but not the highest) of mycorrhizal colonization (in all their forms, hyphae,

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arbuscular and vesicles) and spore production process takes place. Levels of 20 to 70% of colonization are reported and spore production reach from hundreds to thousands of spores per 100 g of rhizosphere soil.

In plants with large life cycles, such as trees, few studies have been carried out to examine that symbiotic relationship[6]. In banana plantations, it is likely that agronomical management and environmental factors have a stronger influence on AM fungal phenology, distribution and abundance because a plant phenological state can lost a long time and the phenological state can be reached at different times during a year.

Naturally, a banana plant has an eight to twelve-month life cycle, from seedling to fruiting, depending on the clone type used. The plants have only one blooming or fruiting period during the life-cycle and remain alive after this period[7].

In banana trees, new shoots emerge from the plant before the fruiting stage is reached. Even though sprouts emerge from the same rhizome, they develop their own root system independently from the mother plant. The latter will use these new root systems when its own system senesces.

When the fruiting and harvesting period are completed, the plant is cut off to allow for sprout development. A banana commercial crop remains productive for a long period of time but for several reasons, the plants are not developed in synchronicity.

Unlike grain and cereal crops, different phenology stages can be found in banana plantations in all seasons of the year allowing for the study of the influence of the environment and agricultural practices on AMF colonization and spore production in a pre-selected phenological stage.

Although few reports exist regarding the established relationship between the AMF and banana tree plants, it is possible that this behaves similar as reported in other agricultural systems[1,5]. Phosphorous fertilization[8-10], farming systems[8,11,12], tillage practices[11,13,14], the employment of fungicides[15] and temperature and humidity of soil[8], they affect the effectiveness of the AMF.

On the other hand, it is not known if the environmental and external controls are important for if same or they are hidden by what seems to be the decisive mechanism: the readiness of carbon for the development of the AMF.

The main objective of this research was study the influence of the agronomical practices and seasonal changes on intraradical colonization and spore production patterns of Arbuscular Mycorrhizal Fungi (AMF) of Grand Nain banana clone plantations with contrasting management. We selected the fruiting phenological stage of banana plants as the reference point because this is expected to be the one where usually the maximum degree of root colonization has been achieved and fungal sporulation has been induced.

MATERIALS AND METHODS

This study was conducted for two years (2002-2003), in two 20 year-old Grand Nain banana clone plantations with similar climate and edaphic conditions but with differ agronomical management. These plantations are located in Tapachula, Chiapas, Mexico, between 14°30' and 15°N and 92° and 92°30' W, with an average temperature of 27.1°C and an average annual precipitation of 2,800 mm. The first plantation (AC) uses agrochemicals for weed, pest and disease control and excessive chemical fertilization (240 g N, 40 g P, 900 g K per plant); whereas, the second plantation (LI) use a low input cultural management for their control and fertilization. Both farms have a drainage system in order to keep out the water excess accumulated during the rainy season. To avoid edaphic variations, assays were performed on a one-hectare area within each plantation. Ten samples of 2 kg soil and roots were taken in January, at the Dry Season (DS), in May, at the Beginning of the Raining Season (BRS); and in September, at the late Rainy Season (RS). The samples were taken from the rhizosphere of those banana plants where the fruiting stage was just starting and from a 30 cm wide and 30 cm deep semicircle, towards the mother plant[16].

Root colonization percentages were determined by microscopy[17] from 100 root segments previously stained following the Phillips-Hayman's method[18]. The AMF spores were extracted using the Gerdemann and Nicolson's wet sieving and decanting method[19]. Typical characteristics of spores, subtending hyphae connections and wall numbers and characteristics were used to determine genera of the different types of AMF, according to Schenck and Pérez[20]. A variance analysis test was used to determine if the agrochemical employment or the climatic condition has bigger impact on the studied variables.

RESULTS

Effect of agronomic handle and season in the colonization of the Grand Nain banana roots by AMF: The root colonization results showed that the sampling season and the agronomic handling influences the degree of root colonization in banana plants (Table 1). For both plantations, the lowest degree of colonization was found

Table 1: Total root colonization of the Grand Nain banana clone observed during the study. Average of 10 samples, 100 rootlets per sample. Plantation with use of Agrochemicals (AC); plantation with Low Input management (LI)

	Plantation	
Season	AC	LI
DS	49.0±5.2b	58.6±2.9a
BRS	29.8±7.4cd	57.8±3.2ab
RS	25.0±6.3d	38.6±5.0c

Table 2: Number of spores found in the rhizosphere of the Grand Nain banana clone during the study. Plantation with use of Agrochemicals (AC); plantation with Low Input management (LI)

	Plantation	
Season	AC	LI
DS	54.0±15.4d	211.0±18.4b
BRS	39.8±13.7d	277.4±23.5a
RS	49.2±10.3d	100.8±17.4c

in the RS, whereas the highest was reached in the DS. In the AC plantation, a decrease from 49.0 to 24.0% was observed in root colonization when passing from the DS to the RS, whereas in LI plantation the decrease was from 58.6 to 38.6% for the same period.

No statistical differences in colonization were found between the BRS and the RS well as between DS and BRS in AC and LI plantations, respectively. However, the differences between the RS and the DS, was statistically different ($p<0.05$).

Also, independently of the season in which the sample was taken, the colonization of the banana roots was higher in LI than in AC (Table 1) and these differences were statistically significant ($p<0.05$). This difference can be been that the AC plantation uses a bigger quantity of fungicides and other agrochemicals, than the LI plantation.

Effect of agronomic handle and season in AMF spore production: In order to compare the AMF spore production, they were quantified separately as individual spores and as sporocarps. The content of spores in the rhizosphere of the plantation AC practically remained constant (non statistically significant) during the study, while this parameter was variable highly in the plantation LI (Table 2). In this last one, it was an increment of 37% in the content of spores when passing from the DS to the BRS and later on, in the RS, a drastic decrement was observed (64%) of them. These variations were statistically significant ($p<0.05$).

The sporocarps showed similar behavior to the individual spores (data not shown), meaning that the highest sporocarp numbers were found in LI plantation at the beginning of the rainy season and quantities changed with the seasons in a similar way.

Table 3: General characteristics of individuals that form different AMF groups isolated from the rhizosphere of the Grand Nain banana clone

Group	Color	Size (μm)
GC-1Ban	Brown	59.22-89.00
GC-2Ban	Magenta	159.33-238.40
AH-1Ban	Hyaline	75.18-92.78
SN-1Ban	Black	309.81-480.97

Table 4: Distribution (in percentages) of different spore groups found in the rhizosphere of the Grand Nain banana clone during the study

	Season					
	RS		DS		BRS	
Spore group	AC	LI	AC	LI	AC	LI
GC-1BAN	61.6	57.6	68.1	56.6	84.0	46.0
GC-2BAN	27.8	29.8	22.6	24.3	10.7	29.6
AH-IBAN	1.4	2.8	7.8	2.3	1.9	10.1
SN-1BAN	9.2	9.8	1.5	16.8	3.4	14.3

Table 5: Diversity indexes of AMF groups on the Grand Nain banana clone culture

	Season					
	RS		DS		BRS	
Diversity index	AC	LI	AC	LI	AC	LI
D	0.4411	0.4340	0.5212	0.4095	0.7184	0.3293
(1-D)	0.5590	0.5660	0.4790	0.5905	0.2820	0.6710
N_2	2.2670	2.5455	1.9190	2.4420	1.3920	3.0370
H'	0.9610	1.1040	0.8580	1.0510	0.5740	1.2260

D = Simpson's index; (1 – D) = Simpson's Complement; N_2 = Hill's index: H' = Shannon – Wiener's index

The differences in spore and sporocarps numbers between the LI and AC plantations were, very probably, due to the different handling agronomic and these variations were significant statistically.

Genera of AMF in Grand Nain banana rhizosphere soil: The criteria used to identify AMF species are under development, therefore many studies find individuals sharing many characteristics of a genus, but they have no similarities with reported species. To avoid confusion, in the present work, general criteria of the genus were used such as size, color, basal structure type of the subtending hyphae, ornamentation and wall's number and structure, to define the AMF spore community of rhizosphere soil from these plantations. Using these morphological characteristics, the different AMF individuals were gathered in four different groups. Those with characteristics closed to the *Glomus* genus were grouped as type GC-1Ban and GC-2Ban, those characteristics closed to *Acaulospora* genus were grouped as AH-1Ban and those related to the *Sclerocystis* genus were grouped as SN-1Ban. The characteristics of color and size range of individuals which form the different AMF spore types are shown in Table 3.

Regarding the distribution of spore types observed during the study, we observed that the type GC – 1Ban is dominant over the other three and this is prevalent through all seasons (Table 4). The supremacy of the type GC-1Ban can be explained by the fact that the plantation LI up to five years before beginning the study, also used an agronomic process based on chemical fertilization and control of wed, pest and diseases with agrochemicals, for what one can think that this type of AMF was selected and it has remained as dominant. Also, it can be observed that the distribution of the different types of spores this better one conserved in the LI plantation.

Diversity indexes were calculated to examine that differential use of agrochemicals between the plantations was the factor determining the distribution of the different spore groups in Grand Nain banana rhizosphere (Table 5). All indexes, Simpson (D) and its complement (1 – D), Hill (N_2) and Shannon – Wiener (H'), demonstrated higher diversity in LI than in AC.

DISCUSSION

Following the onset of fructification as an indicator, this study found that either the degree of root colonization (Table 1), as well as the number of produced spores (Table 2) depended so much on the season when the samples were taken like of the agronomic handling of the cultivation. Consequently, climate and agrochemicals has an important role in root-AMF relationship in Grand Nain banana clone.

As the RS is getting established in the region, soil moisture is gradually increasing, reaching saturation levels and very often soils are flooded. Therefore, it is safe to assure that in this season the rhizospheric environment in both plantations is micro-aerobic or, even, anaerobic. The lowest level of root colonization was found in both plantations under these conditions. This result confirms that previously reported by Bowen[21] and Sylvia and Williams[22] regarding that the AMF are aerobic and that the excess of water have considerable impact in its distribution and effectiveness.

When the dry season starts, the reverse process occurs, this means that it will pass from a permanent saturation condition, to lower humidity. Samples from both plantations at the dry season showed the highest root colonization percentages.

If we consider that the increment observed in the colonization level by AMF of the roots of both plantations, when passing from to RS to DS, was exclusively (what is not necessarily certain) due to the interaction among the spores that germinate and the not having colonized roots, it would be thought that the

Table 6: Spores produced per 1% of colonized root of the Grand Nain banana clone tree cultivated with different agronomic handling and in the different seasons

	Season		
Plantation	RS	DS	BRS
AC	1.9	1.1	1.3
LI	2.6	3.6	4.8

spores of the AC plantation were more effectiveness than the present in the LI plantation, since for each two present spores in the rhizosphere of the AC plantation the colonization level was incremented in 1% while, in the LI plantation to achieve an 1% of colonization was required for the presence of five spores. However, this appreciation is not correct since the effectiveness of the spores cannot establish under this complex context.

On the other hand, the drastic reduction in the level of colonization of the roots of the plantation AC was not the result of passing from the DS to the BRS, since this parameter remained without change in the plantation LI. This reduction was probably due to an increment in the agrochemicals concentration, since with the first rains the material stocked in the leaves of the banana was washed and crawled toward the soil.

The negative impact of the agrochemicals used in the industrial cultivation of the banana tree (represented in great measure by fungicides) can be established when comparing the colonization levels and the produced spores. This way, if we take the values of the LI plantation as base, we find a 13.6, 11.6 and 28.0% as average negative impact in the colonization efficiency and 0.14, 3.27 and 3.67 times less produced spores per 1% of root colonized (Table 6) during the RS, DS and BRS, respectively, in AC plantation..

The deleterious effect of the agrochemicals found in this study confirms the previously reported for the fungicides[23,24] and herbicides[25] in other crops.

Although diversity indexes were developed to compare populations of plants and animals in different ecosystems and to a less extent in insect populations, their use in comparing AMF populations in the studied plantations suggested that the plantation using agrochemicals had lower species diversity than the one that does not use them.

Despite of the promiscuous behavior of AMF, present results show to strong relationship between GC –1BAN type and the rhizosphere of the Grand Nain banana clone, since such population reached values up to 84% in the AC and more of 50% in LI. In a future this group of spores can be domesticated for its employment as inoculum either in the established banana tree fields or in the propagation process at nursery stage or in the greenhouse level, previous development of a production process.

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