Article

Abundance and genetic diversity of *Fusarium oxysporum* and *Trichoderma* sp. in muse AAB

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Abstract

The occurrence and diversity of *Fusarium* and *Trichoderma* species was studied in 29 points of a banana plot (Musa AAB) in Veracruz, Mexico, during November 2017 and April 2018. The plot was divided into two parts; in the first one, fallow and weed removal were carried out (Procedure in culture. 1) while in the second one these activities were not carried out (Procedure in culture; and 2). Fungi were isolated from the soil collected at these sites using potato dextrose and agar (PDA) and K2. At the time of collecting the samples, temperature, precipitation and solar radiation were recorded. The results of the analysis showed 68% more presence of colony forming units (CFU) in soils where no cultivation work was carried out using K2 unlike soils where they were performed using PDA (32%). A small amount of CFU was also observed when the temperature, precipitation and radiation values were high. Molecular analyzes showed 65% more abundance of species in soils without labor compared to cultivated soils (35%), six of 16 *Fusarium* isolates were *Fusarium oxysporum* f. sp. *melonis* and six of 13 *Trichoderma* isolates were *Trichoderma longibrachiatum* in the first sampling while nine of 14 *Trichoderma* isolates were *Trichoderma spirale* in the second sampling. The analysis of the intrapopulation diversity of seven *Trichoderma spirale* isolates showed bands of 75 to 500 bp in three loci of the genome in the VB28IT1 isolate with five ISSR.

Keywords: banana, populations, CFU.

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Introduction

Fusarium is a genus of fungi that includes a large number of plant pathogenic species. Its high coevolutive capacity with cultivated plants has given it greater genetic variability and increased species diversity. Only the *Fusarium oxysporum* group includes 120 special forms that cause disease in specific host plants (Bentley *et al*., 1998; Leong, *et al*., 2010; Benaouali *et al*., 2014). Of these special forms *F. oxysporum* Schlechtend f. sp *cubense* (E.F. Smith) Snyd and Hans, was the cause of one of the most catastrophic diseases worldwide in bananas (Ploetz, 2015).

It is estimated that between 1960 and 1970 in Mexico, about 40 000 ha of banana of the manzano or Silk variety (Musa AAB) were destroyed by this disease (Orozco *et al*., 2009) and most of the soils of the producing areas of plantain were infested with *F. oxysporum* f. sp *cubense* (Orozco *et al*., 2009) which made the production of susceptible varieties such as Silk or manzano tree difficult.

Fusarium is a pathogen that forms resistance structures called clamidospores that give it the ability to survive for long periods in the soil, and its main forms of dispersion are by movement of infested soil and runoff water (Retana *et al*., 2018); however, its abundance and diversity in the soil also depends on other factors such as crop variety, physicochemical characteristics of the soil, climatic conditions and human activity (Bateman and Murray 2001; Bernhoft *et al*., 2012).

All this interaction of factors, make necessary the study of the behavior, abundance and diversity of the different races and special forms *Fusarium oxysporum* with the crop and *Trichoderma* species, which have shown properties in their control (Maina *et al*., 2016), as well such as the impact of cultural practices that can alter this behavior.

At present, most of the studies carried out directly with diversity of species show the history and evolutionary potential of a population or infer in practical aspects such as the virulence gene flow to other geographical areas of both fungi (McDonald and Linde, 2002); however, few studies have studied the impact of cultural practices at different times of the year and climatic conditions on the abundance and genetic diversity of *Fusarium* species in the soil (Bernhoft *et al*., 2012) and their interaction with *Trichoderma* species, therefore, there is little understanding in this aspect (Maina *et al*., 2016).

Due to the above, the present study was proposed with the objective of knowing the effect of cultural work (fallow and weed control) and climatic conditions (temperature, relative humidity and precipitation) on the abundance and genetic diversity of populations of *Fusarium oxysporum* and *Trichoderma* sp., in the AAB banana crop, using the PDA and K2 culture media for their isolation and the polymerase chain reaction (PCR) technique for identification.

Materials and methods

Site location and sampling

A total of 58 soil samples weighing 500 g were collected on a plot of manzano or Silk banana (Musa AAB) in Platanozapan, Municipality of Tlapacoyan Veracruz, Mexico, 29 were collected in the month of November 2017 and the rest in April 2018. For the collection of samples, sampling

sites were georeferenced in the plot, which was previously divided into two parts, in one fallow and weed control was performed, while in the other no work was performed. Each site was marked with wooden stakes every five meters, and in each of them at 0.2 m depth a soil sample was taken which was analyzed in the laboratory of the Faculty of Agro-Hydraulic Engineering of the Benemérita Autonomous University of Puebla, Mex.

Isolation and morphological characterization of the *Fusarium* and *Trichoderma* genus

10 g of soil were taken from each sample which were diluted in 90 mL of 0.05% Tween 80, of the dilution obtained, 1 mL was deposited in 9 mL of 0.05% Tween 80 to obtain a second dilution. Of both dilutions 100 µL were extended with a glass triangle on Komana culture medium (K2) and potato dextrose and agar (PDA). From each dilution and culture medium, three repetitions were performed, which were incubated at 30 °C for 48 and 72 hours in the dark. After this time, each and every one of the colonies with morphological characteristics of trichoderma and Fusarium were counted and isolated in a new medium (Chaverri *et al*., 2003; García-Núñez *et al*., 2016; Sánchez-López *et al*., 2012).

Quantification of *Fusarium* and *Trichoderma*

Amount of CFU g^{-1} of soil in PDA and K2 medium: before isolation in new medium and to facilitate counting *Trichoderma* sp. and *Fusarium oxysporum* of each repetition made and for each culture medium used (PDA and K2), each of the Petri dishes was divided into 4 equal parts and in each quadrant the existing colonies were counted, then the CFU calculation was performed by box and evaluated the effectiveness of each of the media.

Amount of CFU g^{-1} of soil with respect to cultural work: the amount of isolated colonies was also analyzed with respect to cultural work done (fallow and weed control) against any work performed.

Amount of CFU g^{-1} with respect to the seasons of sample collection and climatic conditions: the month of December was chosen as the first season of sample collection and the month of April as the second season in order to compare the effect of the climatic conditions, precipitation, temperature, relative humidity and solar radiation present in these two seasons on the amount of CFU existing in soil in each of the sampled parts of the plot. For the analysis of results obtained from CFU g^{-1} in each case, a comparison test of means with a variance analysis (Anova) was performed using the statistical package SAS 9.0 for Windows.

Determination of the presence of *Fusarium oxysporum* f. sp. *cubense* race 1

To corroborate the presence of race 1 of *Fusarium oxysporum*, manzano banana plants (AAB) were inoculated for each isolate with 1X 10^6 CFU. The plants were protected in the greenhouse, 7 days after infection they were evaluated qualitatively on a scale of 1-5 (1= no damage; 2= isolated points in the vascular tissue; 3= discoloration of up to one third of the vascular tissue; 4= discoloration greater than one third of the tissue; and 5= total discoloration of the tissue (Dita *et al*., 2011), for which the base of the corm was cut transversely and observed in the discoloration of vascular tissues.

Molecular identification and determination of *Trichoderma* and *Fusarium* diversity between species and within the most abundant species

For identification, determining the abundance and diversity of the genus studied began with the extraction of DNA from the 14-day-old isolates, seeded in cellophane disk on ADS medium, and incubated at 30 °C, the mycelium obtained was stored in 2 mL eppendorf tubes and lyophilized in a Labconco® lyophilizer..

Portions of 0.05 g of lyophilized dry mycelium were macerated with a pistil in tubes cooled with liquid nitrogen. Subsequently, we proceeded according to the instructions of the Dneasy plant mini kit of the Qiagen brand. The DNA quality obtained from each isolate was determined with a Thermoscientific nanodrop, Nanodrop 2000C model, visualized by an agarose gel in a UVP transilluminator, model: 3UV-LMS26 and was stored in a freezer at -7 °C, for amplification by PCR.

The DNA obtained from each isolate of *Fusarium oxysporum* and *Trichoderma* sp. was amplified by polymerase chain reaction (PCR) for the elongation factor gene EF1-∝. In the case of *Fusarium oxysporum*, the primers: TEF1 and TEF2 were used, with a kit for PCR amplification of the GoTaq® brand, Promega Corporation, Madison, WI, USA, for which 9.5 µL of 10% trehalose was used, 5 µL of 5x Buffer, 10 mM of dNTPs, 2.5 mM μL^{-1} of $MgCl_2$, 1 µM of each first, 5 U μL^{-1} of Taq DNA polymerase and 15 to 20 ng mL^{-1} of DNA, for a final volume of 25 µL mixture, which was amplified at 1 cycle of 95 °C for 2 min, 10 cycles of 94 °C for 30 s, 66 °C (Touchdown -1 °C → 56 °C) 30 s and 72 °C per 1 min, 36 cycles of 94 °C for 30 s, 56 °C for 36 s and 72 °C for 1 min and a cycle of 72 °C for 10 min.

In the case of *Trichoderma* sp., the initiators EF1-728F and EF1-986R described by Carbone and Kohn (1999) were used using a mixture similar to that described for *Fusarium oxysporum*, with the 94 °C 1 cycle amplification program for 5 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min and a cycle of 72 °C for 10 min (Sanchez Lopez *et al*., 2012) All samples were amplified in a personal Mastercycler thermocycler (Eppedor®) and its obtained PCR products were visualized in 2% agarose gel, TAE buffer (1X) plus 10 µL of ethidium bromide which was exposed to 80 V in a Thermocientific electrophoresis chamber .

The amplified products were monitored with a 100 bp DNA Ladder® molecular weight marker, Promega Corporation, Madison, WI, USA, purified and sequenced by Macrogen Inc. laboratories (Geumchen-gu, Seoul, Korea). The sequences were edited with the BioEdit v 7.1.9 program (Hall, 1999). The phylogenetic trees were generated by a maximum likelihood analysis (Maximum Likelihood, ML) and nearest neighbor (Neighbor Joining) using Mega v.5 (Tamura *et al*., 2011), followed by a bootstrap analysis with 1 000 repetitions.

The calculation of the means of interpopulation as well as intrapopulation diversity were obtained with a bootstrap of 1 000 replicas, including in the analysis 21 nucleotide sequences for *Trichoderma* sp. and 20 for *Fusarium oxysporum* with the MEGA v.5 program. To have a molecular approximation of the genetic variability within the most abundant species (*Trichoderma spirale*) the polymorphism of eight of its isolates was measured: VB7IT1, VB28IT1, VB2IT2,

VB8IT2, VB11IT2, VB24IT2, VB25IT2, VB29IT2, by genotyping the ISSR markers: $(GA)_9C$, $(GA)_9T$, $(CA)_8RG$, $(ACC)_9$ and $(GTG)_5$, which were previously selected for their polymorphism (66 to 100%) according to Thangavelu *et al*. (2011); Nirmaladevi *et al*. (2016). ISSR markers were synthesized by Integrated ADN Technologies.

The amplifications were performed in a final volume of 25 μL of reaction, containing 1X buffer for PCR, 0.2 mM dNTPs, 1.5-2.5 mM MgCl, 0.5 μM of each first, 1 unit of Taq DNA Polymerase (GoTaq®, Promise Corporation, Madison, WI, USA) with 20 ng of genomic DNA. The thermal conditions of the reactions were 94 °C for 5 min followed by 40 cycles of 30 s of denaturation at 94 °C and 48 to 64 °C for 45 s of alignment, 1.3 min of extension at 72 °C and a final extension at 72 ºC for 7 min. The PCR products were separated by electrophoresis on a high resolution molecular grade agarose gel (Sigma-Aldrich®, Sigma-Aldrich Corporation, Inc.) at 3% plus 10 μL of ethidium bromide at 45 V for 4 h, using a 50 bp molecular marker (HyperLadder®, BioLine USA Inc. Tauton, Ma, USA).

Results and discussion

Isolation and morphological characterization of the *Fusarium* and *Trichoderma* genus

They were able to obtain 29 isolates in the first season, 16 belonging to *Fusarium* sp. and 13 belonging to *Trichoderma* sp., while in the second sampling season a total of 20 isolates were obtained, six belonging to *Fusarium* sp., and 14 belonging to *Trichoderma* sp. and one to *Hypocrea lixii* (Table 3 and 4).

In the *Fusarium oxysporum* isolates, isolated clamidospores, short conidiophores in monophialides, macroconidia with three or four fusiform or canoe-shaped transverse septa, microconidia abundant in aerial mycelium were observed, while for the *Trichoderma* genus, subglobital conidiophores, attenuated at the tip , the unicellular clamidospores of globose form found in intercalar and terminal form, conidia of ellipsoidal form and green tonality. Characteristic that coincide with those described by Chaverri *et al*. (2003).

Quantification of *Fusarium* and *Trichoderma*

Amount of CFU g^{-1} soil in PDA and K2 medium: the presence of *Trichoderma* sp. in the samples taken in both seasons and both media; however, it was difficult to count due to its rapid growth, which caused the loss of the shape of the colony, generating doubt if it came from one or more spores. In this regard, Al-Sadi *et al*. (2015) state that *Trichoderma* sp. species are generally characterized by their rapid growth and ability to survive in variable environmental conditions, in addition to varying their diversity of species from one substrate to another.

On the other hand, the analysis of variance showed significant differences ($p< 0.001$) between the averages obtained from the treatments. According to the minimum significant difference test (LSD), the PDA was identified as the medium that showed the best result in isolation in both samples (with and without labor) compared to the K2 medium in terms of growth and development

of *Fusarium oxysporum*; that is, there was an average value greater than 200 CFU g^{-1} of soil, while treatments with K2 medium the average was between 50 and 100 CFU g^{-1} of soil (65% higher in PDA than the amount found in K2 medium) (Figure 1).

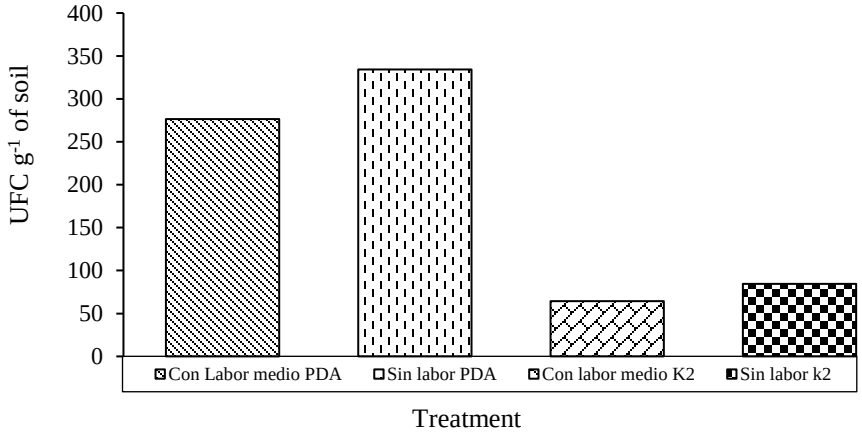

Figure 1. Amount of CFU according to the work done in the crop.

This suggests that the PDA medium provides the appropriate levels of nutrients, as well as the appropriate conditions during the development of both fungi (López *et al*., 2004), while the K2 although it has a large number of nutritional elements It is affected by other factors such as pH, fungicides and antibiotics as mentioned by Komada (1975) limiting its development.

However, although authors such as Bragulat *et al*. (2004) mention that *Fusarium* sp., does not have large limitations in growth in artificial media there are components that affect its characteristic sporulation (López *et al*., 2004). In this case, the tone of the *Fusarium* colonies obtained in PDA was very similar between genders in the first 48 hours of emergency, which made the UFC count difficult.

Similar studies were obtained by Benaouali *et al*. (2014) when working with *Fusarium oxysporum* f. sp. *radicis lycopersici*, who observed better spore growth and pigmentation in the PDA medium compared to other media evaluated. Hence the importance of rapid growth and sporulation in the culture media used because they favor the early identification of fungi preventing CFU from joining.

Although in this study there was fluctuation in the number of isolated CFU per sampled site, it was presented in both media used during the isolation process. These results suggest that there are other factors not analyzed in the study that affect the amounts of CFU specifically per soil site that have no relation to the culture medium. Similar results were obtained by Jiménez-Fernández *et al*. (2010) who when doing studies of identification and quantification of *Fusarium oxysporum* in soil finding that, in samples obtained directly from field soils, populations varied in some of their replicas of isolation compared to artificially infested soils.

Amount of CFU g^{-1} of soil with respect to cultural work: the statistical analysis showed significant differences (p= 0.0352) between treatments, and at least two of them were different (Table 1). The treatment without labor and with PDA medium obtained the highest concentration of CFU g^{-1} of soil with a difference of 68% greater than treatment four.

Table 1. Analysis of the effect of cultural work on the amount of CFU g^{-1} of soil.

Treatment	(CFU g^{-1} of soil)
1. Without labor +PDA	420 a
2. Without labor +K2	270 ab
3. With labor +PDA	270 ab
4. With labor +K2	135 b
LSD	176.28

Means with equal letters are not statistically different, according to the LSD test.

However, although the concentration obtained in the treatments varied among them, statistically the treatments one, two, three and two, three, four were similar indicating the lack of values that show the effectiveness of the cultural work. The effect of agricultural management practices was observed, specifically re-incorporation of senescent plant residues and fungicide application, changing the structure and genetic diversity of the *Fusarium* communities.

Decomposing plants as a source of nutrients for *Fusarium*, which can grow saprophytically generating greater abundance and distribution of species in the soil. However, it is necessary to mention that in their study they sampled these soils for five years, unlike the present study where only two seasons were sampled in one year, resulting in a difference in the data obtained.

Amount of CFU g^{-1} with respect to the seasons of sample collection and climatic conditions: the greatest amount of CFU in the soil was detected in the sampling carried out in the month of December (first season) compared to the month of April where there is a drastic decrease in the amount of CFU, and even in this last season no spores were found in some sampled points (Table 2).

Table 2. Quantity CFU g^{-1} present in PDA medium and climatic variables of the municipality.

Características	December (2017)	April (2018)
CFU g^{-1} of soil (medium PDA)	1098.62	297.93
CFU g^{-1} of soil (medium K2)	180	74.48
Precipitation (mm $monthly^{-1}$)	116.2	276.8
Average temperature (°C)	19.38	24.34
Relative humidity (%)	86.09	86.83
Radiation (W m^{-2})	323.14	386.14

These results coincide with the increase in temperature, precipitation and solar radiation in the second season of sample collection. The temperature and precipitation present in the sampling seasons can have a significant influence on the diversity of fungal species in a given area.

In this regard, Benaouali *et al*. (2014), also emphasizes the importance of temperature in the optimal growth of *F. oxysporum* f. sp. *radicis lycopersici* on the soil. However, although in both seasons of study the changes in relative humidity were almost nil, there was more solar radiation in the second season which also coincided with a decrease in the amount of CFU, in this context Pinkerton, (2000) mentioned that the amount of absorbed solar radiation reduces the population density of fungi found over 30 cm of the soil surface.

Determination of the presence of *Fusarium oxysporum* f. sp. *cubense* race 1

Three plants were observed with slightly necrosed corms, one of these was inoculated by *F. oxisporum* f. sp. *cubensis*, while the remaining two were inoculated with *F. oxisporum* f. sp. *melonis* according to the identification result of the PCR tests of these isolates. According to Dita *et al*. (2018) susceptible cultivars such as the AAB group show more severe symptoms or damage than varieties with intermediate resistance when grown under similar environmental conditions and depending on the inoculum pressure and environmental conditions the disease in the plant can be observed early.

Molecular identification and determination of genetic diversity of *Trichoderma* and *Fusarium* between species and within the most abundant species

The amplification of the PCR products allowed to visualize in the agarose gel 700 bp for the genus *Fusarium* sp. and 350 bp for *Trichoderma* sp. The sequences of identified species were recorded in the National Center for Biotechnology Information (BCNI) (Table 3 and 4). Greater abundance of species was observed in the first season, unlike the second.

Table 3. Isolates obtained in the first sampling season.

Sample	Isolated	Species	NCBI
1	VB1IF1	*Fusarium oxysporum* voucher	MK087011
2	VB2IF1	*Fusarium oxysporum* f. sp. *radicis-cucumerinum*	MK087012
	VB2IIF1	*Fusarium oxysporum* f. sp. *pisi*	MK087013
6	VB6IT1	*Trichoderma spirale*	MK086990
7	VB7IF1	*Fusarium oxysporum* f. sp. *melonis*	MK087014
	VB7IT1	*Trichoderma spirale*	MK086984
8	VB8IT1	*Trichoderma longibrachiatum*	MK086985
9	VB9IT1	*Trichoderma harzianum*	MK086986
10	VB10IF1	*Fusarium oxysporum* f. sp. *melonis*	MK087015
	VB10IT1	*Trichoderma parareesei*	MK086987
13	VB13IF1	*Fusarium oxysporum* f. sp. *melonis*	MK087031
14	VB14IF1	*Fusarium oxysporum* f. sp. *radicis-cucumerinum*	MK087016

Sample	Isolated	Species	NCBI
15	VB15IF1	*Fusarium oxysporum* f. sp. *melonis*	MK087017
	VB15IIF1	*Fusarium oxysporum* f. sp. *pisi*	MK087018
	VB15IT1	*Trichoderma longibrachiatum*	MK086988
16	VB16IT1	*Trichoderma longibrachiatum*	MK086989
19	VB19IF1	*Fusarium* sp.	MK087019
20	VB20IF1	*Fusarium* sp.	MK087020
21	VB21IF1	*Fusarium oxysporum* f. sp. *melonis*	MK087021
	VB21IT1	*Trichoderma orientale*	MK086997
22	VB22IF1	*Fusarium* sp	MK087022
	VB22IIF1	*Fusarium oxysporum* f. sp. *melonis*	MK087023
25	VB25IT1	*Trichoderma longibrachiatum*	MK086998
26	VB26IF1	*Fusarium oxysporum*	MK087024
	VB26IT1	*Trichoderma andinense*	MK086991
27	VB27IF1	*Fusarium oxysporum* f. sp. *radicis-cucumerinum*	MK087025
	VB27IT1	*Trichoderma longibrachiatum*	MK086992
28	VB28IT1	*Trichoderma spirale*	MK086999
29	VB29IT1	*Trichoderma longibrachiatum*	MK086993

National Center for Biotechnology Information (BCNI).

Table 4. Isolates obtained in the second sampling season.

Sample	Isolated	Species	NCBI
2	VB2IT2	*Trichoderma spirale*	MK087001
	VB2IIT2	*Trichoderma spirale*	MK087002
3	VB3IF2	*Fusarium oxysporum* f. sp. *cubense*	MK087032
6	VB6IF2	*Fusarium* sp.	MK087026
	VB6IT2	*Hypocrea lixii*	MK087033
7	VB7IF2	*Fusarium oxysporum* f. sp. *vasinfectum*	MK087027
8	VB8IF2	*Fusarium* sp.	MK087028
	VB8IT2	*Trichoderma spirale*	MK087003
9	VB9IF2	*Fusarium oxysporum*	MK087029
10	VB10IT2	*Trichoderma parareesei*	MK087004
	VB10IIT2	*Trichoderma* sp.	MK087005
11	VB11IT2	*Trichoderma spirale*	MK087006
15	VB15IT2	*Trichoderma spirale*	MK086994
18	VB18IT2	*Trichoderma koningiopsis*	MK086995
21	VB21IT2	*Trichoderma hamatum*	MK086996
22	VB22IF2	*Fusarium oxysporum*	MK087030
	VB22IT2	*Trichoderma koningiopsis*	MK087007
24	VB24IT2	*Trichoderma spirale*	MK087008
25	VB25IT2	*Trichoderma spirale*	MK087000
27	VB27IT2	*Trichoderma spirale*	MK087009
29	VB29IT2	*Trichoderma spirale*	MK087010

National Center for Biotechnology Information (BCNI).

The species that was found in greater abundance in the first season was *Fusarium oxysporum* f. sp. *melonis* with 20%, while in the second one this special form was not found and only four samples were obtained with the *Fusarium* genus, being isolated from one of them *Fusarium oxysporum*. F. sp. *cubense* (Figure 2 and 3). According to Suárez-Estrella (2004), this fungus is able to survive in crop residues, in this case it was found in May, and in soil where no cultivation work was performed.

However, in the second sampling season there was a reduction of both the *Fusarium* genus and the aforementioned species (Figure 2 and 3) and the maintenance of the population density of the *Trichoderma* genus was observed with 20% presence of *Trichoderma longibrachiatum* in the first season and 40% of *Trichoderma spirale* in the second; that is, twice the presence of the latter species. In this context, the species of the *Trichoderma* genus have a high saprophytic capacity while the majority of Fusarium species are phytopathogenic, so their degree of adaptation is different (Maina *et al*., 2016).

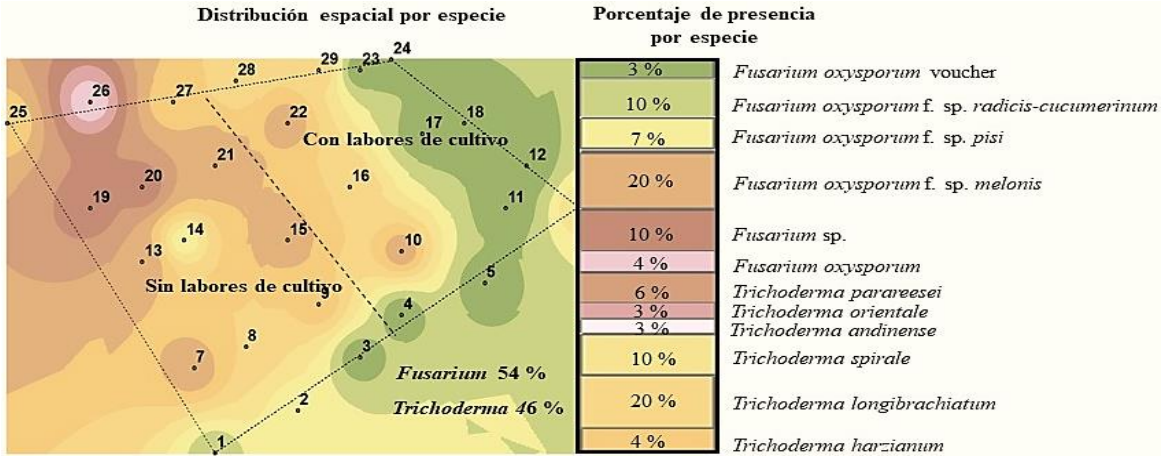

Figure 2. Species of the *Trichoderma* and *Fusarium* genus detected in the soil in the first sampling season.

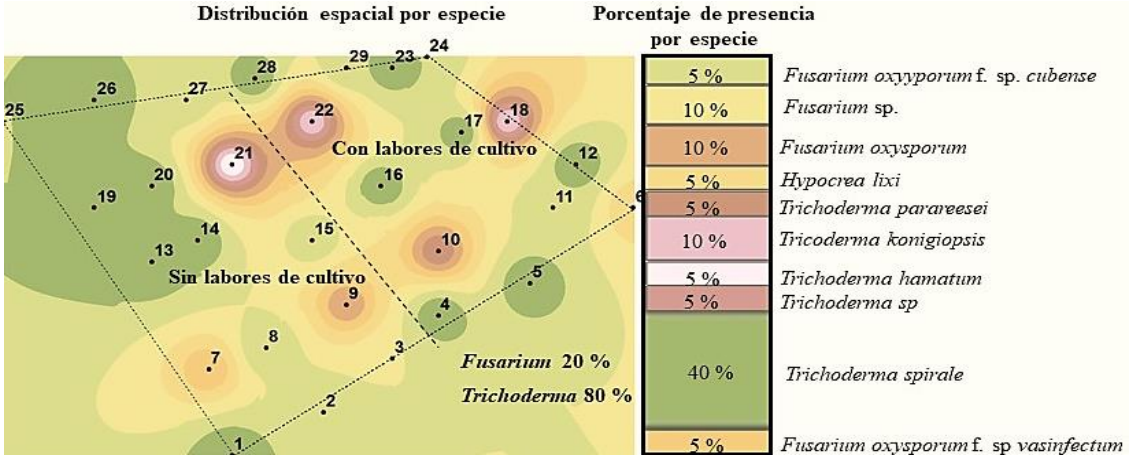

Figure 3. Species of the *Trichoderma* and *Fusarium* genus detected in the soil in the second sampling season.

The DNA sequences analyzed for a Bootstrap above 80% inferred from maximum parsimony (MP), maximum Likelihood (ML) and Neighborhood Joining (NJ) showed a phylogenetic arrangement for both genus (Figure 4 and 5) which grouped isolated sequences of both seasons with 99% similarity; however, it did not show the relationship between kinship and its presence by season or with the cultivation work.

Figure 4. Phylogeny of *Fusarium oxysporum* species as a result of data analysis of EF1-∝. Bostrap values above 80% inference of maximum parsimony (MP), maximum likelihood (ML) and Neighborhood Joining (NJ). The blue circle indicates that the isolate was obtained from the area where cultivation work was carried out, while the green circle indicates that the isolate was obtained from soil without cultivation work.

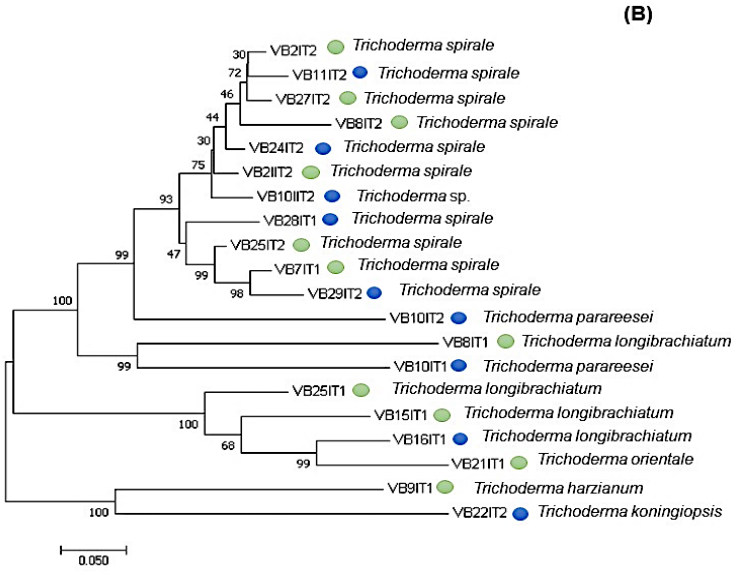

Figure 5. Phylogeny of species of *Trichoderma* sp. as a result of the data analysis of EF1-∝. Bostrap values above 80% inference of maximum parsimony (MP), maximum likelihood (ML) and Neighborhood Joining (NJ). The blue circle indicates that the isolate was obtained from the area where cultivation work was carried out, while the green circle indicates that the isolate was obtained from soil without cultivation work.

These results also showed a greater genetic diversity of species of the *Trichoderma* genus with an average of the interpopulation diversity of 5.75 obtained from 20 sequences of seven populations of the *Trichoderma* genus and a differentiation coefficient of 4.27 obtained from a bostrap of 1 000 replicas. While for *Fusarium*, interpopulation diversity averaged 2.14 with a differentiation coefficient of 4.66 obtained from 21 sequences from eight populations belonging to the genus.

According to studies conducted by Maina *et al*. (2016) found a negative correlation between the occurrence and diversity of *Trichoderma* sp and *Fusarium* sp., in undisturbed areas they observed a high abundance of *Trichoderma* sp. and low occurrence of *Fusarium* and conversely in disturbed areas where there was greater occurrence of *Fusarium* than *Trichoderma* sp. In this sense we observe a greater number of species from both *Fusarium* and *Trichoderma* sp. in soils without cultivation, there is a reduction for the *Fusarium* genus when doing cultivation work and little influence of these tasks on the amount of *Trichoderma* species (Figure 4 and 5).

The diversity results of the eight *Trichoderma spirale* isolates, which was the species that presented the greatest abundance, showed a low intrapopulation diversity observed in the expression of the locis, because only in the VB28IT1 isolate obtained from site 33 bands were amplified which they showed a size between 75 to 500 bp.

In this case, a total of seven bands were amplified from five ISSR; one of the GA9T fragment, five of the ACC6 fragment and one of the GA9C fragment while the CA_8RG and GTG_5 fragments did not show amplification in any of the isolates. Two populations or more that descend from a common ancestor, have allelic frequencies identical to their ancestor. This is possible when analyzing populations living in moderately small spaces in which there is little incidence of factors or barriers that influence the pressure of genetic selection within the species.

Conclusions

This study reiterates the importance of the proper use of the culture medium for the isolation of species of the *Trichoderma* and *Fusarium* genus in soil, because the truthfulness of the results when doing population density studies, as well as population density of abundance and diversity of species. However, in open-field studies the effect of climatic factors, as well as that of cultural activities and times of sample extraction, also influence this type of study.

Finding in these results a greater diversity and abundance of species of both genus in soils where no cultivation work is carried out, in addition to the scarce influence of such work on the amount of *Trichoderma* species, which showed a succession of *T. longibrachiatum* by *T. spirale* from the first to the second season. This sequence and domain expose the biocontrol activity, which could maintain the stability of the populations of the *Fusarium* genus, especially *F. oxysporum* f. sp. *cubense* in banana.

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