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CHARACTERIZATION OF *Phytophthora cinnamomi* Rands ISOLATES FROM THE AVOCADO-PRODUCING AREA OF MICHOACAN, MEXICO

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Michoacan State concentrates the greatest area for avocados in Mexico and worldwide. Nevertheless, one of the main phytosanitary problems on avocado culture is the fungal disease avocado root rot, caused by *Phytophthora cinnamomi* Rands. The genetic diversity of the causing- pathogen is unknown; this has limited the generation of effective pathogen control strategies. With the aim of determining the genetic diversity of this phytopathogen, avocado roots affected by *P. cinnamomi* Rands were collected; oomycete isolates were obtained on PDA (Potato-Dextrose-Agar medium) and re-cultured on V8 medium. A total of 60 isolates from the whole avocado-producing area of Michoacan were analyzed. The morphological characterization indicated variations in the colony growth shape of different isolates: cotton-like with sharp tips (20 %), rose-like without sharp tips (15 %), cotton and rose-like without tips (20 %), rose-like (10 %), open camellia-like (5 %), no-camellia formation (5 %), open-camellia forming a flower (10 %) and concentric rose-like (20 %). In order to perform a molecular DNA characterization, a set of oligonucleotides designed for the amplification of the Internal Transcribed Spacer 1 (ITS1) region of the nuclear ribosomal DNA of *Phytophthora* spp. was employed. Products of amplification of approximately 750 pb were obtained. Sequenced amplicons presented a maximum identity of 95 % with *P. cinnamomi* according to a BLAST (Basic Local Alignment Search Tool, NCBI) analysis of the nucleotide sequences.

Keywords: avocado, avocado sorrow, *Phytophthora cinnamomi.*

CARACTERIZACIÓN DE AISLADOS DE *Phytophthora cinnamomi* Rands DE LA FRANJA AGUACATERA DE MICHOACÁN, MÉXICO.

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En el estado de Michoacán se concentra la mayor extensión de aguacate de México y del mundo. No obstante ello, uno de los principales problemas fitosanitarios en el cultivo, es la enfermedad llamada "Tristeza del aguacatero", causada por *Phytophthora cinnamomi* Rands. La diversidad genética del agente causal es desconocida; esto ha limitado la generación de estrategias eficientes de control. Con el propósito de conocer la diversidad genética del fitopatógeno,

se colectaron raíces de árboles de aguacate afectados por *P. cinnamomi* Rands, los aislamientos del oomycete se realizaron en el medio nutritivo PDA (Papa-Dextrosa-Agar), y se recultivaron en el medio nutritivo V8. Un total de 60 aislados provenientes de toda la franja aguacatera, fueron analizados. La caracterización morfológica indicó que la forma de la colonia en picos algodonosa fue del 20 %, arrosetada sin formar picos 15 %, algodonosa arrosetada 20 %, arrosetada 10 %, camelia abierta 5 %, sin formar la flor de la camelia 5 %, camelia abierta formando la flor 10 % y arrosetada concéntrica 20 %. Se llevó a cabo una caracterización molecular a nivel de ADN, para lo cual se utilizó un juego de oligonucleótidos diseñados para la amplificación de la región espaciadora interna 1 (ITS1) del ADN nuclear ribosomal de *Phytophthora* spp. Se obtuvieron productos de amplificación con un tamaño aproximado de 750 pb. Los amplicones secuenciados presentan una identidad máxima de 95% con ITSs de *P. cinnamomi* al realizar un análisis tipo BLAST de las secuencias de nucleótidos (Basic Local Alignment Search Tool, NCBI).

Palabras claves: aguacate, tristeza del aguacatero, *Phytophthora cinnamomi.*

1 Introduction

In the state of Michoacan, Mexico, avocado culture has been established in a "strip" along 30 municipalities, where the cultured area varies among municipalities. This region is the world largest producer of avocado. However, there are different factors that limit its production and affect the quality of avocado fruits, emphasizing fungal diseases like the one named “avocado sorrow”, caused by *Phytophthora cinnamomi*, a phytosanitary problem constrainting production of this important fruit tree. The first reports of this disease in Michoacan are from 1979, where the disease damaged 13,000 trees in soils poor in organic matter. By 1994 it was established that “avocado sorrow” was distributed in nearly 100,000 trees in andosol type soils. In 1999 there were 550,000 trees affected in the municipalities of Uruapan, San Juan Nuevo, Tingüindín, Los Reyes, Tancítaro, Peribán and Ziracuaretiro, with losses reckoned in about 64,000,000 USD (Vidales, 1999; Mora *et al*., 2000).

Despite its great importance, no accurate information on the genetic and pathogenic diversity of the causal agent of “avocado sorrow” is available to date. This information is fundamental in order to develop efficient strategies of its control, as well as to be able to devise programs for the genetic improvement of rootstocks with attributes of resistance to this disease. The aim of this study is to generate knowledge on the genetic and pathogenic diversity of *P. cinnamomi* that can be used to design efficient control strategies in the avocado strip of Michoacán.

2 Materials y Methods

Roots infected with *P. cinnamomi* were collected among areas with contrasting environmental conditions, previously determined by agroclimatic characterization

of the avocado strip in Michoacan state. Roots were collected from trees that showed the characteristic symptoms of the disease (Figure 1), according to the protocols described by Zentmyer (1980) Téliz and Mora (1997). Isolation and multiplication were done in agreement to the criteria and methodology described for *P. cinnamomi* by Zentmyer (1980).

Morphological characterization of *P. cinnamomi*. Protocols for the characterization were as described by Zentmyer (1980), Donald and Olaf (1996), Santiago (1992) and Hernández (1999), they include characteristics of the colony and mycelium, as well as the presence of radial rings, the stumps were placed under constant 24 ºC to determine their optimum development.

Molecular characterization of *P. cinnamomi.* For the extraction of *P. cinnamomi* genomic DNA a total DNA extraction protocol was modified from the previously reported by Liu et al. (1995). The main variation to the original protocol consisted of precipitating the DNA with 0.25 volumes of AcNa 3M and five volumes of cold absolute ethanol and to incubate to -20 ºC during an hour before recovering by centrifugation at 13,000 rpm for 10 minutes.

PCR amplification of the *P. cinnamomi* ITS 1 from rDNA. The conserved primers ITS1 ad ITS4 (White et al., 1990) used to obtain the ITS1 amplification in diverse species, due to be design in a highly conserved region; as well as a pair of primers designed for the specific amplification of the ITS1 region of the rDNA of *Phythophthora* (Phy1 and Phy2), were used.

The PCR reactions contained 10 to 50 ng of DNA; 0.8 mM each dNTPs; 2mM $MgCl_2$; 1X *Taq* buffer (Invitrogen); 1 U *Taq* DNA polymerase and 10 pmol each pair of primers used. Amplification conditions involved an initial 94ºC (2 min) denaturing incubation followed by 40 cycles of 94ºC (30 s), 60ºC (30 s), 72ºC (1 min) and a 72ºC (10 min) final extension.

In some cases a previous *EcoR*I DNA digestion was performed using 100 to 200 ng DNA and 2 U of enzime in a 10 µl reaction. Once digested, 5 µl were used in the PCR reaction.

PCR products were resolved in 1 to 1.2 % agarose at 80-100 volts, during 30 to 60 min.

3 Results y Discussion

60 stumps were morphologically characterized. A great variability of morphological shapes of colony growth were observed among stumps, including open camellia-like, camellia with sharp tips, and some closed cotton-like growth; closed camillia with sharp tips; concentric rose-like. There were no changes on colony color (either on top or below agar) or changes on the media color. Mean growth speed was 14.5 days to occupy all the Petri dish, or 3.4 cm per day. Figure 2 shows an example of six different shapes observed on diverse *P. cinnamomi* stumps grown on agar medium.

Amplification of an ITS 1 fragment of rDNA with conserved primers. Total DNA was obtaind from the 60 stumps of *P. cinnamomi* aisolated. When conserved primers ITS1 and ITS4 were used, a 900 bp amplicon was obtained (Figure 2A), nevertheless this pair of primers only lend to amplification on approximately 50% of the reactions, in spite of the diverse conditions of amplification practiced. In addition amplification efficiency of the primer pair Phy1 and Phy2 was evaluated, obtaining an amplicon of approximately 750 bp from DNA samples of ten stumps (Figure 3B).

In the remaining 50 stumps, amplicons presented apparent small variation in size when resolving in agarose (Figure 4). Seven amplicons of diverse sizes were sequenced (Figure 4, lanes marked with asterisk), the sequences obtained presented a maximum identity of 95% with ITSs of *P. cinnamomi* according to a BLAST analysis (Basic Local Alignment Search Tool, NCBI).

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