AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Morphological and molecular characterization of *Podosphaera xanthii*, causal agent of powdery mildew in husk tomato and watermelon

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ABSTRACT

Objective: To determine the causal agent of powdery mildew in husk tomato and watermelon, as well as its morphological and molecular characterization.

Design/methodology/approach: Leaves with powdery mildew symptoms were collected from husk tomato (*Physalis ixocarpa*) and watermelon (*Citrullus lanatus*) in Iguala, Guerrero, Mexico in 2018. From two isolates (Phyxa1 and Phyxa2) of husk tomato and two of watermelon (Citrus1 and Citrus2), the morphological characterization was carried out by assembling morphological structures and visualizing them under an optical microscope. For molecular characterization, the ITS region was amplified with the use of primers ITS1 and ITS4, PCR was performed and the products obtained were sequenced in the company Macrogen®. A phylogenetic analysis was performed with the resulting sequences and they were compared with other sequences available in GenBank.

Results: It was determined that there is morphological and genetic variability between isolates from husk tomato and watermelon. The largest sizes of conidiophores and conidia were from Phyxa1 and Phyxa2 isolates, the smallest sizes were found in Citrus1 and Citrus2. The isolates presented a tendency to group according to the host, the Phyxa1 and Phyxa2 isolates were associated with Solanacea isolates, while the Citrus1 and Citrus2 isolates were grouped with isolates of the Cucurbitaceae family.

Findings/conclusions: *Podosphaera xanthii* was shown to be the agent associated with powdery mildew in husk tomato and watermelon. The morphological and genetic variability of *P. xanthii* was determined, which was associated with the host of origin.

Keywords: Fungal diseases, *Physalis ixocarpa*, *Citrullus lanatus*.

Citation: Reyes-García, G., Ortega-Acosta, S.A., Palemón-Alberto, F., Domínguez-Monge, S., Rojas-García, R.A., Maldonado-Peralta, M. de los Á., Toledo-Hernández, E., Terrones-Salgado, J., Cruz-Lagunas, B., Damián-Nava, A. (2022). Morphological and molecular characterization of *Podosphaera xanthii*, causal agent of powdery mildew in husk tomato and watermelon. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i12.2250

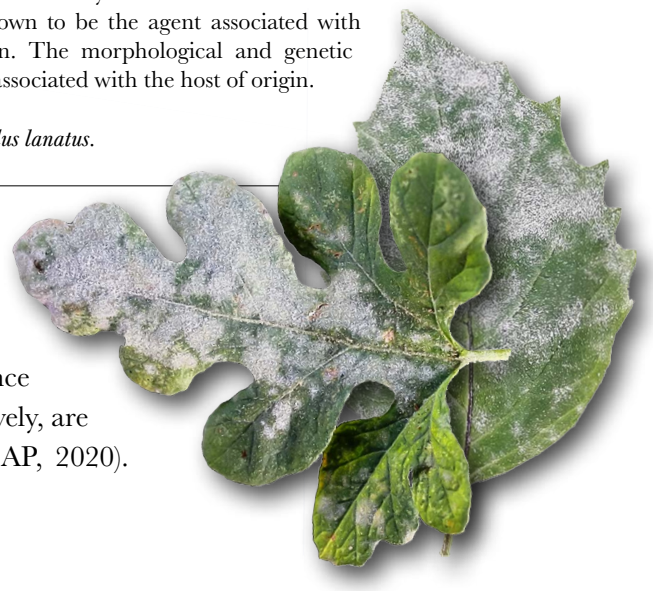
Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: April 12, 2022.
Accepted: November 25, 2022.
Published on-line: January 19, 2023.

Agro Productividad, 15(12). December. 2022. pp: 65-71.

INTRODUCTION

In Mexico, the crops of husk tomato (*Physalis* spp.) and watermelon (*Citrullus lanatus*) are of economic importance, since surfaces of 40,116 and 39,735 ha, respectively, are destined to their cultivation annually (SIAP, 2020).



However, these crops are susceptible to the disease known as powdery mildew. Globally, *Podosphaera xanthii* has been reported as an agent associated to powdery mildew in the crops of husk tomato and watermelon (Kousik *et al.*, 2011; Meeboon *et al.*, 2016). The most common species of powdery mildew in cucurbits are *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Leão *et al.*, 2019). In Mexico, *P. xanthii* has been reported in Sinaloa as the causal agent of powdery mildew in *Physalis ixocarpa* and *Citrullus lanatus* (Félix-Gastélum *et al.*, 2005; Félix-Gastélum *et al.*, 2007).

On the other hand, grouping fungi taxa by molecular phylogeny has shown a general relationship with grouping by infectivity, which suggests that the separation of niches caused by specialization of the host unleashes the genetic divergence of these fungi (Hirata *et al.*, 2000). In this sense, Takamatzu *et al.* (2013) performed a phylogenetic analysis through the sequence analysis of ITS and 28S regions of the rDNA of species of powdery mildew of the genus *Golovinomyces* and determined a high evolutionary relationship with their host plants. Other studies have shown the usefulness of the nuclear sequencing of ribosomal DNA such as the ITS and 28S regions to determine the phylogenetic relationships of species of powdery mildew (Saenz and Taylor, 1999; Bradshaw *et al.*, 2020). Recently, in the tropical environmental conditions of Iguala de la Independencia, Guerrero, considerable damage was detected in husk tomato and watermelon crops, with impact of 100% in that municipality; based on a literature review, the species of powdery mildew in husk tomato and watermelon are unknown.

Therefore, this study had the objective of determining the causal agent(s) of powdery mildew in husk tomato and watermelon, as well as their morphological and molecular characterization.

MATERIALS AND METHODS

Collection of plant material

In production family gardens of Iguala, Guerrero, leaves were collected in January 2018 with symptoms of powdery mildew in husk tomato (*Physalis ixocarpa*) and watermelon (*Citrullus lanatus*) plants during the phenological stage of flowering and fruit development, finding an impact of 100%.

Morphological characterization

Samples of husk tomato and watermelon leaves infected with powdery mildew were collected and placed in plastic bags. With the use of a binocular stereoscopic microscope, small portions of mycelium were transferred to a slide and tinted with cotton lactophenol blue. An optical microscope was used (Nikon®) for the morphological characterization. From each isolate/host, 100 conidiophores and conidia were measured with an AmScope® chamber. Microphotographs of conidia and conidiophores were captured.

Molecular characterization

Based on two isolates (Phyxa1 and Phyxa2) of leaves with symptoms of powdery mildew in husk tomato and two in watermelon (Citrus1 and Citrus2), mycelium was scraped directly from the symptomatic tissue of each isolate with the help of a stereoscopic

microscope and was deposited in sterile Eppendorf tubes of 1.5 ml. The DNA was extracted through the CTAB method (Doyle and Doyle, 1990) and the concentration was determined in a Nanodrop® 2000 spectrophotometer (Thermo Scientific, MA, USA). The region of internal transcribed spacer (ITS) of the nuclear ribosomal region of DNA was amplified for all the isolates with the use of universal primers ITS1 and ITS4 (White *et al.*, 1990). The PCR mixture consisted of: 2 μL buffer (1 X), 0.6 μL MgCl2 (2.0 μM), 0.2 μL dNTP's (0.2 mM), Primer (10 μM) forward 0.6 μL and reverse 0.6 μL, 0.1 μL Taq DNA polymerase (0.5 U) (Promega®), and 20 ng of the DNA extracted from each isolate. The mixture was adjusted with ultra-pure sterile water at a final volume of 10 μL. The PCR amplification was carried out in a thermocycler (Techne-TC-512®), for which the following amplification program was used: initial denaturalization of 5 min at 94 °C, followed by 35 cycles that consisted of 40 s of denaturalization at 94 °C, aligning of 1 min at 62 °C, and final extension of 1.5 min at 72 °C with a final extension of 5 min at 72 °C. The amplified PCR products were purified with the Wizard®SV Gel kit and PCR Clean-Up System (Promega®) following the instructions from the manufacturer. The products were verified in agarose gels at 1.5% in a horizontal electrophoresis system during 85 min at 45 V (Thermo Scientific™ Owl™ Horizontal Gel Electrophoresis Systems) and visualized in a UV light trans illuminator (First Light® Illuminator), and sequenced at the Biotechnology Institute in UNAM. The sequences were edited and assembled with the DNABaser® ver 4.0 software. The sequences obtained in this study were deposited in GenBank.

Phylogenetic analysis

A phylogenetic analysis of the ITS region was conducted with the sequences of the isolates obtained from husk tomato (Phyxa1 and Phyxa2) and watermelon (Citrus1 and Citrus2). The sequences were compared with other isolates available in GenBank of *Podosphaera xanthii*, and related species (*P. macrospora*, *P. leucotricha* and *P. macularis*), and *Cystotheca lanestri* was used as organism outside the group. The sequences were homologated at 489 pb. The phylogenetic analysis was carried out with the Maximum Likelihood method, and the Tamura-Nei model (Tamura and Nei, 1993), for which 1000 bootstrap replicates were used, the analyses were conducted with MEGA® ver. X. (Kumar *et al.*, 2018).

RESULTS AND DISCUSSION

In this study *Podosphaera xanthii* was identified as the agent associated to powdery mildew in husk tomato and watermelon in Iguala, Guerrero, Mexico. The symptoms present in leaves were white powdery colonies, with circular or irregular shape, which invaded 100% of the leaf surface, and caused necrosis and premature senescence (Figure 1A and 1B).

Morphological characterization

Morphological variability was detected in the isolates from husk tomato and watermelon, which was influenced by the host of origin (Table 1).

Podosphaera xanthii isolated from husk tomato presented flexuous, ramified and septate hyphae. The conidiophores were straight to slightly curved, non-ramified and cylindrical and measured on average 181.0×12.7 and 178.1×12.2 μm (length×width), for the

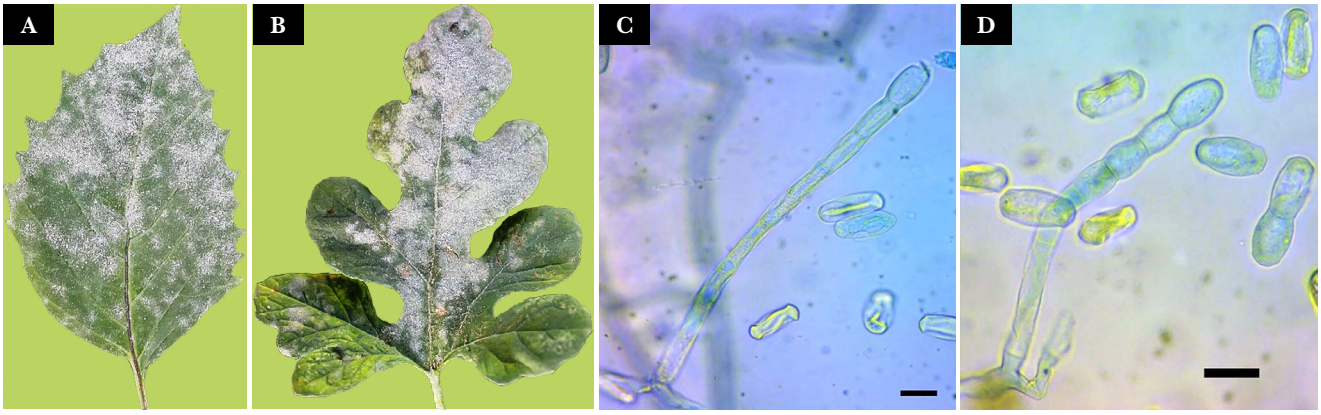


Figure 1. Symptoms of powdery mildew; A=Powdery mildew in husk tomato (*Phyxalis ixocarpa*). B=Powdery mildew in watermelon (*Citrullus lanatus*). C=Conidia and conidiophores of *Podosphaera xanthii* isolated from husk tomato. D=Conidia and conidiophores of *P. xanthii* isolated from watermelon. Bar=20 μm.

isolates Phyxa1 and Phyxa2, respectively. They presented doliform and ellipsoid conidia, and measured 37.3×18.2 and 36.6×18.4 μm (length×width), for the isolates Phyxa1 and Phyxa2, respectively. The length×width rate was 2.1 and 2.0 for Phyxa1 and Phyxa2, respectively.

P. xanthii isolate from watermelon produced flexuous, ramified and septate hyphae. The conidiophores were straigth to slightly curve, non-ramified and cylindrical, measures 158.0×11.7 and 157.6×11.6 μm (length×width) for Citrus1 and Citrus2, respectively. The conidia were doliform and ellipsoid, and measured 29.69×16.0 and 30.7×15.9 μm (length×width) for Citrus1 and Citrus2, respectively, and an average length×width rate of 1.9 (Citrus1) and 2.0 (Citrus2). The isolates Phyxa1 and Phyxa2 presented statistically the largest size of conidiophores and conidia when compared with the isolates Citrus1 and Citrus2 (Table 1).

Based on the morphological characteristics, the fungi identified from leaves of husk tomato and watermelon correspond to *P. xanthii* (Braun and Cook, 2012). The conidia and conodiophores of larger size were observed in the isolates Phyxa1 and Phyxa2 from husk tomato; these same isolates presented the highest length/width rate of conidia. The isolates analyzed from watermelon presented the smaller sizes regarding the characteristics

Table 1. Morphological characteristics of *P. xanthii* isolated from husk tomato and watermelon (*Citrullus lanatus*), from Iguala, Guerrero, Mexico.

Isolation	Conidiophores						Conidia						LWR
	Length			Width			Length			Width			
	Min[y]	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	
Phyxa1	92.7	274.3	181.0 a[z]	9.8	15.4	12.7a	28.5	46.7	37.3 a	13.7	24.5	18.2 a	2.1
Phyxa2	122.7	274	178.1 a	11	15.09	12.8a	26.2	44.9	36.6 a	12.2	23.8	18.4 a	2.0
Citrus1	113.1	212.6	158.0b	9.4	15.3	11.7b	22.4	35.1	29.7 b	13.3	19.0	16.0b	1.9
Citrus2	120.8	210.2	157.6 b	10.1	13.8	11.6 b	25.2	35.5	30.7 b	13.1	20.8	15.9b	2.0

[y]=Min=minimum size of conidiophores or conidia in μm. Max=maximum size of conidiophores or conidia in μm. Avg=average in μm. LWR=Length/width rate.
[z]=Means with the same letter in the same column are not significantly different (P=0.01) based on Duncan's multiple range test.

aforementioned (Table 1). The results suggest that there is morphological variation of *P. xanthii* based on their host of origin. In a study carried out in Taiwan, Yeh *et al.* (2021) reported notable morphological variability of *P. xanthii* in the anamorph stage, which was influenced according to the host where it was isolated; similar results were determined in this study.

Molecular characterization

The sequences obtained from amplification of rDNA of the ITS region from the isolates of husk tomato and watermelon were deposited with the following numbers of access: Phyxa1 (GenBank: MH238506), Phyxa2 (MH238507), Citrus1 (MH238508) and Citrus2 (MH238509).

The phylogenetic analysis demonstrated that there is genetic diversity of *P. xanthii* at the level of amplified region (ITS), according to the host from which it was isolated; for their part, the isolates obtained from husk tomato (Phyxa1 and Phyxa2) presented a tendency to group with isolates from *P. xanthii* isolated from *Petunia×hybrida* (Solanaceae) from the United States of America. Meanwhile, the isolates Citrus1 and Citrus2 grouped with diverse species of Cucurbitaceae and other families, for example *Sechium edule*, *Cucurbita moschata*, *Cucumus sativus*, *Cucumis melo*, *Momordica charantia*, among others (Figure 1). In this regard, in USA Xiang *et al.* (2020) evaluated the genetic variability of *P. xanthii* isolated from *Cucurbita* spp. and *Lagenaria siseraria* through the amplification of the ITS region, this variation was strongly influenced by the place of collection of isolates and the host. In addition, in Italy, through a RAPD analysis, Miazzi *et al.* (2011) reported high genetic variability of *P. xanthii* isolated from *Cucurbita* spp., while in this study only the sexual phase of *P. xanthii* was detected; however, the sexual phase has been reported in Sinaloa, Mexico,

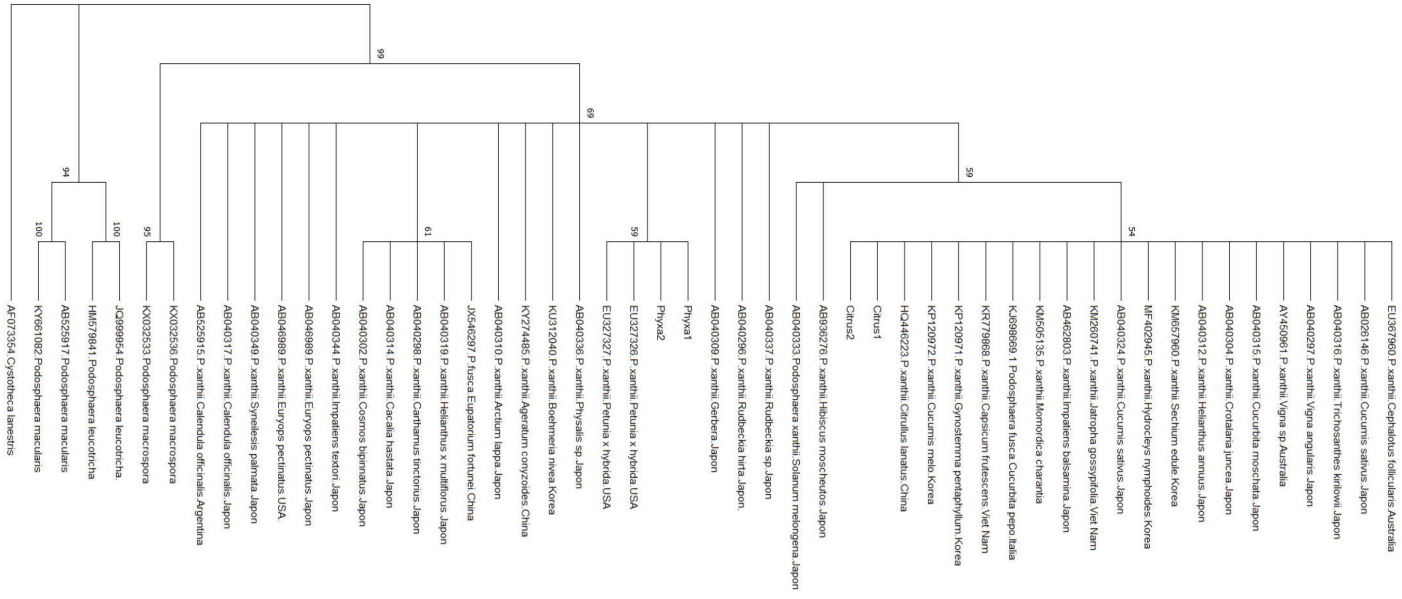


Figure 2. Phylogenetic tree based on sequences of the ITS region of *P. xanthii* isolates Phyxa1, Phyxa2, Citrus1 and Citrus2, and their relationship with other isolates. *Cystotheca lanestri* was used as organism outside the group. The phylogenetic tree was constructed in MEGA X, using the Maximum Likelihood method and the Tamura-Nei model with 1,000 bootstraps.

in hosts of squash and cucumber (Félix-Gastélum *et al.*, 2005). Considering the lack of sexual reproduction, the genetic variability mechanism of *P. xanthii* could happen through the presence of parasexuality in the hyphae, and in addition for powdery mildew its control is generally carried out through the application of fungicides, which is why the mutation to develop resistance to fungicides is another possibility for developing genetic variability (Vielba-Fernández *et al.*, 2020; Xiang *et al.*, 2020). It is necessary to perform other research studies to generate greater knowledge on the biology of *P. xanthii* in Guerrero, Mexico, in order to contribute useful information for the management of powdery mildew in husk tomato, watermelon and other crops of importance for the diet that are susceptible to this pathogen.

CONCLUSIONS

This study shows that *P. xanthii* is the agent associated with powdery mildew in husk tomato and watermelon. The morphological and genetic variability of *P. xanthii* was determined, which was associated to the host of origin. The conidiophores and conidia were of larger size in husk tomato, while the smaller sizes were determined in watermelon. The phylogenetic analysis demonstrated a tendency of the isolates from husk tomato to be associated to isolates from Solanacea, while the isolates from watermelon to be grouped with isolates from Cucurbitaceae.

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