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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF POWDERY MILDEW DISEASE INFECTING ZUCCHINI *CUCURBITA PEPO* OF MIDDLE REGION IN IRAQ

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Abstract

Powdery mildew spread was investigated in zucchini protected culture located at middle of Iraq during 2018 growing season. Three Iraqi provinces, namely, Baghdad, Babylon and Diyala shown the highest disease incidence were included in this study. Symptomatic zucchini samples were microscopically examined. The conidiophore length and width ranged 47-48and 11-12 µm, respectively. Conidia length and average number scoredan average between 12-13 µm and 5-7 spores, respectively. Powdery mildew infection and severityscored 84.7 and 88.7 % highest infectivity and severity in Baghdad, respectively. The disease scored 74.2 and 65.13 lowest infectivity and severity percent in Diayla, respectively. Conidial stage was present, while teleomorph and the chasmothecium could not be formed. Polymerase chain reaction (PCR) using species specific primer setsconfirmed powdery mildew is a single infection of *Podosphaera xanthii* in all zucchini samples collected from Baghdad, Babylon and Diayla. Sequence analysis confirmedthe detection when ~450 DNA fragments amplified from powdery mildew fungus mycelia were identical to IIS gene of *P. xanthii* from the Gen Bank. Besides. All sequences obtained shared 95.81-99.51% maximum nucleotide identities with equivalent Genbank sequences from China (MK357402) and Iran (MF663780). The high disease incidence, fruiting bodies absence, high nucleotide identify and phylogenetic relatedness suggested *P. xanthii*, impacting zucchini in Baghdad, Babylon and Diayla, may have been introduced to Iraq through plant material movement.
Keywords: Morphology, molecular characterization, of powdery mildew disease

Introduction

Zucchini squash *Cucurbita pepo* L. is one of the most important cucurbits within the Family Cucurbitaceae worldwide. Due to the high nutritional and medicinal values its fruit, zucchini is grown in many country for the (Food and Nutrition solution, 1993). Besides, mature zucchini seeds were found to contain 46% oils ,34% proteins, 10% carbohydrates and 2.8 o fibers (Whitaker & Bemis, 1993). It is thought, zucchini has been originated from Middle and South America then spread worldwide (Dilson, 2002). Zucchini squash is an herbaceous annual plant and requires a worm growing season (Hassan, 2001). In Iraq, zucchini has two growing seasons, the spring season starts in March and the autumn season in the late August. In winter, it is grown under plastic tunnels (Alsaeedi, 2003). Cucurbits are infected by many biotic and a biotic diseases causing serious losses in production. Powdery mildew is one of the most important fungal diseases impacting cucurbits, including cucumber *Cucumis sativus*, zucchini squash *Cucurbita pepo* L., marrows *C. maxima*, muskmelon *Cucumis melo* and watermelon *Citrullus vulgaris* (Stephane *et al*., 2013; Margaret, 2013). This disease is highly spread in greenhouses because of the temperature and humidity levels suitable for its growth and spread (Andrivon, 1993). Braun in (1995) identified three fungus genera, *Erysiphe*, *Sphaerotheca* and *Leveillula* that infecting cucurbits and causing powdery mildew diseases in different European countries. Whereas, Alqasim and Abu Ballan (1986) reported *Erysiphe cichoracearum* species causing powdery mildew on many cucurbits in Jordan. In Libya, powdery mildew disease was reported to be caused by three different fungal species on cucurbits, namely, *S. fuliginea, E. cichoracearum* and *L. taurica.* The species *S. fuliginous* (formally known as *P. xanthii*) was the most abundant among the other species on cucurbits in green houses and fields. Both anamorphic andteleomorphic stages were found on zucchiniwhereas anamorphic stage only was found on other cucurbites infected with *S. fuliginous* (El-Ammari and Khan, 1987; Khan, 1987). In Sweden, powdery mildew on cucurbit was found to be caused by the species *E. polyphaga*, while it was caused by two species *E. cichoracearum* and *S. fuliginea* on cucumber in Germany (Junell, 1967). In Syria, (Almaghribi and Tabash,1991) reported the species *E. cichoracearum* was infecting some cucurbits in Latakia province, while(Al-Baghdadi *et al*., 2001) reported the same species on cucurbits in Damascus. *E. cichoracearum* and *S. fuliginea* were reported on many cucurbits on different locations of the Syrian beach (Youins, 2004). (Ali, 2005) showed the infection of cucurbits was by *E. cichoracearum* and *S. fuliginea* and the last was the most prevalent as it had the highest conidia production percent. Powdery mildew disease on zucchini squash is caused by two fungi species, namely, *Podosphaera xanthii* (synonym *Sphaerotheca fuliginea*) and *Golvinomyces cichoracearum* (synonym *Erysiphe cichoracearum*) (Kristova *et al.*, 2009). Both *S. fuliginea* and *E. cichoracearum* were found to infect cucurbits together especially on cucumber and zucchini squash, as conidial or anamorphic stage only was noticed, but teleomorphic stage was absent and no fruiting bodies were formed (Omran *et al*., 2015). Ruey *et al*. (2008) applied PCR technique to differentiate three fugal species causing powdery mildew disease; namely, *E. cichoracearum*, *P. xanthii* and *Levellula taurica* using PN_{23}/PN_{23} primer set targeting ITS region in rDNA. Three primer set were designed; namely, S_1/S_2, G_1 / G_2 and L_1 / $L_2$from ITS region of *E. cichoracearum*, *P. xanthii* and *L. taurica*, respectively. These primers could amplify 454 and 391 bp DNA fragments, respectively, from 4 fungus isolates infecting sunflower plants.

Aim Research

Given the lack of a diagnostic study of the cause of powdery mildew disease on squash percussion in Iraq, therefore this study aimed to diagnose the cause of powdery mildew disease on squash in appearance and in part using the technology PCR.

Material and Methods

The investigation of the fungus causing zucchini powdery mildew under greenhouse conditions

Symptomatic zucchini leaves exhibiting powdery mildew disease were collected from greenhouses in Baghdad (Abu Ghraib, Al-Jadrriya, Ridwaniyah and Yusufiya), Diyala (Al-Khalis and BaniSa'ad) and Babylon (Al Midhatiya and Al Muhaweel) (table 1). Infectivity and disease severity percentages were estimated separately and samples were collected in polyethylene bags, labeled and transferred to the lab for microscopic examination and molecular detection. Infectivity percent was calculated as follows:

$$Infectivity\ percent = \frac{No.\ of\ infected\ leaves\ in\ one\ plant}{Total\ plant\ leaf\ number} \times 100$$

Disease severity was calculated based on McKinney formula (1923):

$$Disease\ severity = \frac{\sum L \times C}{T \times H} \times 100$$

Where L: Number of leaf tested of each class, C: class number, T: total number of leaf tested, H: the heist value of evaluation scale.

The following scale was used to evaluate disease severity based on the present of disease spots covering leaf surface (0-4):

0= Healthy leaves, 1=1-25% of leaf area infected with disease, 2= 26-50% of leaf area infected with disease, 3= 51-75% of leaf area infected with disease and 4=76-100% of leaf area infected with disease.

Table 1: sampling locations of zucchini squash

Province	Location	Sampling date
Baghdad	Abu Ghraib	29 / 11 / 2018
	Yusufiya	30 / 11 / 2018
	Al-Jadrriya	10 / 11 / 2018
Babylon	Al Muhaweel	25 / 11 / 2018
	Al Midhatiya	26 / 11 / 2018
Diyala	Al-Khalis	21 / 11 / 2018
	BaniSa'ad	22 / 11 / 2018

Morphological identification of the pathogen

Leaf tissues from samples of zucchini squash exhibiting typical powdery mildew symptoms were directly examined by microscope at 10x magnification power. The pathogen was identified morphologically following the taxonomic key constructed by Pawar and Chavan (2010) based on structures formed by the fungus.

Molecular identification of powdery mildew pathogen

Total DNA was extracted from the fungus mycelium of selected three isolates, following (Ruey *et al*., 2008) and using AccuPrep® Plant DNA Extraction Kit from (Bioneer, S. Korea) following the manufacturer instructions. PCR was performed using AccuPower PCR PreMix kit from (Bioneer, S. Korea) and S1 (5`-GGATCATTACTAAGCGCGA GGC CC CG-3`)/S2 (5`- CGCCGCCCTGGCGCGAGATA CA-3`) and G1 (5`-TCC GTAGGTGAACCTGCGGAAGGA T-3`)/G2(5`-CAACACCAAACCACACACACGGCG-3`) primer sets amplifying 454 and 391 bp DNA fragment size from ITS region of *P. xanthii* and *E. cichoracearum*, respectively (Ruey *et al*., 2008). PCR amplification was performed using 1 cycle pre-denaturation step for 5 min at 95 ºC, 35 cycles of denaturation for 20 sec at 95 ºC, annealing for 30 sec at 65 ºC and extension for 1 min at 72 ºC and 1 cycle final extension for 5 min at 68 ºC. PCR products were analyzed by ethidium bromide gel electrophoresis using 2.5% agarose for 15 min at 125 m Amp (Sambrook& Russell 2006). PCR products including DNA fragments of expected size were sent to MacrogenInc., South Korea for sequencing in both directions, to generate consensus sequences. Sequences obtained were analyzed and compared to equivalent GenBank sequences performing BLAST comparison using MEGA X (Kumar *et al*., 2018) and STD v1.2 (Muhire *et al*., 2014) software. GenBank accession numbers (MN561051–MN561054) were assigned to powdery mildew pathogen sequences isolated in this study.

Results and Discussion

The investigation of powdery mildew disease in protected culture:

This study was conducted tovalidate the powdery mildew disease dissemination on zucchini squash in Baghdad, Babylon and Diyala Provinces during autumn season, 2018. The infection rate of this disease on zucchini was quite high when ranged 65.13-88.7 and 74.2-84.7% infectivity and disease severity percent, respectively (table 2). The highest infectivity and disease severity were scored in Abu Ghraib-Baghdad protected culture, while the lowest were in BaniSa'ad-Diyala (Table 2). These variations may be ascribed to the differences in environmental conditions among the growing areas. Many previous works indicated of temperature at 25C° and 80% humidity enable infection, development and the spread of powdery mildew disease (Manners and Hossain, 1963; Margaret, 2013; Agrios, 2005).

Table 2: Infectivity and disease severity percentages of powdery mildew infecting zucchini squash in mid-Iraq growing area 2

Province	Location	Disease severity%	Infectivity %
Baghdad	Abu Ghraib	84.7	88.70
	Yusufiya	81.4	85.50
	Al-Jadrriya	80.5	82.03
Babylon	Al Muhaweel	81.4	82.27
	Al Midhatiya	79.4	80.20
Diyala	Al-Khalis	77.2	68.70
	BaniSa'ad	74.2	65.13
L.S.D. 5%		0.252	0.256

Morphological characterization:

Microscopic examination of samples exhibiting powdery mildew symptoms was based on anamorphic or conidial stage only as the pathogen did not form the teleomorphic stage (Amari & Fadil, 2003). Examination revealed that plant tissue contained a colorless mycelium, forming erect conidiophores baring chains of hyaline conidia (Fig. 1). Conidiophores ovoid in shape which is characteristic to the species *P. xanthii* (El–Ammari& Khan, 1986). The conidiophore height and width ranged 47-48 and 11-12µm, respectively (Table 3). Conidiophores length and width ranged 30-35 and 12-13.3 µm, respectively. Number of conidia ranged 5-6 per conidiophore, whereas conidiophore height with conidia was 183-197 µm. Similar data was obtained by Diego *et al*. (2010) when identifying *P. xanthii* infecting zucchini. Many studies indicated powdery mildew

pathogen scan be identified based on anamorphic stage only at different geographical locations including Australia (Clear, 1958), the USA (Kable and Ballantyne, 1963), France (Viennot – Bourgin, 1970) and Libya (Amari & Fadil, 2003). According to Pawar & Chavan (2010) the genus *Podospheara* forms cleistothecia type fruiting bodies which are called chasmothecia. These structures are dark in color, have simple mycelia appendages and include one ascus including 8 ascospores.

Table 3: Some morphological features of the fungus causing powdery mildew on zucchini squash in the Mid-Iraq

Location	Conidiophore height/µm	Conidiophore width/µm	Conidia length/µm	Conidia width/µm	No. of conidia on the conidiophore
Baghdad	47.5	11.6	33	13.3	6
Diyala	48	11	30	12	5
Babylon	47	12	35	13	6

Each value in this table representing an average of 50 leaves randomly collected from zucchini plants grown ingreen houses in the 3 provinces

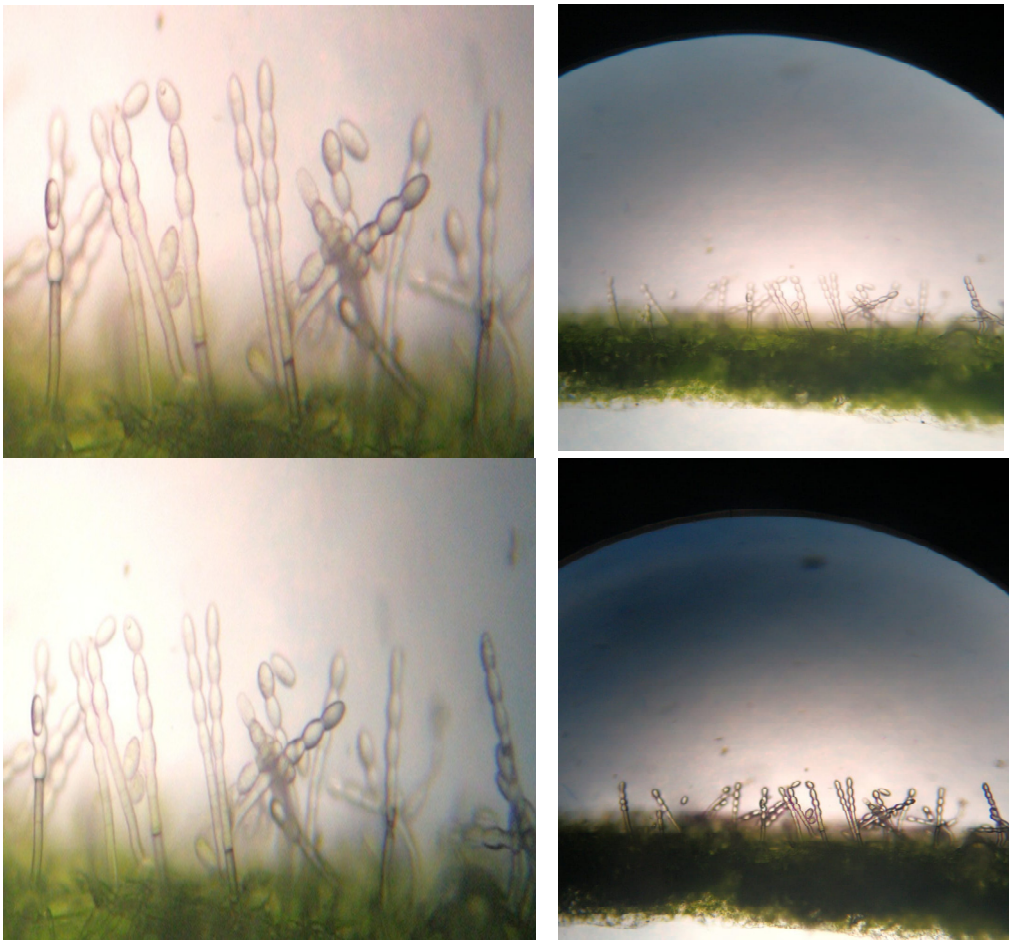

Fig. 1: Asexual stage of mildew fungal pathogen on zucchini showing conidia and conidiophores 10 x

Molecular characterization of powdery mildew pathogenic fungus infecting zucchini

PCR results showed S1/S2 primer set could detect *P. xanthii* in infected samples when amplified ~454 bp DNA fragments from all 4 samples (Fig. 2).

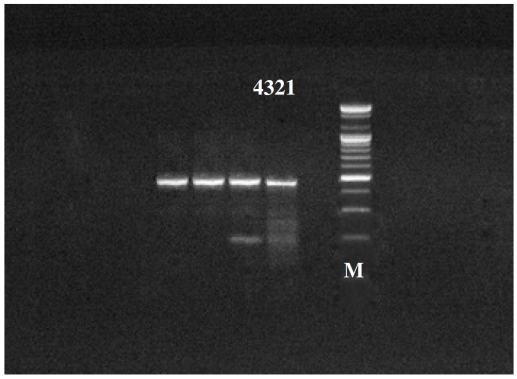

Fig. 2 : Ethidium bromide stained gel pattern showing ~ 454 bp DNA fragments amplified by S1/S2 primer set. (1) Greenhouse sample, (2) Baghdad sample, (3) Babylon sample, (4) Diyala sample and M: 100 bp DNA marker.

Nucleotide sequence analysis revealed ~ 454 bp DNA fragments obtained from powdery mildew infected zucchini samples were amplified from the ITS region of the fungus *P. xanthii.* Sequences obtained shared 95-100% maximum nucleotide sequence identities with the equivalent GenBank sequences from China (Acc. no. MK357402) and Iran (Acc. no. MF663780) (Fig. 3).

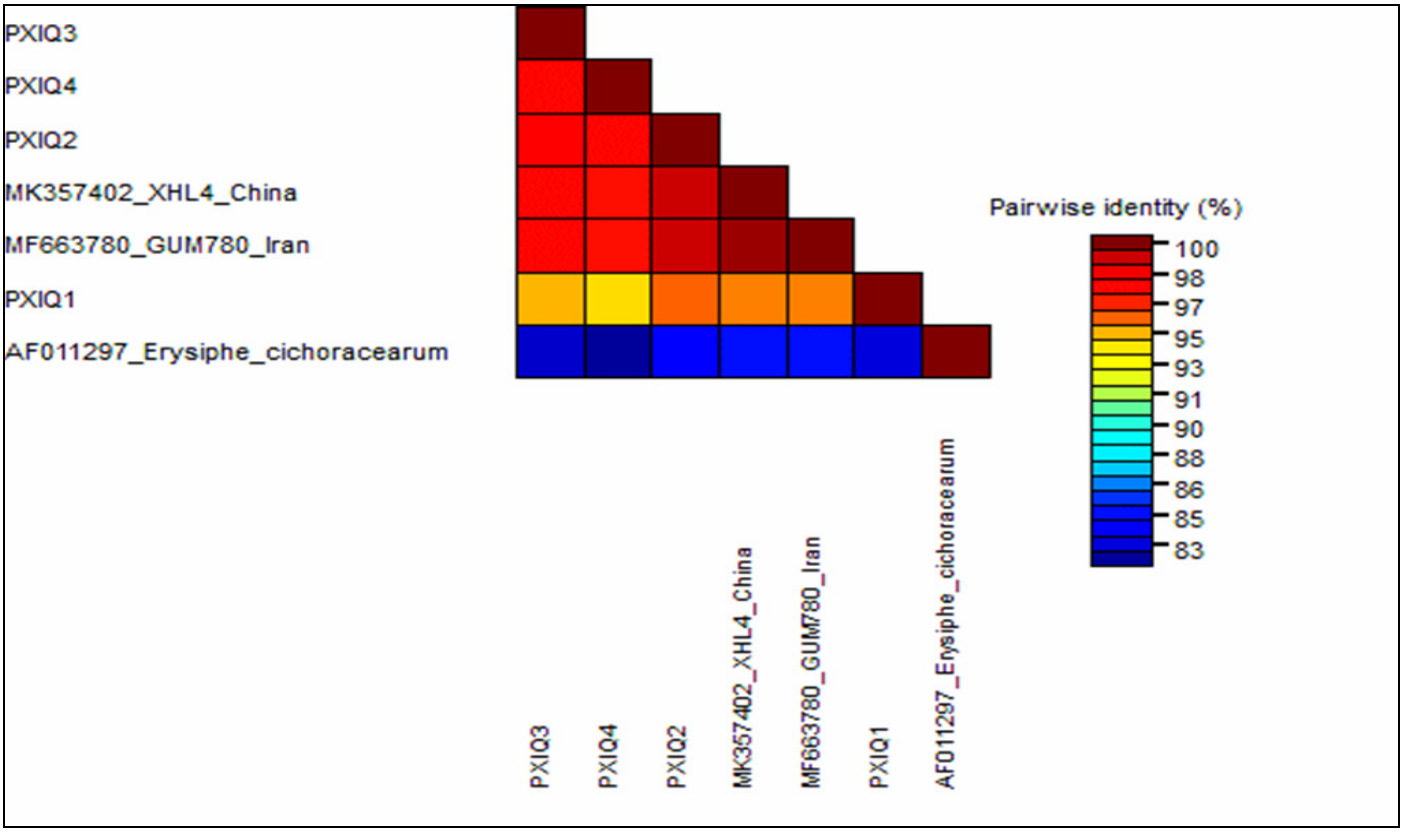

Fig. 3 : Nucleotide identity comparison of partial ITS sequences of *P. xanthii* isolates obtained from Baghdad (PXIQ1-PXIQ2) and Diyala (PXIQ3-PXIQ4) with equivalent GenBank sequences from China and Iran. *Erysiphe cichoracearum* was included as an out-group comparison. Sequences analysis was performed by SDT v1.2 (Muhire *et al*., 2014).

Neighbor-Joining phylogenetic tree confirmed the relatedness when grouped all Iraqi isolates to equivalent *P. xanthii* sequences from the GenBank and separated them from *E. cichoracearum* sequence from the GenBank (Fig. 4). Molecular data and analysis confirmed that the pathogenic fungus isolates causing powdery mildew disease on zucchini squash belong to *P. xanthii* but not to another fungal species or genus. The high nucleotide sequence identities to Chinese and Iranian sequences suggested *P. xanthii* isolated may have a common origin. Conceivably, powdery mildew diseases are spread in many zucchini squash growing area in Iraq and may impact cucurbits and decrease production. Thus precaution control procedures, like applying chemical and cultural controlling methods, may be required to minimize the spread of this disease. The current study provided the first molecular data and confirmation concerning *P. xanthii* in Iraq.

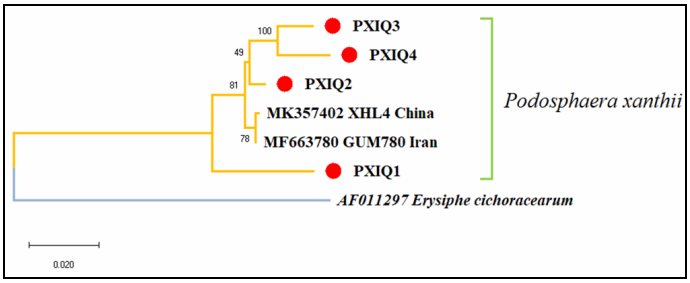

Fig. 4: Phylogenetic relatedness of *Pododsphera xanthii*

Neighbour Joining phylogenetic tree constructed from partial nucleotide sequences of ITS region, including 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2 of P. xanthii isolated from zucchini squash in Baghdad (PXIQ1-PXIQ2) and Diyala (PXIQ3-PXIQ4) provinces (tagged with ●) and equivalent sequences retrived from the GenBank. *Erysiphe cichoracearum* sequence was included as an out-group comparison. Phylogenetic analysis was performed by MEGAX(Kumar et al. 2018).

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