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First Report of *Phaeoacremonium inflatipes* and *Phaeoacremonium mortoniae* Associated with Grapevine Petri Disease in Iran

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ABSTRACT

Petri disease is responsible for grapevine decline and occurs wherever grapevines (*Vitis vinifera*) are cultivated. *Phaeoacremonium* species are among the principal hyphomycetes associated with Petri disease. During 2009, a field survey was conducted throughout different vineyards in the Fars province of Iran in order to determine the fungal pathogens associated with the vine decline observed in the region. Samples were taken from grapevines showing yellowing, interveinal chlorosis, leaf necrosis, reduced growth, wilting, wood necrosis and streaking, and xylem discoloration symptoms in cross section. Isolations were made from symptomatic wood tissues from cordons and trunks on malt extract agar supplemented with 1 mg ml^{-1} streptomycin sulphate (MEAS) and potato dextrose agar (PDA) media. Based on morphological and molecular characteristics two species of *Phaeoacremonium*, *Phaeocremonium mortoniae* and *Pm. inflatipes,* were isolated and identified from grapevines showing yellowing, slow dieback, stunted growth, and reduced foliage in Bavanat (Fars province, south-western Iran). Pathogenicity tests were conducted on rooted grapevine cuttings (cv. Askari) under greenhouse conditions. Based on the results of pathogenicity tests, both tested *Phaeoacremonium* species were pathogenic and caused significant vascular discoloration in inoculated cuttings four months after inoculation. The fungi were reisolated from the margins of the lesion and healthy tissue, completing Koch's postulates. Based on our knowledge, this is the first report of *Pm. mortoniae* and *Pm. inflatipes* causing grapevine Petri disease in Iran.

Keywords: Fars province, Vascular discoloration, Vine decline.

INTRODUCTION

Esca and Petri diseases are responsible for grapevine decline worldwide. Symptoms associated with Petri disease are characterized by stunted growth, shorter internodes, small leaves, interveinal chlorosis, smaller trunks and branches and a general decline of young vines resulting in plant death (Morton, 1995; Bertelli *et al*., 1998; Sidoti *et al*., 2000). Vascular symptoms can be seen by making cross and longitudinal sections in both cordons and trunk and include brown to black streaking of xylem tissues and black spots. The main pathogens associated with grapevine decline symptoms and Petri disease are *Phaeoacremonium* spp., most frequently *Pm. aleophilum* W. Gams, Crous, M.J. Wingf. and Mugnai [teleomorph: *Togninia minima* (Tul. and C. Tul.) Berl.], and *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. and L. Mugnai) Crous and W. Gams (Larignon and Dubos, 1997). These species, in association with some basidiomycetes such as *Fomitiporia mediterranea* M. Fischer and to a lesser extent *Stereum hirsutum* (Willd.: Fr) Pers. are frequently reported as being the cause of esca in mature grapevines (Larignon and

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Dubos, 1997; Mugnai *et al.*, 1999; Ari, 2000; Surico *et al*., 2006). Recently, five *Phaeoacremonium* spp. (*Pm. armeniacum, Pm. globosum, Pm. occidentale* A.B. Graham, P.R. Johnst. and B. Weir (Graham *et al*., 2009), *Pm. cinereum* D. Gramaje, H. Mohammadi, Z. Banihashemi, J. Armengol and L. Mostert and *Pm. hispanicum* D. Gramaje, J. Armengol & L. Mostert) were isolated and identified from grapevines (Gramaje *et al*., 2009a). To date, 25 species of *Phaeoacremonium* have been identified and reported from grapevines worldwide (Crous *et al.,* 1996; Dupont *et al.,* 2000; Mostert *et al*., 2005, 2006; Essakhi *et al*., 2008; Gramaje *et al*., 2009a, Graham *et al*., 2009).

In Iran, esca disease was first reported by Karimi *et al*. (2001). They observed foliar and trunk symptoms similar to esca disease in grapevines cultivated in Bojnourd (in the north of Khorassan province) during 1998-99. *Fomitiporia mediterranea* and *Pa. chlamydospora* were isolated from white wood decay and wood discoloration, respectively (Karimi *et al*., 2001). A similar study was conducted during 2005-06 in Northern-Khorassan province and several fungi including *Fomitiporia* sp., *Phaeoacremonium* sp., *Acremonium* sp. and *Cephalosporium* sp. were found in association with infected grapevines (Karimi-Shahri and Farashiyani, 2006). Thereafter, a general survey was conducted in different vineyards in various provinces of Iran and based on morphological and molecular characteristics, the following fungi were identified to be associated with symptomatic vines: *Pm. aleophilum*, *Pa. chlamydospora*, *F. mediterranea*, *Pm. parasiticum* and *Phaeoacremonium* sp. (Gräfenhan, 2006). Later, isolates of *Phaeoacremonium* sp. were identified as *Pm. iranianum* L. Mostert, Gra¨f., W. Gams & Crous (Mostert *et al*., 2006). In a comprehensive study, a general survey was conducted from different vineyards in various provinces of Iran including Fars, Isfahan, Kohgiluyeh and Boirahmad and Hamedan. Based on morphological and molecular characteristics (PCR-RFLP and partial sequences of the β-tubulin gene) the following fungi were identified to be associated with symptomatic vines: *Pm. aleophilum*, *Pm. parasiticum*, *Phaeoacremonium* sp., *Pa. chlamydospora*, *Diplodia seriata*, *Neofusicoccum parvum*, *Cylindrocarpon liriodendri, Phoma* sp., *Phialophora* like fungi and *Acremonium* sp. (Mohammadi, 2008). Later, isolates of *Phaeoacremonium* sp. were identified as *Pm. cinereum* (Gramaje *et al*., 2009a). Recently, a relatively high occurrence of grapevine decline disease has been observed in different vineyards of Iran (Mohammadi and Banihashemi, 2007; Mohammadi *et al*., 2008; Banihashemi *et al*., 2009; Mohammadi *et al*., 2009). The aim of this study was to identify isolates of *Phaeoacremonium* collected from young vines in Fars province that appeared to be morphologically different from known *Phaeoacremonium* species in Iran.

MATERIALS AND METHODS

Field Survey and Sample Collection

During spring and summer of 2009, a field survey of 28 own rooted grapevine vineyards, between 4 and 35 years old, was conducted in the Fars province (south-western Iran) in order to determine the fungal pathogens associated with vine decline. Five to seven samples were taken from each vineyard from grapevines showing yellowing and necrotic spotting of the leaves, reduced growth of the canes and shoots, defoliation, and different symptoms in wood such as black spots, central brown necrosis, brown and black streaking of the wood and white rot.

Fungal Isolation and Identification

Different parts of grapevines including symptomatic crown, mid-trunk and branches were used for isolation. Cross and longitudinal sections of woody vine parts were examined in order to see the presence of wood discoloration symptoms. Isolation was made from different types of necrotic

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tissues. Small pieces of approximately 4 mm in size of symptomatic tissue were surface disinfected by immersing in 1.5% solution of NaOCl for 30 seconds, rinsed by sterile distilled water (SDW) and plated on malt extract agar (MEA: 2% malt extract, Merck, Germany: 1.5% agar, Merck, Germany) supplemented with 1 mg ml^{-1} streptomycin sulphate (MEAS). Cultures were incubated at 25ºC in the dark. Isolates were transferred to potato dextrose agar (PDA: Merck, Germany) or MEA plates, incubated at room temperature and examined weekly.

Morphological and Cultural Studies

Morphological and cultural characters of single spore *Phaeoacremonium* isolates were studied on four media including MEA, PDA, water agar (WA, 2% agar; Merck, Germany) and oatmeal agar (OA: 30 g oatmeal; 12.5 g agar; Merck, Germany) (Dupont *et al*., 2000). To induce sporulation, isolates were cultured onto MEA and PDA and placed at 25°C in the dark for about three weeks. Microscopic mounts were made from aerial mycelia 2–3 cm from the colony margin. Micro-morphological characters such as conidiophore structure and size, phialide types and size, extent of wart formation, and conidial shape and size were measured/recorded from water mounts. Thirty measurements of each type of structure were made using a light microscope. Water agar was used to examine the presence and size of hyphal warts. Radial growth of the isolates was measured on MEA, PDA and OA after 16 days at 25°C (Mostert *et al*., 2006).

Molecular Identification

DNA Extraction and Polymerase Chain Reaction (PCR)

Isolates were grown on PDA for 10 to 15 days at 25°C in the dark. Fungal mycelia and conidia from pure cultures were scrapped and mechanically disrupted by grinding to a fine powder under liquid nitrogen using a mortar and pestle. Total DNA was extracted using the DNeasy Kit (Qiagen, Germany) following manufacturer recommendations. DNA samples were kept at -20°C until they were used for PCR amplifications.

The specific primers Pm1 and Pm2 for *Phaeoacremonium*, which yielded a fragment of 415 bp for the ITS1 and ITS2 regions of rDNA, were used for direct PCR amplification and detection of the genus *Phaeoacremonium* as described by Aroca and Raposo (2007). In addition, partial sequences of the β-tubulin gene, were amplified using primers T1 and Bt2b for the identification of *Phaeoacremonium* species. The PCR was performed as described by Aroca and Raposo (2007).

Pathogenicity Tests

Two isolates of *Pm. inflatipes* (Pin-1 and Pin-2, GenBank GQ903719 and GQ903720 respectively), *Pm. mortoniae* (PMH1, GenBank Accession No. JF831449 and PMH2) and *Pa. chlamydospora* (Pch-2 and Pch-3, GenBank accession nos. GQ903724, and GQ903725 respectively), as positive control, were selected for pathogenicity tests under greenhouse conditions. Pathogenicity tests were conducted on rooted grapevine cuttings cv. Askari. Cuttings were cut into uniform lengths (about 35 cm) and wounded between the two upper internodes with a 4 mm cork borer. A 4 mm mycelium agar plug from a 16-day-old culture was placed in the wound. Wounds were wrapped with moist cotton and parafilm. Twelve cuttings per fungal isolate were used. Twelve cuttings were inoculated with 4 mm non-colonized MEA agar plugs for a negative control. Inoculated cuttings were planted immediately in individual pots, placed in a greenhouse at 25ºC and watered as needed. Plants were arranged in a completely randomized design. After four months, cuttings were collected and inspected for lesion development. The extent of vascular discoloration was measured upward and downward from the inoculation point. Ten small pieces (about 0.5 cm) of necrotic tissue from the edge of each lesion were cut

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and placed on MEA in an attempt to recover the inoculated fungi and complete Koch's postulates. The fungi were identified as previously described. One-way analysis of variance (ANOVA) in SAS Ver. 9.1 (SAS Institute, Cary, North Carolina, USA) was performed in order to evaluate differences in the extent of vascular discoloration induced by fungal isolates. The LSD test was used for the comparison of treatment means at $P< 0.01$.

RESULTS

Fungal Isolation and Identification

Morphological Identification

In the present study, nine *Phaeoacremonium* isolates were obtained from a 7- year-old-vinyard (cv. Askari) in Bavanat. Of these, four isolates were isolated from black spot of three infected grapevines showing yellowing, slow dieback, reduced foliage and decline symptoms. Based on micromorphological and cultural characteristics the isolates were different from *Pm. aleophilum* and *Pm. parasiticum* which were reported earlier from Iran.

Colonies of these isolates were flat and white to gray on PDA and felty to powdery and gray on OA. Colonies on MEA were flat and brownish gray in the dark at 25°C. Conidia were hyaline, conidiophores short and usually branched. Phialides were terminal or lateral and mostly monophialidic. Based on these morphological characteristics, the isolates were identified as *Pm. inflatipes* (Mostert *et al*., 2006). Morphological characteristics of *Pm. inflatipes* isolates are presented in Tables 1 and 2. Micro- and macro-morphological features are summarized in Figure 1.

Five isolates of a *Phaeoacremonium* sp. were also obtained from discolored vascular tissues of two diseased grapevines showing slow dieback, stunted growth, small chlorotic leaves and reduced foliage. Colonies of these isolates were flat and yellowish white on PDA and OA. Colonies on MEA were white-to-pale gray in the dark at 25°C for 16 days. Conidia were hyaline, conidiophores short, unbranched and often ending in a single terminal phialide. Phialides were terminal or lateral and mostly monophialidic. Based on the morphological characteristics, the isolates were identified as *Pm. mortoniae* (Groenewald *et al.*, 2001; Mostert *et al*., 2006). Morphological characteristics are shown in Tables 3 and 4. Micro and macro-morphological features are summarized in Figure 2.

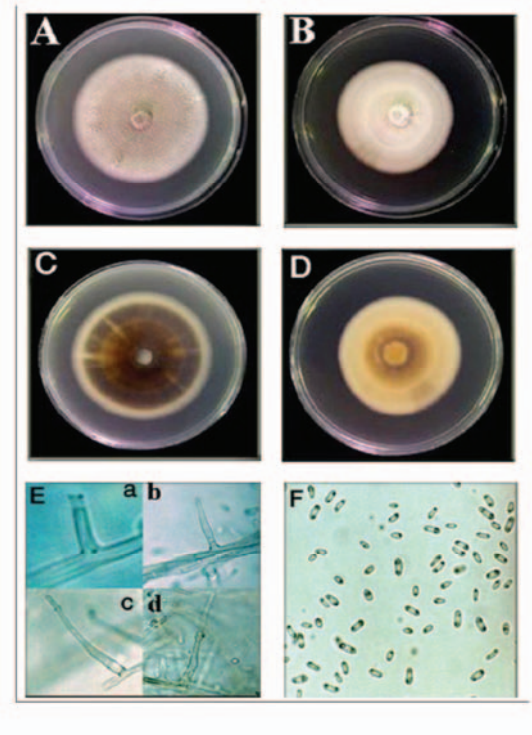

Figure 1. *Phaeoacremonium inflatipes*. 16-day-old colonies on MEA (A) and PDA (B). Colonies reverse on MEA (C) and PDA (D). Type of phialides and conidiophore (E); type I (a),; Type II (b); Type III (c); conidiophore (d), Conidia (F).

Molecular Identification

The DNA extracted from *Phaeoacremonium* isolates found in this study was amplified using the primers pair Pm1 and Pm2. An amplicon of about 415 bp was obtained for *Phaeoacremonium* isolates.

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Table 1. Micro-morphological characters of *Pheoacremonium inflatipes* isolates after 16 days at 25°C in the dark.

Isolate code	Conidial dimension		Phialide size[c] (µm)			Conidiophore
	Conidial size[a](µm)	L/W ratio[b]	Type I	Type II	Type III	size[d](µm)
Pin1	3.0(3.9)5.5 × 1.0(1.0)2.0	3.9	3.0(7.8)15.5	8.0(12.7)16.0	12.0(19.2)27.0	15.0(22.8)30.5
Pin2	3.0(4.3)5.0 × 1.0(1.0)1.5	4.3	3.5(8.0)14.5	7.5(13.0)16.0	12.5(20.2)27.0	16.5(25.3)31.0
Pin3	3.0(3.8)5.0 × 1.0(1.2)2.0	3.2	4.0(7.6)14.0	8.5(12.0)16.0	14.0(20.4)27.0	14.5(25.7)42.0
Pin4	3.0(3.7)5.0 × 1.0(1.2)1.5	3.1	4.0(7.7)12.0	7.0(11.6)18.5	10.5(19.4)27.0	18.0(26.1)39.5
Mean	3.0(3.9)5.1 × 1.0(1.1)1.8	3.60	3.6(7.8)14.0	7.8(12.3)16.6	12.3(19.8)27.0	16.0(25.0)35.8

[a] Minimum, mean value and maximum size for length and width of 30 conidia; [b] Length/Width; [c and d]Minimum, mean (in brackets) and maximum size for 30 phialides and conidiophore measured.

Table 2. Colony growth rates of *Pheoacremonium inflatipes* isolates after 16 days, at 25°C in the dark.

Isolate code	Radial growth[a] (mm)			Daily growth rate (mm)		
	MEA	PDA	OA	MEA	PDA	OA
Pin1	26.5(28–27.8)28	22.5(23–22.8)23	29.5(29.5–29.6)30	1.74	1.43	1.85
Pin2	25.5(27.5–27)28	21(23.5–22.9)24	28.5(28.5–29.1)30	1.69	1.43	1.82
Pin3	27(27.5–27.4)28	21.5(21.5–22.2)24	28.5(29–28.9)29.5	1.71	1.39	1.81
Pin4	28.5(29.5–29.4)30	23.5(24–24.1)25	30(31–31.1)32.5	1.84	1.51	1.94
Mean	26.9(28.1–27.9)28.5	22.1(23–23)24	29.1(29.5–29.7)30.5	1.75	1.44	1.86

[a] Minimum size, most frequent value and mean size (in brackets) and maximum size of radial growth.

Table 3. Micro-morphological characteristics of *Pheoacremonium mortoniae* isolates after 16 days at 25°C in the dark.

Isolate code	Conidial dimension		Phialide size(µm)[c]			Conidiophore
	Conidial size[a](µm)	L/W ratio[b]	Type I	Type II	Type III	size(µm)[d]
PMH1	2.0(5.0)7.0 × 1.5 (1.5)2.0	3.3	3.0(7.8)15.5	8.0(12.7)16.0	12.0(19.2)27.0	15.0(23.5)34.5
PMH2	2.5(5.3)6.5 × 1.0 (1.3)1.5	4.1	3.5(8.0)14.5	7.5(13.0)16.0	12.5(20.2)27.0	14.5(25.5)35.5
PMH3	2.0(4.5)6.5 × 1.0(1.4)1.5	3.2	4.0(7.6)14.0	8.5(12.0)16.0	14(20.4)27.0	13.0(23.5)42.0
PMH4	1.5(4.5)6.5 × 1.0(1.2)1.5	3.8	4.0(7.7)12.0	7.0(11.6)18.5	10.5(19.4)27.0	14.5(23.0)40.0
PMH5	2.0(5.0)6.0 × 1.0(1.6)2.0	3.1	4.0(7.7)12.0	7.0(11.6)18.5	10.5(19.4)27.0	13.0(22.0)40.5
Mean	2.0(4.9)6.5 × 1.1(1.4)1.7	3.5	3.6(7.8)14.0	7.8(12.3)16.6	12.3(19.8)27.0	14.0(23.5)38.8

a = Minimum, mean value and maximum size for length and width of 30 conidia.
b = L/W = Length/width.
c and d = Minimum, mean (in brackets) and maximum size for 30 phialides and conidiophores measured.

Table 4. Colony growth rates of *Pheoacremonium mortoniae* isolates after 16 days, at 25°C in the dark.

Isolate code	Radial growth[a] (mm)			Daily growth rate (mm)		
	MEA	PDA	OA	MEA	PDA	OA
PMH1	19.5(26–24.8)26	18.5(20–19.8)20	25.5(25.5–25.6)27	1.55	1.24	1.60
PMH2	21.5(24.5–23)25	18(19.5–19.9)21	26.5(25–26.3)27	1.44	1.24	1.64
PMH3	20(24.5–23.4)25	19.5(19.5–20.2)22	26(25.5–25.5)27.5	1.46	1.26	1.59
PMH4	21.5(25.5–24.4)26	19.5(20–20.1)21	28(27–29.3)30.5	1.53	1.26	1.83
PMH5	19.0(25.5–23)26	20.5(21–21.1)22	27.5(27.2–29)30	1.44	1.32	1.81
Mean	20.3(23.8)25.6	19.1(20.2)21.2	26.7(27.1)28.4	1.48	1.26	1.69

[a] Minimum size, most frequent value and mean size (in brackets) and maximum size of radial growth.

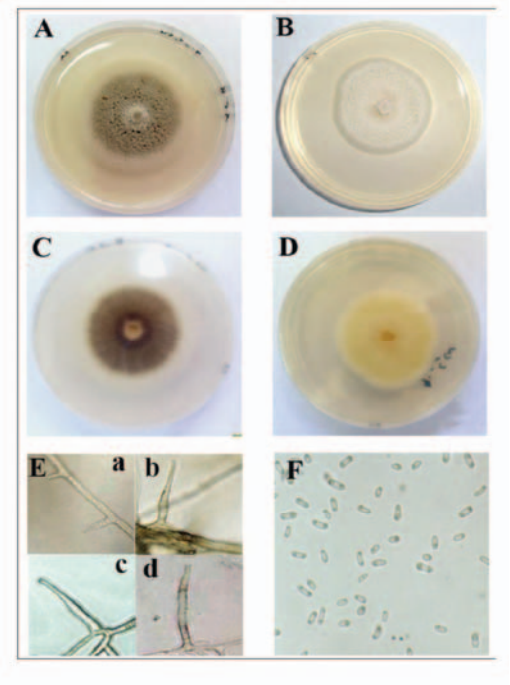

Figure 2. *Phaeoacremonium mortoniae*. 16-day-old colonies on MEA (A) and PDA (B). Colonies reverse on MEA (C) and PDA (D). Type of phialides and conidiophore (E); type I (a); Type II (b); Type III (c); conidiophore (d), Conidia (F).

These isolates were also amplified by using T1 and Bt2b primers. A PCR fragment of about 750 bp was obtained from a partial sequence of their β-tubulin gene. After sequencing and Blast search in the GenBank, four isolates of *Pm. inflatipes* showed 100% similarity to the sequence of *Pm. inflatipes* isolates in GenBank (GenBank Accession No. AY579323, Mostert *et al*., 2005). The sequence of *Pm. mortoniae* isolate showed 100% similarity to corresponding sequence from *Pm. mortoniae* deposited in GenBank (GenBank Accession No. HM116767, Johnston *et al*., 2010, unpublished).

Pathogenicity Tests

Analyses of variance of the lesion length data on grapevine cuttings inoculated with *Phaeoacremonium* species indicated a significant treatment effect (F = 385.11 and P < 0.001; ANOVA tables not shown). All fungal isolates used were pathogenic and

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caused large wood discoloration on inoculated plants, which were significantly longer than the controls. *Pa. chlamydospora* isolates were more virulent and produced significantly ($P< 0.0001$) longer lesions (ranging from 58 to 71 mm) in all inoculated plants than those of *Pm. mortoniae* (ranging from 41 to 56 mm) and *Pm. inflatipes* (ranging from 24 to 37 mm) isolates, while the discoloration of control treatments was scanty (ranging from 15 to 21 mm). *Pm. inflatipes* isolates produced smaller lesions than those caused by *Pa. chlamydospora* and *Pm. mortoniae* isolates in all inoculated branches but still differed significantly from the control. The fungi were reisolated from the margins of the lesion and healthy tissue, completing Koch's postulates, while no pathogens were found in the control plants.

DISCUSSION

Members of the genus *Phaeoacremonium* are known to be cosmopolitan, having a range of woody hosts and wide geographical distribution. They are reported from grapevine from different countries including Italy (Mugnai *et al*., 1999), France (Dupont *et al.,* 2000), Greece (Rumbos and Rumbou, 2001), Argentina (Dupont *et al.,* 2002), Australia (Mostert *et al.,* 2005), Chile (Auger *et al.,* 2005), Austria (Reisenzein *et al.,* 2000), Spain (Armengol *et al.,* 2001), USA (Schek *et al*., 1998), South Africa (Crous *et al*., 1996), Turkey (Ari, 2000) and Iran (Grafenhan and Gams 2004; Mohammadi *et al*., 2008). In recent years, grapevine trunk diseases have gained importance in Iran. In the present study, molecular and morphological studies identified two species of *Phaeoacremonium, Pm. inflatipes* and *Pm. mortoniae,* to be associated with grapevines showing Petri disease symptoms in Bavanat. *Phaeoacremonium* species are often found during general surveys of grapevine trunk pathogens in other grapevine-growing countries (Mostert *et al*., 2006; Essakhi *et al.,* 2008). Petri disease is a major cause of grapevine decline in Iran (Mohammadi *et al*., 2008).

Micromorphological characters, such as cultural characters, size of hyphal warts, conidiophore morphology, and phialide type and shape are useful for the identification of *Phaeacremonium* species (Mostert *et al*., 2005; 2006). However, distinguishing species based solely on morphological characters has proven to be difficult and it has resulted in some misidentifications. The ability to rapidly and accurately identify pathogens that cause Petri disease and esca is a critical first step for epidemiological studies and for a better understanding of the distribution and importance of individual species. Therefore, molecular tools have contributed to identify *Phaeoacremonium* species. In this study based on Pm1 and Pm2 primers and β-tubulin sequencing data amplified by T1 and Bt2b primers, two species of *Phaeoacremonium*, *Pm. inflatipes* and *Pm. mortoniae*, were identified as the causal agents of Petri disease. *Pm. inflatipes* was originally described based on morphological and cultural characteristics by Crous *et al*. (1996). This species has been reported from grapevine in California (Scheck *et al*., 1998; Eskalen and Gubler, 2001), Costa Rica (Mostert *et al*., 2006) and Spain (Gramaje *et al*., 2009b). *Pm. mortoniae* Crous and W. Gams, was also identified and described based on morphological characters, the internal transcribed spacer (ITS) regions 1 and 2, the 5.8S rDNA (Dupont *et al*., 2000) and the β-tubulin gene (Groenewald *et al*., 2001). This species has been reported from grapevines in Hungary and Croatia (Essakhi *et al*., 2008), Spain (Gramaje *et al*., 2007), Sweden and USA (Mostert *et al*., 2006), from kiwifruit in Italy (Prodi *et al*., 2008) and from *Prunus salicina* in South Africa (Damm *et al*., 2008).

In this work, both tested *Phaeoacremonium* species caused significant vascular discoloration on wood in inoculated grapevines 4 months after inoculation, although none of them was more virulent than *Pa. chlamydospora*,

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which caused the largest vascular discoloration affected inoculated area. Several previous studies also indicated higher symptom expression by plants inoculated with *Pa. chlamydospora* than *Phaeoacremonium* spp. (Adalat *et al*., 2000; Gramaje *et al*., 2010). *Pa. chlamydospora* produced larger areas of vascular discoloration than *Phaeoacremonium* spp. under field (Mugnai *et al*., 1999; Halleen *et al*., 2007) and greenhouse (Halleen *et al*., 2007; Aroca and Raposo, 2009) conditions. In different pathogenicity studies, *Pa. chlamydospora, Pm. aleophilum* and *Pm. inflatipes* have been shown to induce decline of young grapevines (Scheck *et al*., 1998). *Pm. inflatipes*, *Pm. aleophilum* and *Pa.chlamydospora* are reported as the causal agents of young vine decline in California. These three species were shown to be pathogenic to grape seedlings and 1-year-old rooted grapevine cuttings (Scheck *et al*., 1998). In seedlings cv. Malvar, all *Phaeoacremonium* species caused defoliation with the exception of *Pm. inflatipes*, which caused stem necrosis (Aroca and Raposo, 2009). To date, two new species of *Phaeoacremonium* as *Pm. iranianum* (Mostert *et al*., 2006) and *Pm. cinereum* (Gramaje *et al*., 2009a) have been described from Iranian vineyards. This study provides evidence for the presence of two other *Phaeoacremonium* spp., *Pm. inflatipes* and *Pm. mortoniae*, as the causal agents of vine decline in Iran, and thus; future field surveys in this country may reveal the presence of other fungal pathogens especially within the *Phaeoacremonium* genus in addition to the fungi reported here.

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اولین گزارش از *Phaeoacremonium inflatipes* و *Phaeoacremonium mortoniae* همراه با بیماری پتری انگور در ایران

ح، محمدی و ض. بنی هاشمی

چکیده

بیماری پتری یکی از بیماری‌های زوال انگور(*Vitis vinifera*) است که در بیشتر مناطق کشت انگور دیده می‌شود. گونه‌های *Phaeoacremonium* یکی از اصلی‌ترین هیفومیست‌های همراه با این بیماری هستند. در سال ۱۳۸۸ به منظور تعیین قارچ‌های بیمارگر همراه با بیماری زوال انگور از باغات مختلف در استان فارس بازدید به عمل آمد. از درختان بیمار که دارای علائم زردی، زردی بین رگبرگی، نکروز برگی، کاهش رشد، پژمردگی و قهوه‌ای شدن چوب و بافت آوندی در برش عرضی بودند، نمونه برداری انجام شد. جداسازی عوامل قارچی از بافت‌های آلوده شاخه و تنه درختان بیمار با به کار گیری محیط کشت‌های عصاره مالت-آگار حاوی یک میلی گرم در میلی لیتر سولفات استرپتومیسن (MEAS) و عصاره سیب زمینی-آگار (PDA) انجام گردید. بر اساس خصوصیات مورفولوژیکی و مولکولی دو گونه *Phaeoacremonium* به عنوان *Pm. inflatipes* و *Pm. mortoniae* از درختان بیمار با علائم زردی، سرخشکیدگی، کاهش رشد و کم برگی در بوانات (استان فارس، جنوب غربی ایران) جداسازی و شناسایی گردیدند. آزمون بیماری‌زایی تحت شرایط گلخانه‌ای و بر روی قلمه‌های ریشه‌دار شده انگور (رقم عسکری) انجام شد. بر اساس نتایج حاصل از آزمون بیماری زایی، هر دو گونه *Phaeoacremonium* بر روی قلمه‌های مایه زنی شده بیماری‌زا بوده و پس از چهار ماه باعث ایجاد تغییر رنگ بافت آوندی در قلمه‌ها شدند. قارچ‌های مایه‌زنی شده از حاشیه بافت آلوده و سالم در لکه‌ها مجددا جداسازی و شناسایی شدند در حالی که از گیاهان شاهد قارچی جداسازی نگردید. بر اساس اطلاعات موجود این اولین گزارش از *Pm. inflatipes* و *Pm. mortoniae* به عنوان عوامل بیماری پتری انگور در ایران است.

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