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Identification and pathogenicity of fungal species associated with grapevine trunk diseases in Khorasan–Razavi province, Iran

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Abstract: Grapevine trunk diseases (GTDs) are the most important factors in crop reduction and cause considerable economic problems in grapevines worldwide. From 2016 to 2018, several field surveys were conducted on numerous vineyards in Khorasan–Razavi province to study fungal species associated with grapevine trunk diseases. In this study, samples were collected from the trunk and branches of trees showing yellowing, stunted growth, dieback and wood discoloration in cross–sections. In this study, 258 fungal isolates were obtained and identified based on morphological characteristics and comparison of DNA sequence data (ITS–rDNA region and a part of *β–tubulin* gene). These isolates were identified as *Phaeoacremonium minimum* (75 isolates), *P. parasiticum* (19 isolates), *P. iranianum* (52 isolates), *P. tuscanum* (8 isolates), *Fomitiporia mediterranea* (56 isolates) and *Seimatosporium vitis* (48 isolates). Pathogenicity of the selected isolates was verified by inoculation of potted grapevines shoots under greenhouse conditions. Based on the mean length of wood discoloration in the wood, *P. minimum* and *F. mediterranea* were the most and least virulent species, respectively. Our findings indicated that known fungal trunk pathogens such as *Phaeoacremonium* species and *F. mediterranea* occur on grapevine in Khorasan–Razavi province. This study is the first report of *S. vitis* associated with grapevine decline in Iran.

Keywords: Dieback disease, morphology, sequencing, *Fomitiporia*, *Phaeoacremonium*, *Seimatosporium*

INTRODUCTION

Vitis L., with more than 100 species, is one of the oldest and most important fruit crop genera all over the world (Mullins et al. 1992). *Vitis vinifera* L. (grapevine) is among the most widely cultivated species in Iran and Khorasan province is also considered as one of the most important grape-growing areas in the country. In the past few decades, several studies have shown that more than 30 fungal diseases can affect the health of grapevines worldwide (Wilcox et al. 2015). However, in recent years, great emphasis has been laid on grapevine trunk diseases (GTDs) in Iran (Arabnezhad & Mohammadi, 2012, Arabnezhad et al. 2013, Mohammadi & Banihashemi 2007, Mohammadi et al. 2013a, b, Narmani & Arzanlou, 2017, Abed-Ashtiani et al. 2019) as well as many other countries in the world (Larignon & Dubos 1997, Mugnai et al. 1999, Armengol et al. 2001, Gramaje & Di Marco 2015). Currently, GTDs are known as one of the most destructive fungal diseases and serious threats to the future economic sustainability of viticulture in the world (Bertsch et al. 2013, Gramaje et al. 2016, 2018, Guerin-Dubrana et al. 2019). The term GTDs was first introduced by Chiarappa for a range of leaf disease symptoms and necrosis in the wood tissues of affected trees (Gramaje et al. 2018). GTDs are caused by different groups of fungal pathogens (Bruez et al. 2016). Based on the available references, more than 138 species of 35 different fungal genera have been isolated and reported from a grapevine showing trunk diseases worldwide (Gramaje et al. 2018, Raimondo et al. 2019). Several fungal species belonging to the family Botryosphaeriaceae (Van Niekerk et al. 2004, Úrbez-Torres 2011, Xie et al. 2010, Yang et al. 2017), Togniniaceae (Edwards & Pascoe 2004, Mostert et al. 2006, Gramaje et al. 2011, Raimondo et al. 2014, Gramaje & Di Marco 2015, Da Silva et al. 2017),

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Diatrypaceae (Dumot et al. 2004, Butterworth et al. 2005, Trouillas & Gubler, 2010, Trouillas et al. 2010, Sosnowski et al. 2016, Pitt et al. 2013), *Phaeomoniella* (*Pa.*) *chlamydospora* (Edwards & Pascoe 2004, Gramaje et al. 2009b, Fleurat-Lessard et al. 2010), *Phomopsis* spp. (Phillips 2000, Baumgartner et al. 2013, Úrbez-Torres et al. 2013, Senanayake et al. 2015), *Pleurostoma* (*Pl.*) *richardsiae* (Carlucci et al. 2015, Pintos Varela et al. 2016), *Cadophora* spp. (Gramaje et al. 2011, Travadon et al. 2015) and basidiomycetous species (Fischer 2002, Cloete et al. 2015) have been reported as components of the grapevine trunk-disease complex, although pathogenicity tests have not been completed for all of these taxa. Several studies conducted in Iran have also shown that grapevine cultivars can be affected by various fungal trunk pathogens, including Botryosphaeriaceae, *Pa. chlamydospora*, *Phaeoacremonium* species, *Pl. richardsiae*, *Cylindrocarpon liriodendri* and basidiomycetous species (Karimi et al. 2001, Mohammadi & Banihashemi 2007, Mohammadi et al. 2009, Mohammadi 2012, Arabnezhad et al., 2013, Arzanlou et al. 2013, Mohammadi et al. 2013a, b). Although various fungal species have been isolated and reported from grapevines in Iran, the identity of the fungal trunk pathogens on this host plant have not yet been investigated in Khorasan-Razavi province. During the survey carried out on some vineyards in Khorasan-Razavi province, 258 fungal isolates were obtained from grapevines showing trunk disease symptoms. The aim of this study was to identify these isolates and to evaluate their pathogenicity on the grapevine.

MATERIALS AND METHODS

Field survey, sampling and fungal isolation

In order to study fungi associated with grapevine trunk diseases, several field surveys were conducted on various vineyards in Khorasan-Razavi province (northeastern Iran) between May 2016 and August 2018. Samples were collected from the trunk and branches of affected trees showing disease symptoms, including yellowing, stunted growth, dieback and internal wood lesions in cross-sections. Fungal isolation was conducted from each collected sample. Small pieces of tissue (about 5 × 5 mm in size) were cut from the margin between necrotic and apparently healthy wood tissues, surface-disinfected for 2 min in a 0.5 % sodium hypochlorite solution, washed twice with sterile distilled water and then cultures on potato dextrose agar plates (PDA, Merck, Darmstadt, Germany) supplemented with 100 mg l^{-1} streptomycin sulfate (PDAS). All the plates were incubated at 25 °C and the emerging colonies were transferred to fresh PDA. Fungal colonies were purified using single-spore or hyphal-tipping methods.

Morphological identification

All fungal isolates were initially grouped by comparing the main morphological characteristics and cultural appearance. Cultures characters and pigment production on PDA, malt extract agar (MEA, 2% malt extract, Merck, Darmstadt, Germany) and oatmeal agar (OA, 60 g oatmeal, 12.5 g agar, Difco, France) as well as the main microscopic structures including conidiophore morphology, size of hyphal warts, phialide type and shape and conidial size and shape were used to conduct morphological identification of *Phaeoacremonium* isolates (Crous et al. 1996, Mostert et al. 2006, Spies et al. 2018). *Seimatosporium* isolates were identified based on the colony and cultural characteristics on PDA and pycnidia and conidial morphology described by Senanayake et al. (2015). Morphological and microscopic analyses of mycelial culture of basidiomycetous isolates were studied according to Fischer (2002). For these isolates, general colony characteristics on cultures including shape, color, surface aspect and reverse were evaluated and recorded.

DNA extraction, amplification and sequencing

Representative fungal isolates of each group were selected for molecular identification. These isolates were grown on PDA and incubated in the dark at 25 °C. Produced mycelia and conidia were scraped with a sterile scalpel from the surface of 10-to 15-day-old cultures and then total genomic DNA was extracted using CTAB method (Doyle & Doyle 1987). All samples were incubated at −20 °C until conducting PCR amplification. The internal transcribed spacer 1 and 2, including the intervening 5.8S nrDNA gene (ITS) and a part of *β-tubulin* gene (BT) were amplified using primer sets ITS1/ITS4 (White et al. 1990) and T1 and Bt_2b (Glass & Donaldson 1995), respectively. All the polymerase chain reactions (PCRs) were performed in Techne TC-312 Thermal Cycler (Techne, Cambridge, U.K.) as described by Hashemi & Mohammadi (2016). DNA samples and PCR products were evaluated on 0.1% agarose gels stained with ethidium bromide and visualized under ultraviolet light. A 100-bp ladder (GeneRuler DNA Ladder Mix, Fermentas, Vilnius, Lithuania) was used for the evaluation of the band sizes. Purification and sequencing of the PCR products were performed using Bioneer Corporation (Daejeon, South Korea). All the sequences were read and edited by BioEdit Sequence Alignment Editor v. 7.0.9.0 (Hall 2006) and then run through the MegaBLAST function of the National Center for Biotechnology Information's GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/) for initial identification.

Phylogenetic analysis

Phylogenetic analysis was performed only for *Phaeoacremonium* isolates. Beta-tubulin sequences of Iranian *Phaeoacremonium* isolates *were* obtained from grapevine in this study and reference sequences of *Phaeoacremonium* species taken from the GenBank were aligned using default settings of Clustal W algorithm (Thompson et al. 1994) included within MEGAX software package (Kumar et al. 2018).

Pleurostoma richardsiae (CBS 270.33, accession no. AY579334) was used as an out-group. The alignment results were checked and manually improved where necessary. Maximum Likelihood (ML) analysis was performed in MEGAX. Bootstrap support was estimated using 1000 replicates to assess the robustness of each clade.

Pathogenicity tests

The pathogenicity of one isolate from each identified species was tested on two-year-old rooted cuttings of grapevine (cultivar Kolahdari) under controlled greenhouse conditions (at 23 ±2 °C). The inoculated region was surface-disinfested with 75% ethyl alcohol for 10 min. After air drying, a wound was made between two nodes of each cutting using a cork borer of 5 mm diameter. Wounds were inoculated with mycelial plugs (5 mm in diameter) taken from 2-week-old cultures on PDA and then protected by moist cotton and a strip of Parafilm ® (Pechiney Plastic Packaging, enasha, USA). A total number of 10 rooted cuttings per fungal isolate were used and 10 rooted cuttings were inoculated with sterile PDA plugs as the control treatments. All the inoculated cuttings were arranged in a completely randomized design and disease symptoms and the length of wood discoloration were measured after 10 months. Re-isolations of the inoculated isolates were conducted from the symptomatic wood tissues of the treatments and control plants and cultured on PDA plates. Petri dishes were incubated at 25 °C and re-isolated fungi were identified based on the colony and conidial morphology. One-way analyses of variance (ANOVA) in SAS v 9.1 (SAS Institute, Cary, NC, USA) was performed in order to assess differences in the extent of wood discoloration induced by the inoculated isolates. Moreover, the LSD test was used for comparison of treatment means at $p < 0.05$.

RESULTS

Sample collection and disease symptoms

In this study, the wood samples were collected from the trunk and branches of 115 diseased grapevines showing yellowing, stunted growth and dieback. Various wood lesion types include of wedge-shaped necrosis, irregular wood necrosis, central necrosis, black spots and wood decay, were also observed and recorded in cross-sections of collected trunk and branches (Fig. 1).

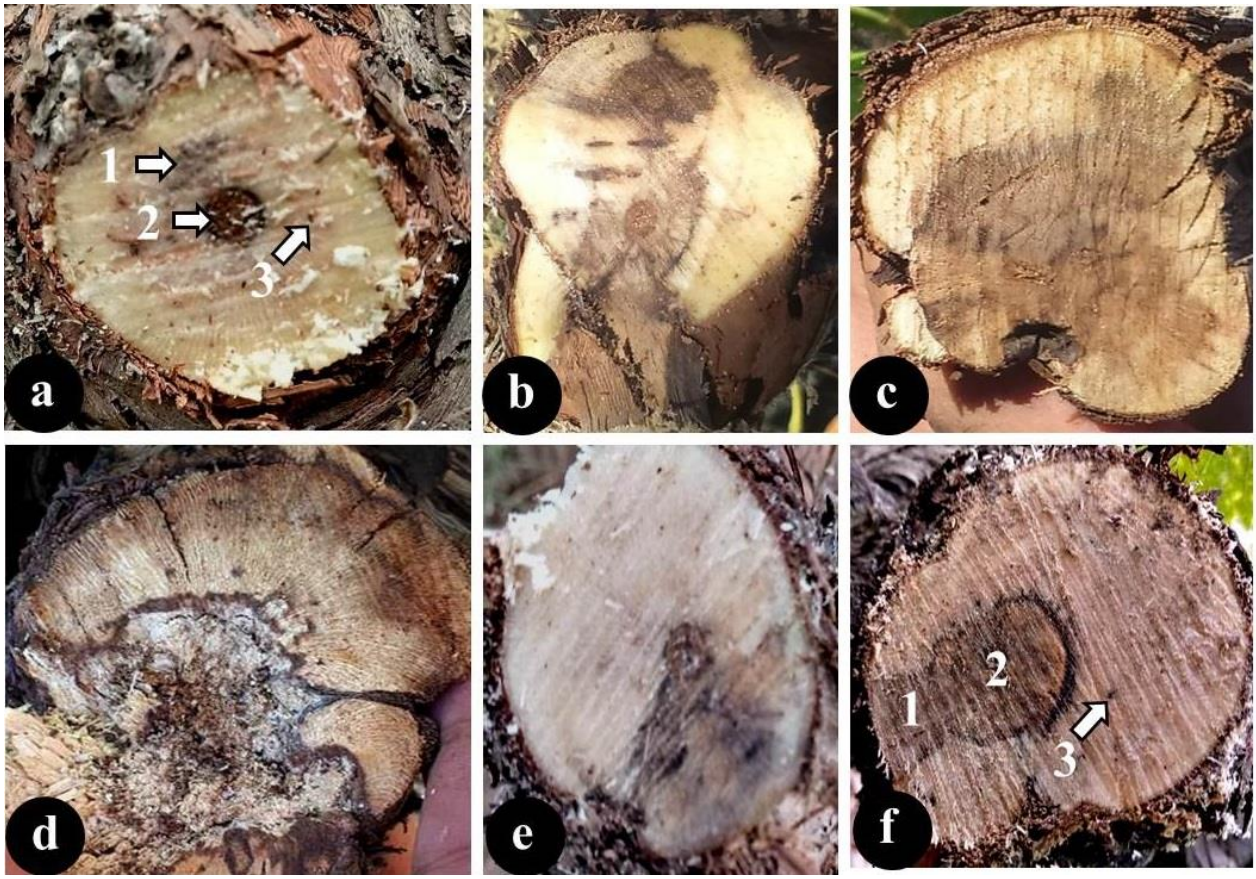

Fig 1. Internal wood disease symptoms on grapevine in Khorasan-Razavi province: Co-occurrence of irregular wood necrosis (1), central necrosis (2) and black spot (3) symptoms (a); irregular wood necrosis (b and c); wood decay (d); wedge-shaped necrosis (e); co-occurrence of wedge-shaped necrosis (1), central necrosis (2) and black spot (3) symptoms in cross-section of an infected branch.

Table1. Geographical origin and number of the fungal isolates recovered from diseased grapevines collected from Khorasan-Razavi province (Iran) during 2016-2018.

Fungal species	Region	Number of the isolates	
		From each region	Total and percentages (%) *
Phaeoacremonium minimum	Neghab	34	75 (29.1)
	Quchan	15	
	Neyshabur	7	
	Kashmar	19	
P. iranianum	Neghab	24	52 (20.1)
	Quchan	9	
	Neyshabur	7	
	Kashmar	12	
P. tuscanum	Neghab	8	8 (3.0)
P. parasiticum	Neghab	19	19 (7.4)
Fomitiporia mediterranea	Neghab	27	56 (21.8)
	Quchan	8	
	Neyshabur	3	
	Kashmar	18	
Seimatosporium vitis	Neghab	27	48 (18.6)
	Quchan	4	
	Neyshabur	3	
	Kashmar	14	

* percentage based on the total number of fungal isolates.

Fungal isolation and morphological identification

In this study, a total number of 258 fungal isolates were obtained. Isolation, frequency and geographical distribution of the isolates recovered from diseased grapevines in Khorasan-Razavi province are shown in Table 1. Based on colony appearance, culture characteristics, and microscopic structures, the main fungal isolates were divided into three groups.

Group one: One hundred and fifty-four isolates (59.7% of total isolates) were classified in this group. These isolates were characterized by beige medium brown, pale to pale brown to reddish-brown flat slow-growing cultures on PDA. Sporulation was abundant, conidia were hyaline and aseptate, hyphae were single or fasciculate, and three types of phialides (I, II, and III types) were different in size and shape were observed in these isolates. These isolates were tentatively identified as *Phaeoacremonium* (Crous et al. 1996, Mostert et al. 2006, Spies et al. 2018).

Group two: Forty-eight isolates (18.6 % of total isolates) as 'pestalotioid fungi' were placed in this group. These isolates were characterized by white to medium-brown, margin uneven and flat slow-growing cultures on PDA. Hyphae hyaline, straight, branched, and septate. Conidiomata pycnidioid, solitary or aggregate, dark, 95−345 µm diam. Conidiophores 7−18 × 2−3 µm, Conidia abundant, single, obovoid to fusiform, straight to slightly curved, 12−19 × 4−6 µm, with 3 dark brown transverse septa, hyaline to sub-hyaline basal and apical cell and two hyaline, unbranched and short flexuous appendages (Fig. 2). These morphological features were compatible with the description of *Seimatosporium* spp. (Senanayake et al. 2015, Wijayawardene et al. 2015, Lawrence et al. 2018).

Group three: Fifty-six isolates (21.7 % of total isolates) were classified in this group. These isolates were characterized by white and medium-growing mycelium on PDA (28 mm at 25 within eight days) that became yellow-orange and brown with age. These morphological features were not sufficient to identify these isolates at a generic level; however, they were considered as basidiomycetous fungi (Sigler & Abbott 1997, Markakis et al. 2017. Brown et al. 2019).

Molecular identification and phylogenetic analysis

To confirm the identification based on morphology and cultural characteristics, BLASTn searches in GenBank showed that ITS sequences of basidiomycetous isolates had 100 % identity with *Fomitiporia mediterranea* (isolate: CB6, accession no. EU477476). Beta tubulin sequences of *Seimatosporium* isolates were also 100 % identical to *Seimatosporium vitis* Napa776 (accession no. KY706252). For phylogenetic analyses, the BT sequences of 17 suspected *Phaeoacremonium* isolates were aligned with 34 sequences of reference isolates and *Pleurostoma richardsiae* (CBS270.33) was used as an out-group. The alignment consisted of 628 characters (including gaps), of which 215 were constant and 292 parsimony informative. Maximum parsimony analysis resulted in 4 equally most parsimonious trees (TL=1303, CI=0.475, RI=0.765, RC=0.363). Analysis of the BT clearly separated Iranian isolates in 4 clades including *P. iranianum* (52 isolates), *P. tuscanum* (8 isolates), *P. minimum* (75 isolates) and *P. parasiticum* (19 isolates). One of the ML trees is shown in Fig. 3 with bootstrap support values at the nodes.

Pathogenicity tests

Mean lengths of wood discolorations caused by inoculated isolates on rooted grapevines are shown in Fig 4. All the inoculated isolates produced wood lesion lengths were significantly different from those in the control plants ($P<0.05$). *Phaeoacremonium minimum* was the most aggressive fungal species and produced the longest necrotic wood lesions (29.8 mm) on the

inoculated shoots, followed by *S. viticola* (27.8 mm) and *F. mediterranea* (12.1 mm). No significant difference was observed between *P. minimum* and *S. viticola*. Re-isolation percentages were between 60.0 % (*F. mediterranea*) and 100 % (*P. minimum* and *S. viticola*) on inoculated plants, and no fungal isolates were isolated from control treatments.

DISCUSSION

Nowadays, GTDs are considered as the most destructive disease of grapevine (Gramaje et al. 2018, Guerin-Dubrana et al. 2019) and to date, more than 138 fungal species have been associated with GTDs (Gramaje et al. 2018, Raimondo et al. 2019) worldwide. The results of this study showed that grapevines in Khorasan-Razavi province are infected with known fungi causing decline diseases. In this province, morphological and molecular data provided evidence that at least *Phaeoacremonium* spp. *F. mediterranea* and *S. vitis* occur on grapevines in this region. In addition to these taxa, several isolates of *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Bipolaris* and *Coniothyrium* species were also isolated from affected grapevines in this province, which were not considered in this paper. *Phaeoacremonium* isolates were the most abundant fungal groups isolated from affected trees. More than 60 species of *Phaeoacremonium* have been isolated and reported from different woody plant hosts around the world, among which grapevine is known as one of the most important hosts of this group of fungi (Crous et al. 1996, Mostert et al. 2006, Essakhi et al. 2008, Graham et al. 2009, Ariyawansa et al. 2015, Gramaje & Di Marco 2015, Crous et al. 2016, Da Silva et al. 2017, Spies et al. 2018). Throughout this work, four *Phaeoacremonium* species, including *P. parasiticum*, *P. minimum*, *P. iranianum* and *P. tuscanum* were recovered from grapevines showing decline symptoms. The first three species are relatively widespread and have been reported from grapevine in various countries around the world (Mostert et al. 2006, Spies et al. 2018), while *P. tuscanum* is reported only from grapevines in Italy (Essakhi et al. 2008) and Iran (Mohammadi 2012, Arabnezhad & Mohammadi 2014).

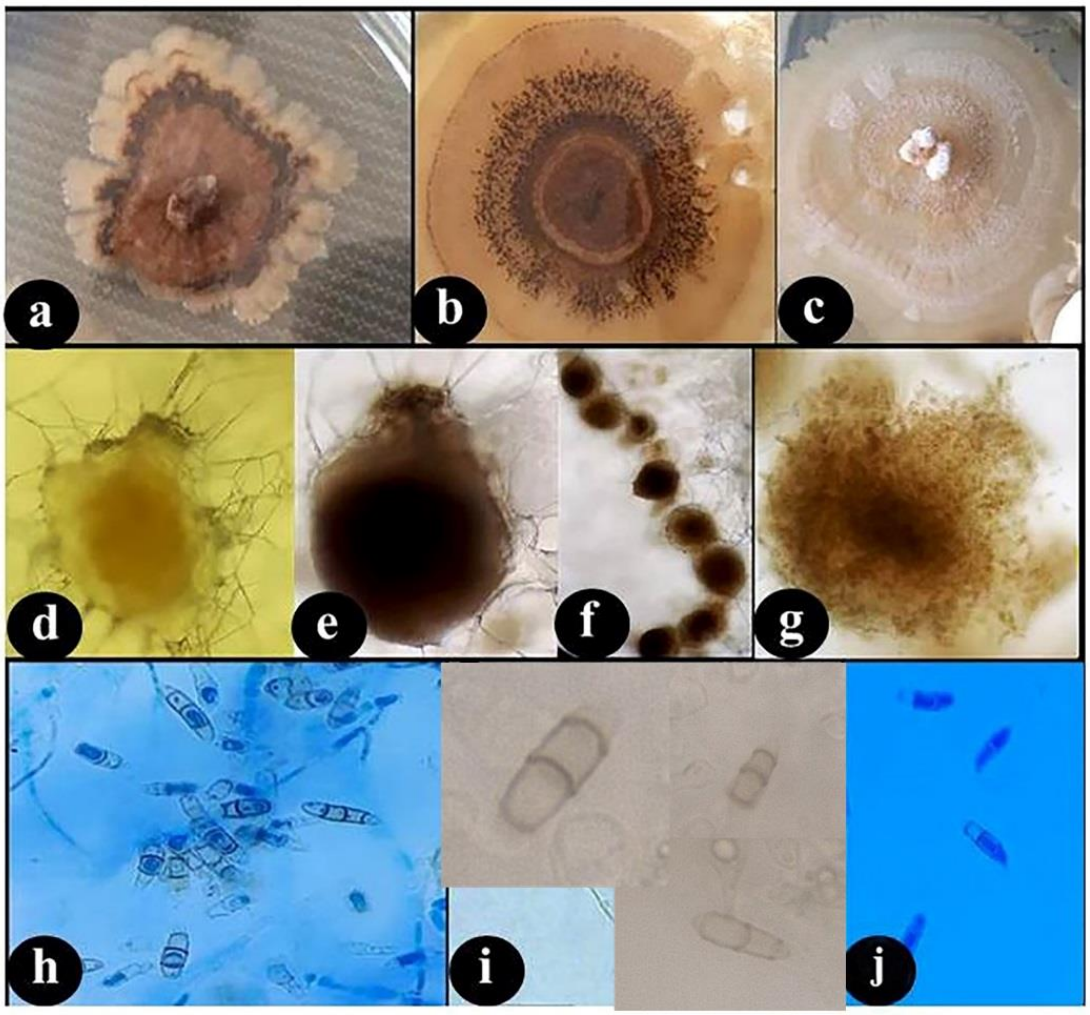

Fig 2. *Seimatosporium vitis:* Colonies on PDA (a-c), Pycnidia on PDA at 25°C in the dark conditions (d-g) and Conidia (h-j).

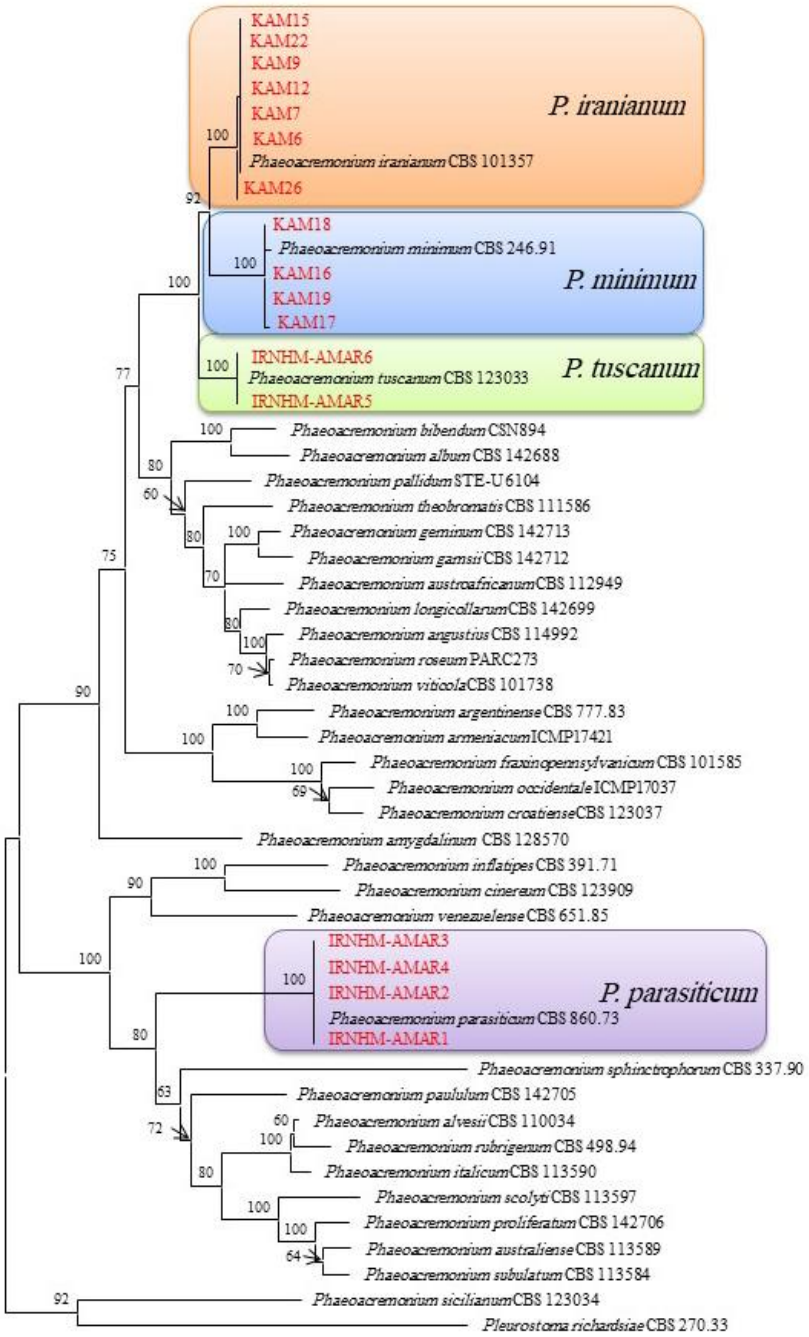

Fig 3. Maximum likelihood tree generated based on beta-tubulin (BT) gene sequences data for *Phaeoacremonium* isolates. Bootstrap values are given at the nodes. The tree was rooted by *Pleurostoma richardsiae* (CBS 270.33). Iranian isolates indicated by red color in each colored rectangle.

In this study, *P. minimum* was the dominant *Phaeoacremonium* species obtained from diseased grapevines. This is in agreement with the previous studies on grapevine in France (Larignon & Dubos 1997), Italy (Mugnai et al. 1999), Australia (Pascoe & Cottral 2000), South Africa (Groenewald et al. 2001), Spain (Armengol et al. 2001), Argentina (Dupont et al. 2002), Chile (Auger et al. 2005) as well as Iran (Mohammadi & Banihashemi, 2007, Arabnezhad & Mohammadi, 2012. Mohammadi et al. 2013a, Arabnezhad et al. 2013). All of the four species of *Phaeoacremonium* isolated during this study have previously been reported from grapevines and other woody plants in various provinces of Iran. For instance, *P. minimum* has previously been reported affecting grapevines in Fars, Hamadan, Isfahan, Yazd, Kerman, Kohgiluyeh and Boirahmad, and West and East Azerbaijan provinces (Arabnezhad & Mohammadi, 2012, 2014, Arzanlou et al. 2013, Mohammadi et al. 2013a, Narmani et al. 2014, Faraji & Mohammadi, 2015). *Phaeoacremonium parasiticum* was associated with grapevine trunk diseases in Fars and Kerman provinces and stone and pome fruit trees

(Arzanlou et al. 2014, Sami et al. 2014, Soltaninejad et al. 2017), cypress (Mohammadi et al. 2014), date palm (Mohammadi, 2014), poplar trees (Hashemi & Mohammadi, 2016, Kazemzadeh Chakusary et al. 2017) and some other forest trees (Kazemzadeh Chakusary et al. 2017) in Iran. *Phaeoacremonium iranianum* was isolated and recorded from grapevines in Fars and South Khorasan and also from alder (Kazemzadeh Chakusary et al. 2017), hawthorn, quince (Sami et al. 2014), apple (Arzanlou et al. 2014, Sami et al. 2014) and pomegranate (Kazemzadeh Chakusary et al. 2017) trees in various provinces of this country. *Phaeoacremonium tuscanum* has previously been reported affecting grapevines in Kohgiluyeh and Boirahmad province (Arabnezhad & Mohammadi 2012) and peach trees in Kerman province (Soltaninejad et al. 2017). Therefore, the current study is the first report on the occurrence of these species on grapevine in Khorasan-Razavi province.

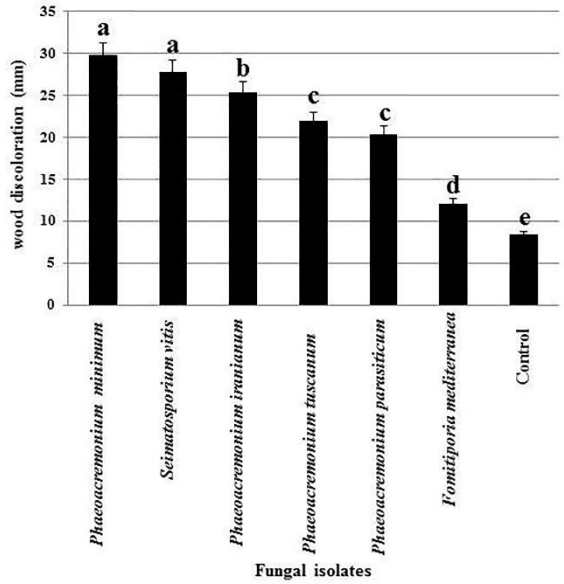

Fig 4. Wood lesion size produced by inoculated fungal species on rooted grapevine shoots, 10 months after inoculation. Means followed by the same letter are not significantly different (at $P < 0.05$) and bars represent standard error of the means.

Fomitiporia mediterranea was another fungal species that was isolated from grapevine in this study. Wood-rotting basidiomycete fungi, including *Fomitiporia* spp., are known as grapevine trunk pathogens (Bertsch et al. 2013, Brown et al. 2019). *Fomitiporia mediterranea* introduced by Fischer (2002) and as a fungal trunk pathogen on the grapevine is widespread in the Mediterranean area (Fischer & Kassemeyer 2003). The importance of this basidiomycete as a fungal trunk pathogen has been widely investigated (Mugnai et al. 1999, Fischer 2002, Fischer & Binder 2004). This fungus is the main wood-decaying pathogen in some wine-growing areas of southern Italy (Ciccarone et al. 2004) and Greece (Markakis et al. 2017). In Iran, this taxon has been previously reported to affect grapevines in Northern Khorasan province (Farashiyani et al. 2010, Rajaiyan et al. 2013), Russian olive (Ahmadyusefi Sarhadi & Mohammadi, 2019) and elm trees (Mirsoleymani & Mostowfizadeh Ghalamfarsa 2019) in Fars province. To the best of our knowledge, this study is the first report of the occurrence of *F. mediterranea* on grapevine in Khorasan-Razavi province.

The genus *Seimatosporium* Corda, with *S. rosae* Corda as the type species, was first described and introduced by Corda (1833). Various *Seimatosporium* species have been isolated and reported from symptomatic or dead parts of grapevines in different countries (Diaz et al. 2012, Vaczy 2017, Farr & Rossman, 2018, Lawrence et al. 2018, Camele & Mang, 2019, Liu et al. 2019). In this study, 48 isolates of *S. vitis* were obtained from necrotic wood tissues of grapevines in Khorasan-Razavi province. This species has also been reported from symptomatic grapevines in Hungary (Vàczy 2017), North Carolina, USA (Lawrence et al. 2018) and Italy (Camele & Mang, 2019). To date, there has only been one report of the sexual morph of *S. vitis* on a dead branch of *Vitis* sp. in Iran (Mehrabi et al. 2017). Therefore, this study is the first report of this species from necrotic wood tissues of grapevine in Iran.

The results of the pathogenicity tests showed that all the inoculated species on the rooted cuttings of the grapevines were pathogenic and produced wood discoloration on inoculated shoots, developing from the point of inoculation. *Phaeoacremonium minimum* showed the longest lesions and were the most virulent species on the rooted cuttings of the grapevines. The findings of this study are in accordance with the results previously reported by other researches on grapevine (Feliciano et al. 2004, Halleen et al. 2007, Aroca & Raposo 2009, Gramaje et al. 2010). Mohammadi et al. (2013a) reported *P. minimum* causing significantly longer lesions than the other *Phaeoacremonium* species tested on grapevine shoots under field conditions in Iran. Similar results were also reported for this fungus on green shoots of grapevine under greenhouse conditions in this country (Mohammadi et al. 2014). Our findings, for the first time in Iran, indicated that *S. vitis* is involved in the grapevine trunk-disease complex. Relatively, little is known regarding the importance of this species on the grapevine. However, pathogenicity tests in Hungary (Vaczy 2017), Italy (Camale & Mang 2019) and the USA (Lawrence et al. 2018) have also shown that *S. vitis* can be considered as a fungal trunk pathogen on the grapevine. Overall, all the fungal species were identified in this study have previously been isolated and reported from grapevine in other provinces of Iran, with the exception of *S. vitis*, which was reported here for the first time associated with wood necrosis of grapevine in this country. Further research is required to provide a better understanding of the role of various fungal species on grapevine GTDs in Iran, especially Khorasan-Razavi province.

REFERENCES

Abed–Ashtiani F, Narmani A, Arzanlou M. 2019. Analysis of Kalmusia variispora associated with grapevine decline in Iran. European Journal of Plant Pathology 154: 787–799.

Ahmadyusefi Sarhadi F, Mohammadi H. 2019. Fomitiporia mediterranea and Phaeoacremonium rubrigenum associated to Russian olive trunk diseases in Iran. 4th Iranian Mycological Congress, 26–28 August 2019, Sari Agricultural Sciences and Nutural Resources University, Sari, Iran. P. 104.

Arabnezhad M, Mohammadi H, Massumi H, Farahmand F. 2013. Fungal flora associated with internal symptoms of grapevine decline in Kerman province. Journal of Agricultural Biotechnology 6: 115–132.

Arabnezhad M, Mohammadi H. 2012. Study of esca and Petri disease of grapevine in Kerman province. Iranian Journal of Plant Pathology 48: 277–283.

Arabnezhad M, Mohammadi H. 2014. Detection of Phaeoacremonium tuscanum and Botryosphaeriaceae species associated with grapevine decline in Iran and the potential role of pruning debris on the survival of the pathogens. Iranian Journal of Plant Pathology 49: 139–148.

Ariyawansa HA, Hyde KD, Jayasiri SC. 2015. Fungal diversity notes 111–252: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 75: 27–274.

Armengol J, Vicent A, Torne L, García–Figueres F, García–Jiménez J. 2001. Fungi associated with esca & grapevine declines in Spain: a three–year survey. Phytopathologia Mediterranea 40: 325–329.

Aroca A, Raposo R. 2009. Pathogenicity of Phaeoacremonium species on grapevines. Journal of Phytopathology 157: 413–419.

Arzanlou M, Moshari S, Salari M, Badali H. 2013. Molecular characterization and pathogenicity of Phaeoacremonium spp. associated with esca disease of grapevine in Northern Iran. Archive of Phytopathology and Plant Protection 46: 375–388.

Arzanlou M, Narmani A, Khodaei S, Moshari S. 2014. Pome and stone fruit trees as possible reservoir hosts for Phaeoacremonium spp., the causal agents of grapevine esca disease, in Iran. Archives of Phytopathology and Plant Protection 47: 717–727.

Auger J, Pérez I, Esterio M. 2005. Fungi associated with grapevine wood decay & young vine decline in Chile. Phytopathologia Mediterranea 44: 89–90.

Baumgartner K. 2013. Susceptibility of cultivated and wild Vitis spp. to wood infection by fungal trunk pathogens. Plant Disease 97: 1529–1536.

Bertsch CM, Ramírez-Suero M, Magnin-Robert P, Larignon J, Chong E, Abou-Mansour A, Spagnolo C, Clèment Fontaine, F. 2013. Grapevine trunk diseases: complex & still poorly understood. Plant Pathology 62: 243–265.

Brown AA, Lawrence DP, Baumgartner K. 2019. Role of basidiomycete fungi in the grapevine trunk disease esca. Frontiers in Plant Science 10: Doi: 10.1111/ppa.13116.

Bruez E, Baumgartner K, Bastien S, Travadon R, Guérin–Dubrana L, Rey P. 2016. Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. Australian Journal of Grape and Wine Research 22: 288–295.

Butterworth SC, Jorda, SA, Schilder AM. 2005. Eutypa dieback: disease progress and losses in 'Concord' grapes. Phytopathologia Mediterranea 44: 106

Camele I, Mang SM. 2019. First report of Seimatosporium vitis associated with grapevine trunk diseases on Vitis vinifera in Italy. Plant Disease 104: 771.

Carlucci A, Lops F, Cibelli F. 2015. Phaeoacremonium species associated with olive wilt and decline in southern Italy. European Journal of Plant Pathology 141: 717–729.

Ciccarone C, Graniti A, Schiaffino A, Marras F. 2004. Molecular analysis of Fomitiporia mediterranea isolates from esca–affected grapevines in southern Italy. Phytopathologia Mediterranea 43: 268–272.

Cloete M, Fischer M, Mostert L, Halleen F. 2015. Hymenochaetales associated with esca–related wood rots on grapevine with a special emphasis on the status of esca in South African vineyards. Phytopathologia Mediterranea 54: 299–312.

Corda ACJ. 1833. Deutschlands Flora, Abt. III. Die Pilze Deutschlands 3–13: 65–96.

Crous PW, Gams W, Wingfield MJ, van Wyk PS. 1996. Phaeoacremonium gen. nov. associated with wilt and decline disease of woody hosts and human infection. Mycologia 88: 786–796.

Crous PW, Wingfield MJ, Burgess TI. 2016. Fungal planet description sheets: 469–557. Persoonia 37: 218–403.

Da Silva MA, Correia KC, Barbosa MAG, Câmara MPS, Gramaje D, Michereff SJ. 2017. Characterization of Phaeoacremonium isolates associated with Petri disease of table grape in Northeastern Brazil, with description of Phaeoacremonium nordesticola sp. nov. European Journal of Plant Pathology. DOI 10.1007/s10658–017–1219–4.

Diaz GA, Elfar K, Latorre BA. 2012. First report of Seimotosporium botan associated with trunk disease of grapevine (Vitis vinifera) in Chile. Plant Disease 96: 1696–1696.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Dumot V, Menard E, Courlit Y, Ouvrie M, Desache F, Boursier N, David S, Dubos B, Larignon P. 2004. Eutypa canker in the Charentes region: results of a 10–year study on Ugni blanc. Phytopathology 568: 4–7.

Dupont J, Magnin S, Césari C. 2002. ITS and β–tubulin markers help delineate Phaeoacremonium

species, and the occurrence of P. parasiticum in grapevine disease in Argentina. Mycological Research 106: 1143–1150.

Edwards J, Pascoe IG. 2004. Occurrence of Phaeomoniella chlamydospora and Phaeoacremonium aleophilum associated with Petri disease & esca in Australian grapevines. Australasian Plant Pathology 33: 273–279.

Essakhi S, Mugnai L, Crous PW, Groenewald JZ, Surico G. 2008. Molecular and phenotypic characteri-zation of novel Phaeoacremonium species associated with Petri disease and esca of grapevine. Persoonia 21: 119–134.

Faraji F, Mohammadi H. 2015. Endophytic fungi associated with healthy and diseased wood tissues of grapevines in Yazd province. 2nd Iranian Mycological Congress, 23–25 August 2015, University of Tehran, Karaj, Iran. P. 172.

Farashiyani A, Mousavi Jorf SA, Karimi MR. 2012. Study of esca disease of grapevine in Bojnourd. Iranian Journal of Plant Pathology 48: 47–49.

Farr DF, Rossman AY. 2016. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved January 11, 2015, from http://nt.ars−grin.gov/fungaldatabases/.

Feliciano AJ, Eskalen A, Gubler WD. 2004. Differential susceptibility of three grapevine cultivars to Phaeoacremonium aleophilum and Phaeomoniella chlamydospora in California. Phytopathologia Mediterranea 43: 66–69.

Fischer M, Binder M. 2004. Species recognition geographic distribution and host–pathogen relationships: a case study in a group of lignicolous Basidiomycetes, Phellinus s.l. Mycologia 96: 799–811.

Fischer M, Kassemeyer HH. 2003. Fomitiporia mediterranea, a new basidiomycete species associated with esca of grapevine in Europe: biology, ecology and distribution. Phytopathologia Mediterranea 43: 144 (abstract).

Fischer M. 2002. A new wood–decaying basidiomycete species associated with esca grapvine: Fomitiporia mediterenea (Hymenochaetales). Mycological Progress 1: 315–324.

Fleurat–Lessard P, Luini E, Berjeaud JM, Roblin G. 2010. Diagnosis of grapevine esca disease by immunological detection of Phaeomoniella chlamydospora. Australian Journal of Grape and Wine Research 16: 455–463.

Glass N, Donaldson G. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61: 1323–1330.

Graham AB, Johnston PR, Weir BS. 2009. Three new Phaeoacremonium species on grapevines in New Zealand. Australasian Plant Pathology 38: 505–513.

Gramaje D, Aguilar MI, Armengol J. 2011. First report of Phaeoacremonium krajdenii causing Petri disease of grapevine in Spain. Plant Disease 95: 615.

Gramaje D, Aroca A, Raposo R, Garcia–Jiminez J, Armengol J. 2009b. Evaluation of fungicides to control Petri disease pathogens in the grapevine propagation process. Crop Protection 28: 1091–1097.

Gramaje D, Baumgartner K, Halleen F, Mostert L, Sosnowski, MR, Úrbez–Torres JR, Armengol J. 2016. Fungal trunk diseases: a problem beyond grapevines? Plant Pathology 65: 355–356.

Gramaje D, Di Marco S. 2015. Identifying practices likely to have impacts on grapevine trunk disease infections: a European nursery survey. Phytopathologia Mediterranea 54: 313–324. http://doi.org/10.14601/Phytopathol.

Gramaje D, García–Jiménez J, Armengol J. 2010. Grapevine rootstock susceptibility to fungi associated with Petri disease and esca under field conditions. American Journal of Enology and Viticulture 61: 512–520.

Gramaje D, Urbez–Torres JR, Sosnowski MR. 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Disease 102: 12–39. http://doi.org/10.1094/ PDIS–04–17–0512–FE.

Groenewald M, Kang J–C, Crous PW. 2001. ITS and β–tubulin phylogeny of Phaeoacremonium and Phaeomoniella species. Mycological Research 105: 651–657.

Guerin–Dubrana L, Fontaine F, Mugnai L, 2019. Grapevine trunk disease in European and Mediterranean vineyards: occurrence, distribution and associated disease–affecting cultural factors. Phytopathologia Mediterranea 58: 49–71.

Hall T. 2006. Bioedit 7.5.0.3. Department of Microbiology, North Carolina State University. http://www.mbio.ncsu.edu/BioEdit/

Halleen F, Mostert L, Crous PW. 2007. Pathogenicity testing of lesser–known vascular fungi of grapevines. Australasian Plant Pathology 36: 277–285.

Hashemi H, Mohammadi H. 2016. Identification and characterization of fungi associated with internal wood lesions and decline disease of willow & poplar trees in Iran. Forest Pathology 46: 341–352.

Karimi MR, Mahmoudi B, Kazemiyan M. 2001. First report of Esca of grapevine in Iran. Phytopathologia Mediterranea 40(supplement): S481.

Kazemzadeh Chakusary M, Mohammadi H, Khodaparast SA. 2017. Decline–associated Phaeoacremonium species occurring on forest trees in the north of Iran. Forest Pathology 47: 1–15.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35: 1547–1549.

Larignon P, Dubos B. 1997. Preliminary studies on the biology of Phaeoacremonium. Phytopathologia Mediterranea 39: 184–189.

Lawrence DP, Travadon R, Baumgartner K. 2018. Novel Seimatosporium species from grapevine in northern California and their interactions with fungal pathogens involved in the trunk–disease complex. Plant Disease 102: 1081–1092.

Liu Z, Liu X , Dong A, Deng S. 2019. First report of Seimatosporium falcatum causing branch and leaf spots of Viscum coloratum in China. Journal of Plant Diseases and Protection 126: 265–267.

Markakis EA, Kavroulakism, Nektarios, Ntougias, Spyridon, Koubouris, Georgios C, Sergentani, Chrysi K, Ligoxigakis, Eleftherios K. 2017. Characterization of fungi associated with wood decay of tree species and grapevine in Greece. Plant Disease 101: 1929–1940.

Mehrabi M, Hemmati R, Abdollahzadeh J. 2017. Description of the sexual morph of Seimatosporium vitis. Cryptogamie Mycologie 38: 3–11.

Mirsoleymani Z, Mostowfizadeh Ghalamfarsa R. 2019. Fomitiporia mediterranea, a new basidiomycete species for mycobiota of Iran. Rostaniha 19: 189–191.

Mohammadi H, 2012. First report of Phaeoacremonium tuscanum associated with grapevine decline disease in Iran. New Disease Reports 25: 21. http://dx.doi.org/10.5197/j.2044–0588.2012.025.021.

Mohammadi H, Banihashemi Z. 2007.Grapevine decline in Fars province. Iranian Journal of Plant Pathology 43: 294–310.

Mohammadi H, Banihashemi Z, Alaniz S, Armengol J. 2009. Characterization of Cylindrocarpon liriodendri associated to grapevine black foot disease in Iran. Journal of Phytopathology 157: 642–645.

Mohammadi H, Banihashemi Z, Gramaje D, Armengol J. 2013a. Fungal pathogens associated with grapevine trunk diseases in Iran. Journal of Agricultural Science and Technology 15:137–150.

Mohammadi H, Banihashemi Z, Gramaje D, Armengol J. 2013b. Characterization of Diplodia seriata and Neofusicoccum parvum associated with grapevine decline in Iran. Journal of Agricultural Science and Technology 15: 603–616.

Mohammadi H. (2014). Phaeoacremonium spp. and Botryosphaeriaceae spp. associated with date palm (Phoenix dactylifera L.) decline in Iran. Journal of Phytopathology 162: 575–581.

Mohammadi H, Kazemi S, Frahmand H. 2014. Phaeoacremonium and Botryosphaeriaceae species associated with cypress (Cupressus sempervirens L.) decline in Kerman province (Iran). Phytopathologia Mediterranea 53: 27–39.

Mostert L, Groenewald JZ, Summerbell RC. 2006. Taxonomy and pathology of Togninia (Diaporthales) and its Phaeoacremonium anamorphs. Studies in Mycology 54: 1–115.

Mugnai L, Graniti A, Surico G. 1999. Esca (black measles) and brown wood streaking: Two old and elusive diseases of grapevines. Plant Disease 83: 404–418.

Mullins MG, Bouquet A, Williams LA. 1992. Biology of the grapevine. G. Mullins, ed. Cambridge University Press, Cambridge, U.K.

Narmani A, Arzanlou M, Babai–Ahari A. 2014. Induction of sexual reproduction and determination of mating types in Phaeoacremonium aleophilum, the causal agent of esca disease of grapevine in East Azerbaijan province. Iranian Journal of Plant Pathology 50: 281–289.

Narmani A, Arzanlou M. 2017. Grapevine Esca disease. Plant Pathology Science 6: 12–21.

Pascoe I, Cottral E. 2000. Developments in grapevine trunk diseases research in Australia. Phytopathologia Mediterranea 39: 68–75.

Phillips AJL. 2000. Excoriose, cane blight and related diseases of grapevines: A taxonomic review of the pathogen. Phytopathologia Mediterranea 39: 341–356.

Pintos Varela C, Redondo Fernandez V, Aguin Casal O, Ferreiroa Martinez V, Mansilla Vazquez JP. 2016. First report of Pleurostoma richardsiae causing grapevine trunk disease in Spain. Plant Disease 100: 2168.

Pitt WM, Trouillas FP, Gubler WD, Savocchia S, Sosnowski MR. 2013. Pathogenicity of diatrypaceous fungi on grapevines in Australia. Plant Disease 97: 749–756.

Raimondo ML, Carlucci A, Ciccarone C, Sadallah A, Lops F. 2019. Identification and pathogenicity of lignicolous fungi associated with grapevine trunk diseases in southern Italy. Phytopathol Mediterr. 58: 639–662.

Raimondo ML, Lops F, Carlucci A. 2014. Phaeoacremonium italicum sp. nov, associated with esca of grapevine in southern Italy. Mycologia 106: 1119–1126.

Rajaiyan M, Karimi Shahri MR, Pirnia M, Pirnia M, Dehvari V, Ghezel Sefloo N. 2013. Detection and identification of some fungal agents causing grapevine decline in Northern Khorasan province vineyards using PCR technique. Applied Entomology and Phytopathology 81: 167–177.

Sami S, Mohammadi H, Heydarnejad J. 2014. Phaeoacremonium species associated with necrotic wood of pome fruit trees in Iran. Journal of Plant Pathology 96: 487–495.

Senanayake I, Maharachchikumbura C, Hyde SSN, Bhat KD, Gareth–Jones JD, McKenzie EB, Dai EHC, Daranagama DQ, Dayarathne DA, Goonasekara MC, Konta ID, Li S, Shang WJ, Stadler QJ, Wijayawardene M, Xiao NN, Norphanphoun YP, Li C, Liu Q, Bahkali XZ, Kang AH, Wang JC, Wen Y, Wendt TC, Xu LJC, Camporesi E. 2015. Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). Fungal Diversity 73: 73–144.

Sigler L, Abbott SP.1997. Characterizing and conserving diversity of filamentous basidiomycetes from human sources. Microbiology and Culture Collections 13: 21–27.

Soltaninejad N, Mohammadi H, Massumi H. 2017. Isolation, identification and pathogenicity of

Botryosphaeriaceae and Phaeoacremonium species associated with decline of Prunus species in Iran. Journal of Plant Pathology 99: 571–581.

Sosnowski M, Ayres M, Scott E. 2016. The influence of water deficit on grapevine trunk disease. Wine & Viticulture Journal 31: 46–50.

Spies CFJ, Moyo P, Halleen F, Mostert L. 2018. Phaeoacremonium species diversity on woody hosts in the Western Cape province of South Africa. Persoonia 40: 26–62.

Thompson JD, Higgins DG, GibsonTJ. 1994. Clustral W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

Travadon R, Lawrence DP, Rooney–Latham S, Gubler WD, Wilcox WF, Rolshausen PE, Baumgartner K. 2015. Cadophora species associated with wood decay of grapevine in North America. Fungal Biology 119: 53–66.

Trouillas FP, Gubler WD. 2010. Pathogenicity of Diatrypaceae species in grapevines in California. Plant Disease 94: 867–872.

Trouillas FP, U´rbez–Torres JR, Gubler WD. 2010. Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. Mycologia 102: 319–336.

Úrbez –Torres JR, Peduto F, Smith RJ, Gubler WD. 2013. Phomopsis dieback: A grapevine trunk disease caused by Phomopsis viticola in California. Plant Dis. 97:1571–1579.

Úrbez–Torres JR, Gubler WD. 2011. Susceptibility to grapevine pruning wounds to infection by Lasiodiplodia theobromae and Neofusicoccum parvum. Plant Pathology 60: 261–270.

Vaczy KZ. 2017. First report of Seimatosporium vitis associated with grapevine trunk disease symptoms in Hungary. Plant Disease 101: 253–254.

Van Niekerk JM, Crous PW, Groenewald JZ, Fourie PH, Halleen F. 2004. DNA phylogeny, morphology and pathogenicity of Botryosphaeria species on grapevines Mycologia 96: 781–798.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: "PCR protocols, a guide to methods and applications". Innis MA, Gelfand DH, Sninsky JJ, White TJ. (Eds.). Academic Press, San Diego, CA, USA, pp. 315–322.

Wijayawardene NN, Hyde KD, Wanasinghe DN, Papizadeh M,Goonasekara ID, Camporesi E, Bhat DJ, McKenzie EHC, Phillips AJL, Diederich P, Tanaka K, Li WJ, Tangthirasunun N, Phookamsak R, Dai D–Q, Dissanayake AJ, Weerakoon G, Maharachchikumbura SSN, Hashimoto A, Matsumura M, Bahkali AH, Wang Y. 2015. Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Diversity 77: 1–316.

Wilcox WF, Gubler WD, Uyemoto JK. 2015. Compendium of Grape Diseases, Disorders, and Pests, 2nd Ed. American Phytopathological Society.

Xie L, Huang SL, Cen ZL, Lu WH, Qin BX, Tang CG, Hu CJ, Qin LP. 2010. First report of Botryosphaeria dothidea causing sweet Osmanthus leaf dieback in China. Agricultural Sciences in China 9: 847–853.

Yang T, Groenewald JZ, Cheewangkoon R, Jami F, Abdollahzadeh J, Lombard L, Crous PW. 2017. Families, genera, and species of Botryosphaeriales. Fungal Biology 121: 322–346.

شناسایی و بیماری‌زایی گونه‌های قارچی همراه با بیماری‌های شاخه و تنه انگور در استان خراسان رضوی، ایران

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چکیده: بیماری‌های شاخه و تنه انگور (GTDs) به عنوان یکی از شاخص‌های مهم کاهش میزان محصول شناخته می شوند که باعث ایجاد مشکلات اقتصادی قابل توجهی در انگور در دنیا می‌شوند. در سال‌های ۱۳۹۵ تا ۱۳۹۷، برای مطالعه گونه‌های قارچی همراه بیماری‌های شاخه و تنه انگور، بازدیدهای متعددی از تاکستان‌های استان خراسان رضوی به عمل آمد. در این مطالعه، از شاخه و تنه درختان با نشانه‌های زردی، کاهش رشد، سرخشکیدگی و تغییر رنگ بافت چوب در برش‌های عرضی نمونه برداری گردید. در این بررسی، ۲۵۸ جدایه قارچی بدست آمد و بر اساس ویژگی‌های ریخت شناختی و مقایسه داده‌های حاصل از تعیین ترادف DNA (ناحیه ITS و بخشی از ژن *β–tubulin*) مورد شناسایی قرار گرفتند. این جدایه‌ها به عنوان *Phaeoacremonium minimum* (۷۵ جدایه)، *P. parasiticum* (۱۹ جدایه)، *P. iranianum* (۵۲ جدایه)، *P. tuscanum* (هشت جدایه)، *Fomitiporia mediterranea* (۵۶ جدایه) و *Seimatosporium vitis* (۴۸ جدایه) شناسایی شدند. آزمون بیماری‌زایی جدایه های انتخاب شده روی شاخه انگورهای کشت شده در گلدان مورد تایید قرار گرفت. بر اساس میانگین اندازه طول لکه‌های ایجاد شده در بافت چوب، گونه‌های *P. minimum* و *F. mediterranea* به ترتیب دارای بیشترین و کمترین شدت بیماریزایی بودند. یافته‌های ما نشان می دهد که عوامل بیماری‌زای شناخته شده شاخه و تنه درختان همچون گونه‌های *Phaeoacremonium* و *F. mediterranea* روی انگور در استان خراسان رضوی قابل مشاهده می باشند. این بررسی اولین گزارش از *S. vitis* همراه با زوال انگور در ایران می‌باشد.

کلمات کلیدی: بیماری سرخشکیدگی، ریخت شناسی، تعیین ترادف، *Phaeoacremonium*، *Fomitiporia*، *Seimatosporium*

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