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Phaeoacremonium tuscanicum, a new fungal pathogen associated with oak decline in Zagros forests, Iran

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Abstract: An extensive survey on phylogeny and pathology of fungi associated with the oak decline in Zagros forests located in Western Azarbaijan, Kurdistan, Kermanshah, Lorestan, and Ilam provinces, a large number of collected fungal isolates seventeen isolates morphologically resembled the members of *Phaeoacremonium*. Similar DNA fingerprinting patterns were generated for all isolates using M13 primer. Thus, one representative isolate (IRAN 4348C) was analyzed based on *tub2* sequence data using maximum parsimony and neighbor-joining algorithms and identified as *Phaeoacremonium tuscanicum*. Pathogenicity was confirmed following Koch's postulates on two-year-old *Quercus brantii* seedlings under greenhouse conditions. To our knowledge, it is the first time *Ph. tuscanicum* is reported from oak trees and confirmed as a pathogenic fungal species on *Q. brantii* in the world.

Keywords: Morphology, phylogeny, pathogenicity, *tub2*

INTRODUCTION

Zagros forests characterized as a semi-arid habitat in the west of Iran are covered by different oak species, including *Quercus brantii* Lindl., *Q. libani* Oliv. and *Q. infectoria* Oliv. (Heydari et al. 2013). In the last two decades, climate changes, drought and biotic agents (fungal pathogens and pests) have affected Zagros forests vegetation and dieback and decline of oak trees are the most serious disease symptoms spreading throughout the Zagros Mountains. The genus *Phaeoacremonium* belongs to the family *Togninaceae* (order *Togniniales*, Ascomycota), containing some 70 species (May 2020, www.indexfungorum.org) is a well-known genus associated with decline and dieback symptoms in different woody plants across the world (Farr & Rossman 2020). *Phaeoacremonium* species are known as the vascular plant pathogens causing wilt and dieback of woody hosts (Mohammadi 2012, Hashemi et al. 2017, Kazemzadeh Chakusary et al. 2017, Spies et al. 2018, Sohrabi et al. 2020). Moreover, *Phaeoacremonium* species are associated with humans, larvae of bark beetles (Mostert et al. 2006) and soil (Crous & Gams 2000, Dupont et al. 2002). This research was conducted to study the fungi associated with the oak decline in Zagros forests located in West Azarbaijan, Kurdistan, Kermanshah, Lorestan and Ilam provinces.

MATERIALS AND METHODS

During July and September 2017, samples of stems and twigs of oak trees showing dieback and decline symptoms were collected. Small pieces of infected woods showing a discoloration of xylem tissues were sterilized in 70% ethanol for 3 min, followed by washing with sterile distilled water and dried on sterile filter paper. The wood segments were plated out on potato dextrose agar (PDA, Quelab) supplemented with 100 mg/L streptomycin sulphate and ampicillin and incubated at 25°C. Fungal isolates were purified using the hyphal tip or single spore techniques on Water Agar (WA, Quelab). Fungal isolates were incubated on PDA, Oatmeal Agar (OA; 30 g boiled grinded oat, 15 g agar, 1 L distilled water) and Malt Extract Agar (MEA 2%, Quelab) at 25°C in the darkness and morphological and cultural characteristics were recorded after 8 and 16 days (Mostert et al. 2006, Essakhi et al. 2008). Type of phialids, structures and dimensions of conidiophores, and conidia recorded in 100% lactic acid. Genomic DNA of pure cultures was extracted according to the modified Raeder and Broda (1985) method as described by Abdollahzadeh et al. (2009). In this study,

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seventeen isolates with *Phaeoacremonium* morphology were obtained. Based on the ISSR fingerprinting patterns generated with M13 primer (data not shown) all isolates belonged to the same species. Therefore, one isolate was selected for phylogenetic analysis and deposited in the culture collection (IRAN) of the Iranian Research Institute of Plant Protection (Tehran, Iran). For molecular identification, partial β-tubulin gene (*tub2*) was amplified using primers T1 (5´-AACATGCGTGA GATTGTAAG-3´) (O'Donnell & Cigelnik 1997) and Bt2b (5´-ACCCTCAGTGTAGTGACCCTTGGC-3´) (Glass & Donaldson 1995). The PCR reaction mixtures were prepared in 25 µL volume containing 1×PCR buffer, 3 mM MgCl2, 200 µM of each nucleotide, 5 pmol of each primer, 1 U of *Taq* polymerase and 1 µL of template DNA (50–100 ng/µL). The PCR conditions were: primary denaturation step of 5 min at 94°C followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 90 s, with a final extension at 72°C for 7 min. The PCR products were purified and sequenced by BGI (China) via BMG (Bio Magic Gene) Co. (Karaj, Iran). The generated sequence was checked and extracted in BioEdit v. 7.0.0 (Hall 2004) and submitted to GenBank. Our isolate sequence, together with 26 *Phaeoacremonium* species sequences retrieved from GenBank and the outgroup species *Pleurostoma richardsiae* CBS 270.33, aligned with Clustal X v. 1.83 (Thompson et al. 1997). Phylogenetic analyses based on maximum parsimony and neighbor-joining algorithms were performed in PAUP v. 4.0b10 (Swofford 2003) as followed by Abdollahzadeh et al. (2009).

A pathogenicity test was performed by inoculation of two-year-old *Q. brantii* seedlings under greenhouse conditions. Seedlings were wounded and inoculated with mycelium plugs from actively-growing cultures on PDA and controls were inoculated with sterile PDA plugs.

RESULTS AND DISCUSSION

According to *tub2* phylogeny, isolate IRAN 4348C (GenBank accession no.: MZ547665) clustered in a clade containing an ex-type strain of *Ph. tuscanicum* (Fig. 1). So far, *Ph. tuscanicum* has been reported from *Juglans regia* (Iran), *Prunus persica* (Iran), *Vitis* sp. (Spain) and *Vitis vinifera* (Iran, Italy and Spain) (Farr & Rossman, 2020).

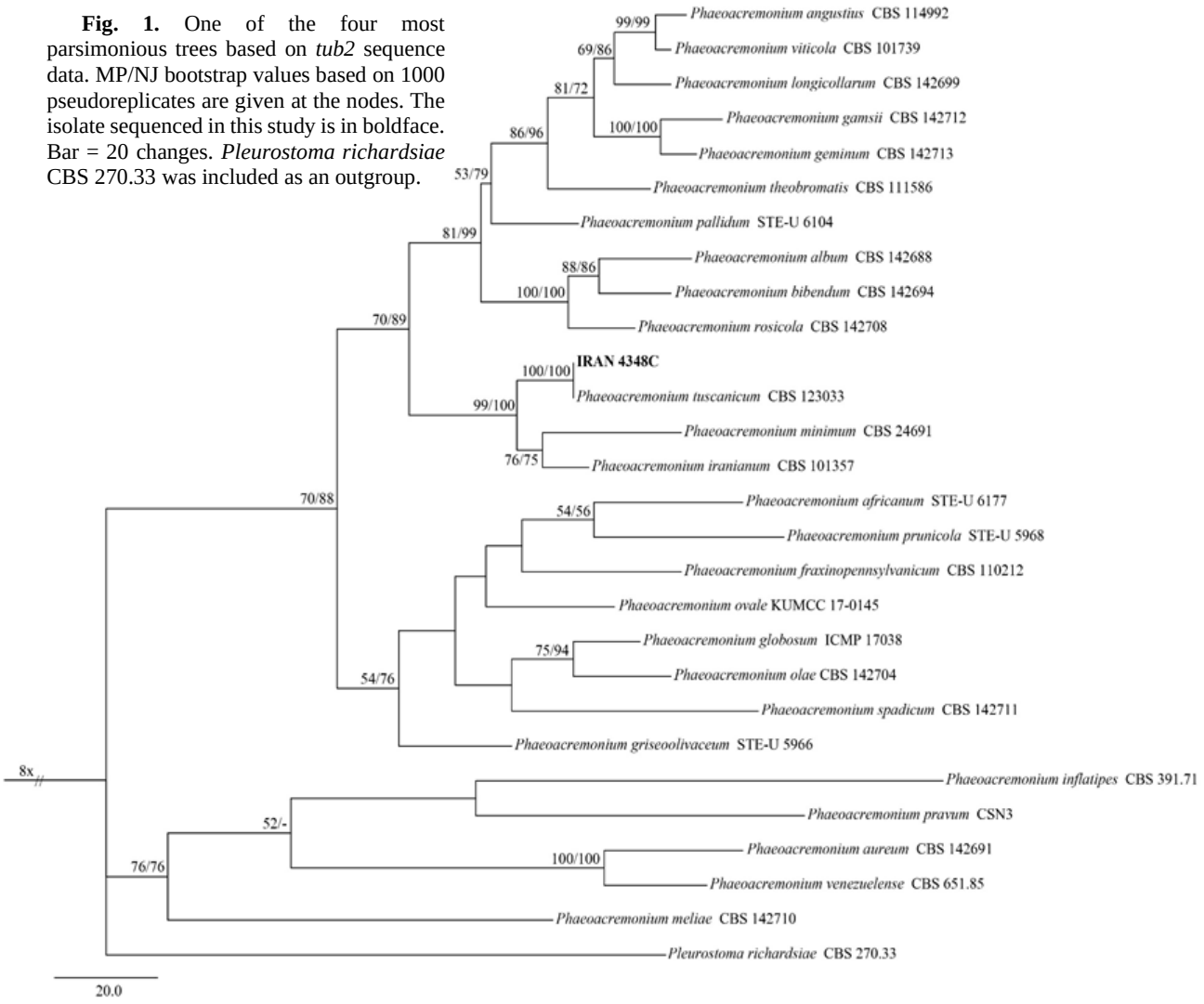

Fig. 1. One of the four most parsimonious trees based on *tub2* sequence data. MP/NJ bootstrap values based on 1000 pseudoreplicates are given at the nodes. The isolate sequenced in this study is in boldface. Bar = 20 changes. *Pleurostoma richardsiae* CBS 270.33 was included as an outgroup.

Pheoacremonium tuscnicum Essakhi, Mugnai, Surico & Crous, Persoonia 21; 119–134 (2008); Fig 2

Colonies on PDA reaching 12 mm within 7 days at 25°C, cottony and flat, with entire margin, after 16 days smoke gray to pale olivaceous gray to buff toward the margin above, reverse dark beige olive; on MEA 10 mm within 7 days at 25°C, cottony and flat, with an entire margin above, after 16 days olivaceous gray to buff toward the margin above, reverse orange; on OA 8 mm within 7 days at 25°C, appressed, fluffy in the middle with entire margin, after 16 days yellowish orange to buff toward margin above, reverse yellowish. Mycelium yellowish to pale brown, septate, branched, single or with a bundle of hyphae. Conidiophores rarely long and branched, straight, simple, septate, pale brown to sub-hyaline, (16–)20–26(–28.6) × (1.21–)1.4–1.9(–2.12) µm (mean 22.62 × 1.65 µm). Phialides mostly lateral and monophialidic, type I phialides subcylindrical, broad at the base, (3.2–)4–15(–17.8) × (1.24–)1.3–2(–2.01) µm (mean 9.17 × 1.65 µm); type II phialides narrowed at the base, widened in the middle part and tapering toward the tip, (8.03–)10–15(–18.23) × (1.2–)1.4–2(–2.16) µm (mean 12.35 × 1.73 µm); type III phialides subcylindrical, (18.3–)19–23(–24.2) × (1.67–)1.8–2(–3) µm (mean 22.62 × 2 µm). Conidia unicellular, hyaline, allantoid or sub-cylindrical, (2.01–)2.5–3.7(–5.37) × (0.8–)0.9–1.3(–2.03) µm (mean 2.94 × 1.18) µm.

Notes. Isolates collected in this study were obtained from Kurdistan (4 isolates from *Q. brantii* and *Q. libani*), Kermanshah (4 isolates from *Q. brantii*), Lorestan (6 isolates from *Q. brantii*) and Ilam (3 isolates from *Q. brantii*) provinces. Sequenced isolate (IRAN 4348C) was collected from Lorestan Province (N33 39.404 E48 16.855).

In the pathogenicity test, after 2 months, necrosis appeared on the leaves of inoculated seedlings. Brown lesions expanded upwards and downwards of the inoculated point on the stem internodes and no symptoms were observed on control seedlings (Fig. 3). To confirm the Koch's postulates, the pathogen was re-isolated from the lesions of seedlings. To our knowledge, it is the first time *Ph. tuscanicum* is reported from oak trees (*Q. brantii* and *Q. libani*) and confirmed as a pathogenic fungal species on *Q. brantii* in the world. It is important to study the pathogenicity of this new pathogen on *Q. libani* and *Q. infectoria*, the other two oak species growing in Zagros forests. Given that the phytotoxic metabolites have been characterized as pathogenicity determinants of fungi (e.g., *Phaeoacremonium aleophilum*) associated with the decline of woody plants (Evidente et al. 2000) to understand the interaction of *Ph. tuscanicum* and oak species in future studies, it is interesting to characterize the phytotoxic secondary metabolites of this fungal species and their role in pathogenicity on the host trees.

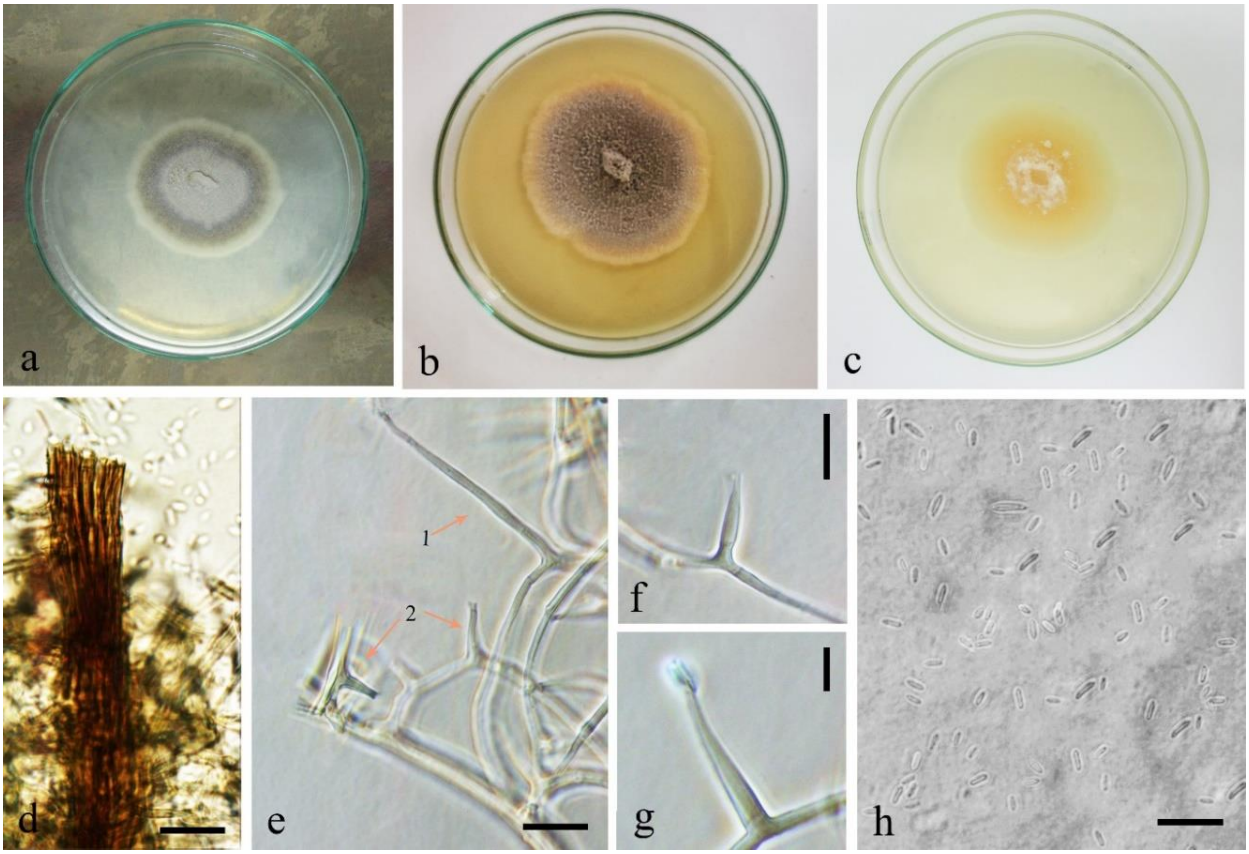

Fig. 2. Sixteen-day-old colonies of *Phaeoacremonium tuscanicum* on a. PDA; b. MEA 2%; c. OA at 25°C; d. Bundle of hyphae; e. (1) conidiophore, (2) phialides type I; f. phialid type II; g. phialide type III; h. conidia. Scale bars: d–f and h = 7 µm, g = 5 µm.

Fig. 3. Disease symptoms caused by *Phaeoacremonium tuscanicum* on oak seedling in greenhouse conditions. a. inoculated plant after 3 months; b. negative control; c. necrotic lesion expanded upwards and downwards of the inoculated point on the stem internodes.

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REFERENCES

Abdollahzadeh J, Goltapeh EM, Javadi A, Shams-Bakhsh M, Zare R, Phillips AJL. 2009. Barriopsis iraniana and Phaeobotryon cupressi: two new species of the Botryosphaeriaceae from trees in Iran, Persoonia 23: 1–8.

Crous PW, Gams W. 2000. Phaeomoniella chlamydospora gen. et comb. nov., a causal organism of Petri grapevine decline and esca. Phytopathologia Mediterranea 39: 112–118.

Dupont J, Magnin S, Césari C, Gatica M. 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.

Essakhi S, Mugnai L, Crous PW, Groenewald JZ, Surico G. 2008. Molecular and phenotypic characterization of novel Phaeoacremonium species isolated from esca diseased grapevines. Persoonia 21: 119–134.

Evidente A, Sparapano L, Andolfi A, Bruno G. 2000. Two naphthalenone pentaketides from liquid cultures of Phaeoacremonium aleophilum, a fungus associated with esca of grapevine. Phytopathologia Mediterranea 39: 162–168.

Farr DF, Rossman AY. 2020. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/.

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

Hall T. 2004. BioEdit version 7.0.0. Department of Microbiology, North Carolina State University.

Hashemi H, Mohammadi H, Abdollahzadeh J. 2017. Symptoms and fungi associated with elm trees decline in Iran. European Journal of Forest Research 136: 857–879.

Heydari M, Poorbabaei H, Rostami T, Begim-Faghir M, Salehi A, Ostad-Hashmei R. 2013. Plant species in oak (Quercus brantii Lindl.) understory and their relationship with physical and chemical properties of soil in different altitude classes in the Arghavan valley protected area, Iran. Caspian Journal of Environmental Sciences 11: 97–110.

Kazemzadeh Chakusary M, Mohammadi H, Khodaparast SA. 2017. Decline-associated Phaeoacremonium species occurring on forest trees in the north of Iran. Forest Pathology 47: e12368.

Mohammadi H. 2012. First report of Phaeoacremonium tuscanum associated with grapevine decline disease in Iran. New Disease Reports 25: 21.

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

O'Donnell K, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous, Molecular Phylogenetics and Evolution 7: 103–116.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1: 17–20.

Sohrabi M, Mohammadi H, León M, Armengol J, Banihashemi Z. 2020. Fungal pathogens associated with branch and trunk cankers of nut crops in Iran. European Journal of Plant Pathology 157: 327–351.

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.

Swofford DL. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

قارچ *Phaeoacremonium tuscanicum*، یک بیمارگر جدید مرتبط با زوال بلوط در جنگل‌های زاگرس ایران

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چکیده: در یک مطالعه وسیع جهت بررسی فیلوژنی و بیماری‌زایی قارچ‌های مرتبط با زوال درختان بلوط در جنگل‌های بلوط زاگرس واقع در استان‌های آذربایجان غربی، کردستان، کرمانشاه، ایلام و لرستان، تعداد زیادی جدایه قارچی جمع‌آوری شد که از میان آنها، هفده جدایه از نظر ریخت‌شناسی مشابه گونه‌های متعلق به جنس *Phaeoacremonium* بودند. الگوی اثرانگشت DNA ایجاد شده توسط آغازگر M13 در همه جدایه‌ها یکسان بود. از این رو، یکی از جدایه‌ها (IRAN 4348C) به عنوان نماینده جهت بررسی‌های فیلوژنتیکی براساس توالی *tub2* انتخاب شد و براساس آنالیزهای فیلوژنتیکی به گونه *Ph. tuscanicum* تعلق داشت. بیماری‌زایی جدایه مذکور بر اساس اصول کخ روی نهال‌های دو ساله *Quercus brantii* به اثبات رسید. براساس اطلاعات موجود، گونه *Ph. tuscanicum* برای اولین بار در دنیا از بلوط جداسازی و به عنوان گونه‌ای بیماری‌زا شناسایی و معرفی می‌شود.

کلمات کلیدی: ریخت شناسی، تبارزایی، بیماری‌زایی، بتاتوبولین

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