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Parasporendocladia bactrospora associated with canker and dieback of walnut trees (*Juglans regia* L.) in Iran

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Abstract: The walnut (*Juglans regia* L.) tree is considered one of the most important fruit trees in Iran. Decline disease of walnut trees is one of the significant problems that have been noticed in different regions of Kerman province (Southeastern Iran) during the last ten years. Therefore, a study was conducted to identify the fungal species associated with walnut trees showing trunk diseases between May 2015 and September 2016. Collected symptomatic wood samples were cut and surface disinfested with NaClO (Sodium hypochlorite) and then plated onto potato dextrose agar (PDA) plates. In this study, 21 isolates of a fungus were isolated from trees showing canker and dieback, and internal wood necrosis in the Bidkhun region of Kerman province. The obtained fungal isolates were first identified and grouped based on the most important morphological characteristics. Total genomic DNA was extracted and identification of the isolates was confirmed by sequence analysis of ITS (the internal transcribed spacer) using the primers ITS1 and ITS4. Based on the most important morphological characteristics and molecular data, these isolates were identified as *Parasporendocladia bactrospora*. This study is the first report of this species associated with canker and dieback symptoms on walnut trees worldwide and the first report of this fungus for the mycobiota of Iran.

Keywords: Dieback, Canker, ITS, *Juglandaceae*, Kerman province, Pathogenicity

INTRODUCTION

Walnut is one of the most important and well-known species in genus *Juglans* L. and the family *Juglandaceae*. The genus of *Juglans* includes 21 species, among which Persian walnut (*Juglans regia* L.) as an economically valuable crop, is grown in many countries. Walnut is an important nut fruit tree grown in the temperate climates and known as a significant source of omega-3 fatty acids and various minerals, and vitamins (Rana et al. 2007). Iran with 150,000 ha and 405,000 tons of walnut, is the third largest producer of this crop in the world (FAO 2021).

Walnut trees may be attacked by various fungal taxa. Species in the *Botryosphaeriaceae* (Li et al. 2016; Gusella et al. 2020), *Diaporthaceae* (Michailides et al. 2012; Chen et al. 2014; Meng et al. 2018), and *Diatrypaceae* families (Eichmeier et al. 2020) and some genera including *Phaeoacremonium* (Spies et al. 2018; Eichmeier et al. 2020), *Cytospora* (Fan et al. 2015; Zhao et al. 2018) and *Cadophora* (Eichmeier et al. 2020) have been reported as the main fungal trunk pathogens on walnut trees. Similar fungal taxa have also been reported from walnut trees in Iran. Several species in the *Botryosphaeriaceae* (Abdollahzadeh et al. 2013; Mohammadi et al. 2013) and *Diatrypaceae* families (Sohrabi et al. 2020), *Cytospora* spp. (Abbasi et al. 2012), *Phaeoacremonium* spp. (Mohammadi et al. 2013; Sohrabi et al. 2020), *Pleurostoma richardsiae*, *Graphium carbonarium* (Sohrabi & Mohammadi, 2020; Sohrabi et al. 2020) and *Cryptosphaeria pullmanensis* (Raoufi et al. 2016; Sohrabi et al. 2020) have previously been reported affecting walnut trees in Iran. These studies show that walnut trees represent a rich catch plant host for many fungal trunk pathogens.

Parasporendocladia bactrospora (W.B. Kendr.) W.P. Wu & Y.Z. Diao comb. nov. (≡ *Phialocephala bactrospora* W.B. Kendr., ≡ *Sporendocladia bactrospora* (W.B. Kendr.) M.J. Wingf.), is considered a wood-inhabiting ascomycetous fungus. This species (previously as *Phialocephala bactrospora*) was initially placed in the *Leptographium* complex (Kendrick 1961, 1963; Wingfield et al. 1987). The genus *Phialocephala* was initially used for fungi with dark conidiophores and conidiogenous head consisting of branches with 1-9 series of terminal phialides (Kendrick 1961). Then, five species of *Phialocephala* were transferred to

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Sporendocladia due to different type of conidiogenesis (Wingfield et al. 1987). In 2022, *Sporendocladia* was transferred to a new genus of *Parasporendocladia* which refers to its similarity to the genus *Sporendocladia*. Thus, *Parasporendocladia* as a new genus is introduced to accommodate *Phialocephala bactrospora*. Morphologically this genus is similar to *Custingophora*, *Sporendocladia*, and *Phialocephala*, while they are placed in different groups from each other in phylogenetic studies (Wu & Diao 2022). *Parasporendocladia* (previously as *Sporendocladia*) species are mostly considered saprophytes on dead plant materials (Kendrick 1961; Barbosa et al. 2009). However, studies have also demonstrated that some species can also be isolated from the wood of living trees (Luque et al. 2000; Jacobs et al. 2003; Roux et al. 2014). *Parasporendocladia bactrospora* (as *S. bactrospora*) was previously reported from some plant species, including *Tilia* sp. in England (Kendrick 1961), trunks of *Quercus suber* in Spain (Luque et al. 2000) and two *Populus* spp. (*P. trichocarpa* and *P. tremuloides*) in Canada (Grünig et al. 2002). Therefore, the role of this fungal species as a trunk pathogen of trees is still unknown. During a survey of walnut orchards throughout the main walnut production regions in Kerman province, some isolates resembling the *Parasporendocladia* species were obtained from trees showing disease symptoms. Therefore, the main objectives of the present study were to identify and characterize these isolates and to evaluate their pathogenicity on walnut

MATERIALS AND METHODS

Fungal isolation and morphological characterization

From May 2015 to September 2016, several field surveys were conducted throughout the main walnut production regions in Kerman province. Wood samples were collected from trunks and branches of diseased trees showing dieback and canker as well as discolored wood tissues in cross sections. All collected wood samples were transferred to the laboratory for further investigation and fungal isolation. In the laboratory, wood pieces (25-30 cm) were prepared from the collected samples showing disease symptoms. Fungal isolations were made from discolored wood tissues. Affected wood tissues were cut into small segments and surface-disinfected with 1.5% sodium hypochlorite solution (NaOCl) for two min, washed twice with sterile distilled water, and finally dried in sterile conditions. Disinfected pieces were plated on potato dextrose agar (PDA) plates (Merck, Germany) supplemented with 100 mg/L streptomycin sulpfate (PDAs). Culture plates were incubated at 25°C in the dark and all colonies were transferred to fresh PDA plates. Pure cultures were obtained from each isolate for further studies.

The initial identification of fungal isolates was made based on the colony and morphological characteristics of the fungal structures characterized by Wingfield et al. (1987). Isolates were transferred onto PDA slants and filter paper and then stored in the culture collection of the Department of Plant Protection of Shahid Bahonar University of Kerman (CCUK).

Molecular characterization and phylogenetic analysis

For DNA extraction, selected fungal isolates were grown on PDA and incubated at 25°C for 10-16 days. Produced mycelia were scraped from the surface of cultures and ground to a fine powder in liquid nitrogen. DNA extraction was made using the CTAB method (Doyle & Doyle 1990). DNA samples were visualized on 1.0% agarose gels (UltraPureTM Agarose; Invitrogen, Carlsbad), stained with ethidium bromide (50 µg/ml), and then kept at –18°C until used for PCR amplification.

For DNA sequence comparisons, two internal transcribed spacers (ITS1 and ITS2) and the intervening 5.8S nrDNA gene region were amplified by ITS1 and ITS4 primers (White et al. 1990). Each PCR reaction mixture (in 25 µL final volume) contained; 1µl of each primer, 0.2 µl *Taq* (5 u/µl), 2.5 µl dNTP (8mM), 1 µl of template DNA obtained from each isolate, 2.5 µl $MgCl_2$ (25mM) and 2.5 µl PCR buffer (10x). The polymerase chain reaction was performed in a Techne TC-312 Thermal Cycler (Techne, Cambridge, UK) according to the following program: one cycle of 3 min at 94°C as the initial denaturation period, 35 cycles of 30 s at 94°C, 30 s at 55 °C, 60 s at 72°C and one cycle of final extension for 10 min at 72°C as final extension period. From each PCR product 3-4 µL was separated using Electrophoresis method in 1.0 % agarose gel, stained with ethidium bromide. Produced products were visualized under ultraviolet (UV) light and a ladder (100-bp, GeneRuler 100 bp DNA Ladder, TMDNA Ladder Mix, Fermentas) was used to evaluate the size of produced bands. PCR products were sent to Bioneer Corporation (Daejeon, South Korea) for purification and sequencing. The sequences were edited with Sequencher software v. 1.8 (Gene Codes Corporation, Ann Arbor, MI), and run through the Basic Local Alignment Search Tool (BLAST), http://blast.ncbi.nlm. nih.gov/Blast.cgi) to determine their preliminary identity.

For phylogenetic studies, a single locus sequence related in our study and those references retrieved from GenBank (Table 1) were aligned by the ClustalW algorithm (Thompson et al. 1994) in the MEGA X software package (Kumar et al. 2018). Phylogenetic analyses were based on Maximum Parsimony (MP) and CI (consistency index), TL (tree length), RC (rescaled consistency index), and RI (retention index) were calculated for trees. The bootstrap analysis method with 1000 replicates was used to evaluate the robustness of the topology and stability of the nodes in generated phylogenetic trees (Felsenstein 1985).

Pathogenicity test

A pathogenicity test was performed using

asymptomatic detached shoots of walnut trees under controlled condition. Pathogenicity tests were conducted in a completely randomized experimental design (CRD) with six replications. The outer bark of each shoot was disinfected with ethanol (70%) before inoculation. Then a 4-mm wound was created at the inoculation site by a sterilized cork borer. Inoculation of the branches was made by placing 4 mm mycelium blocks (from 16 days old colonies on PDA) in the created wounds and then covering them with moist cotton and Parafilm strips (Pechiney Plastic Packaging, Menasha, USA). Six detached shoots were also inoculated with 4 mm sterile PDA plugs as the control treatment. Treatment and control shoots were inserted into the plastic containers, filled with distilled water (800 to 1000 ml), and maintained at 25±2°C. After 40 days, the upward, downward and total lesion length data were measured. Collected data of pathogenicity test was analyzed with variance (one-way ANOVA) using SAS v 9.1 (SAS Institute, Cary, North Carolina, USA). LSD (The least significant difference) test was used for statistical comparison treatment means at P <0.01. Re-isolated fungal species were recognized based on the explanations given in the previous paragraphs, fulfilling Koch's postulates.

Table 1 Origins, host and GenBank accession numbers of the strains used in phylogenetic analyses (Iranian isolate is shown in bold type)

				GenBank accession number
Species	**Code**	**Host**	**Origin**	**ITS**
Ceratocystis fimbriata	C1418	*Ipomoea batatas*	USA	AY157956
Ceratocystis moniliformis	CMW8240	*Cassia fistula*	Bhutan	AY529000
Corollospora fusca	NBRC 32108	-	Japan	JN943385
Corollospora maritima	NBRC 32118	-	Japan	JN943387
Custingophora olivacea	CBS 355.68	-	Germany	MH859151
Gondwanamyces capensis	CBS 119215	*Protea laurifolia*	South Africa	EU552136
Gondwanamyces capensis	CMW1040	-	South Africa	EU660447
Gondwanamyces scolytodis	CCF 3569	*Scolytodes unipunctatus*	Costa Rica	AM267268
Hypocrea americana	CBS 123072	*Piptoporus betulinus*	USA	DQ835410
Lareunionomyces foliicola	CBS 201.95	*Eucalyptus grandis*	Cuba	AF486129
Parasporendocladia bactrospora	CBS 299.62	*Populus trichocarpa*	British Columbia	NR_145202
	CDH004	*Quercus variabilis*	Korea	MF967565
	CBS 378.88	*Populus tremuloides*	Ontario	AF486123
	IRNMSX3	***Juglans regia***	**Iran**	**MW450472**
Petriella setifera	CBS 114629	oak wood	Poland	AJ784398
Petriella sordida	CBS 184.73	wood	Sweden	AY882360
Phialocephala botulispora	DAOM75261	*Picea banksiana*	Canada	AF083198
Phialocephala dimorphospora	CBS 300.62	Wood pulp	Canada	AF486121
Sporendocladia beijingensis	NN077249	*Quercus* sp., dead cupules	China	OL628290
Sporendocladia fumosa	CBS 518.93	*Quercus* sp., dead fruit	China	MH862436

RESULTS

Fungal isolation and morphological characterization

In this study, 21 isolates of a *Parasporendocladia* species were recovered from two trunks and 11 branches of 10 walnut trees showing branch and trunk cankers and dieback in Bidkhan (Kerman Province). In the cross-section of affected samples, irregular wood necrosis, v-shaped necrosis, and black spots were recorded as internal wood lesions (Fig. 1). Isolates were characterized by flat, brownish grey colonies with a white edge on PDA. Colonies reaching a radius of 7.75-9.00 mm in 7 d, 16.50-17.25

mm in 14 d, and 22.00-23.50 mm in 21 d at 25°C. Conidiophores were mononematous, smooth, multiseptate, solitary to aggregated, erect, branched at the apex, and dark brown. The conidiogenous head at the apex of the conidiophores consisted of branches with 1-9 series of terminal phialides and integrated, ampulliform, lageniform, obclavate, and brown, with a deep, cylindrical or subcylindrical collarette. Conidia arranged in false chains were holoblastic, oblong, non-septate, hyaline, smooth, and truncate at both ends (Fig. 2). Teleomorph is Unknown.

Molecular characterization and phylogenetic analysis

The identification of these isolates was confirmed by molecular method and the comparison of the obtained DNA sequences data. The ITS sequence of an Iranian isolate (IRNMSX3) was aligned with 18 reference sequences of isolates and *Hypocrea americana* (CBS 123072) was used as a out-group (Table 1). The alignment consisted of 850 characters (including gaps), of which 256 were constant and 329 were parsimony informative. Maximum parsimony analysis resulted in four equally most parsimonious trees (TL=1237, CI=0.613; RI=0.668, RC=0.410). Analysis of the ITS region clearly clustered the Iranian isolate with the references isolates of *Parasporendocladia bactrospora* (100% bootstrap support) (Fig. 3).

Pathogenicity test

Based on the results of the pathogenicity tests, *P. bactrospora* caused 121-162 mm wood necrotic lesions on inoculated branches of walnut, whereas the control shoots produced no lesions (Fig. 4). The mean of wood lesion lengths caused by IRNMSX3 isolate (143.33 mm) was statistically different (F = 549.94, P < 0.0001) to those recorded on the non-inoculated control shoots (9.67 mm). The percentage of re-isolation of the fungus from symptomatic wood tissues of inoculated shoots was 66.67% while no fungal colonies were re-isolated from control shoots.

DISCUSSION

In this study, we report for the first time the presence of *P. bactrospora* in branches and trunks of walnut trees showing canker, dieback, and internal wood discolorations such as black spots, irregular wood necrosis, and V-shaped necrosis. *Parasporendocladia bactrospora* is mostly isolated and reported as a saprophyte from dead plant materials, however, this species has been isolated from dead material of plants (Kendrick 1961; Barbosa et al. 2009) as well as wood of living trees (Luque et al. 2000; Jacobs et al. 2003; Roux et al. 2014). Based on available references, this fungus was reported to affect *Tilia* in England (Kendrick 1961) and *Populus* spp. such as *P. trichocarpa* and *P. tremuloides* in Canada (Kendrick 1961; Grünig et al. 2002). During a survey conducted by Luque et al. (2000), *P. bactrospora* has been isolated and reported from oak (*Q. suber*) trees showing decline symptoms in Spain. This species has also been reported to affect broadleaved trees such as *Betula* spp., *Populus tremula*, and *Quercus* sp. as well as from logs at sawmills and in loading bays in Norway and Sweden (Roux et al. 2014). Lee et al. (2017) found that *P. bactrospora* was associated with cambium and inner bark of artificially induced wounds on *Quercus variabilis* in Korea. Additionally, this fungus has recently been isolated and identified from dead leaves of *Eucalyptus urophylla* in Brazil (Barreto et al. 2022).

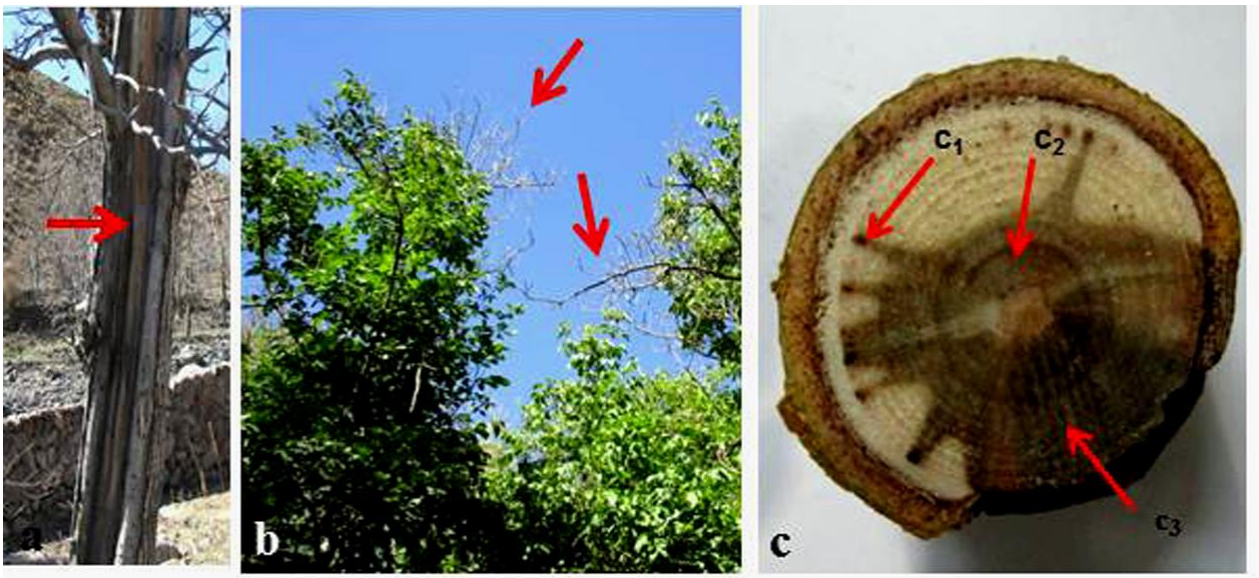

Fig. 1. Main branch canker and trunk disease symptoms found on walnut trees. (a-b) External disease symptoms: (a) Trunk canker, (b) Branch dieback, (c) Internal disease symptoms: (c_1) Black spot, (c_2) Irregular wood necrosis, (c_3) V-shaped necrosis.

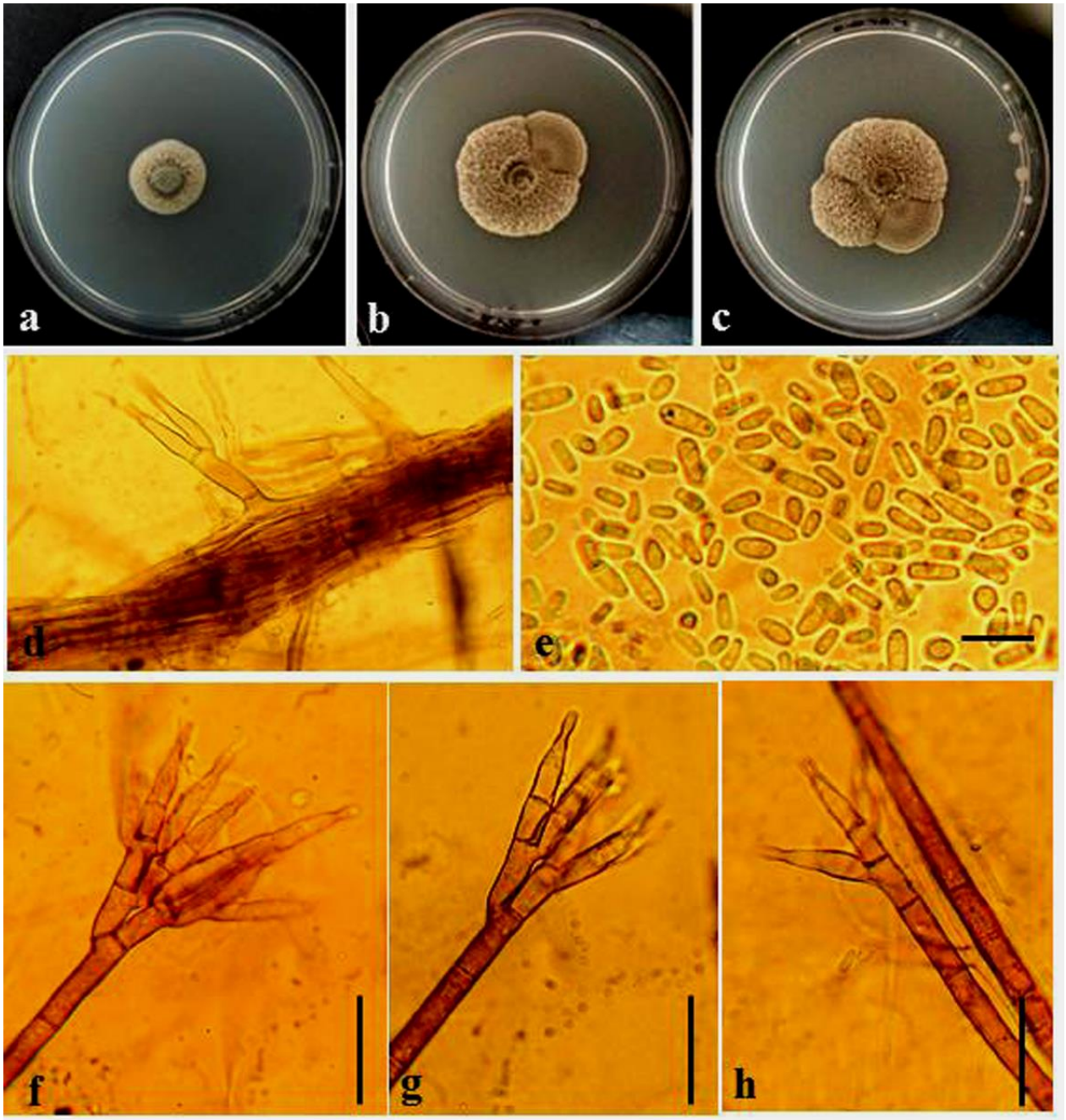

Fig. 2. *Parasporendocladia bactrospora* (IRNMSX3 isolate). Colony on PDA after one (a), two (b), and three (c) weeks, (d) Mycelial bundles, (e) Conidia, (f-h) Conidiophores and phialides, Scale bar: 10µm, (e): 5µm

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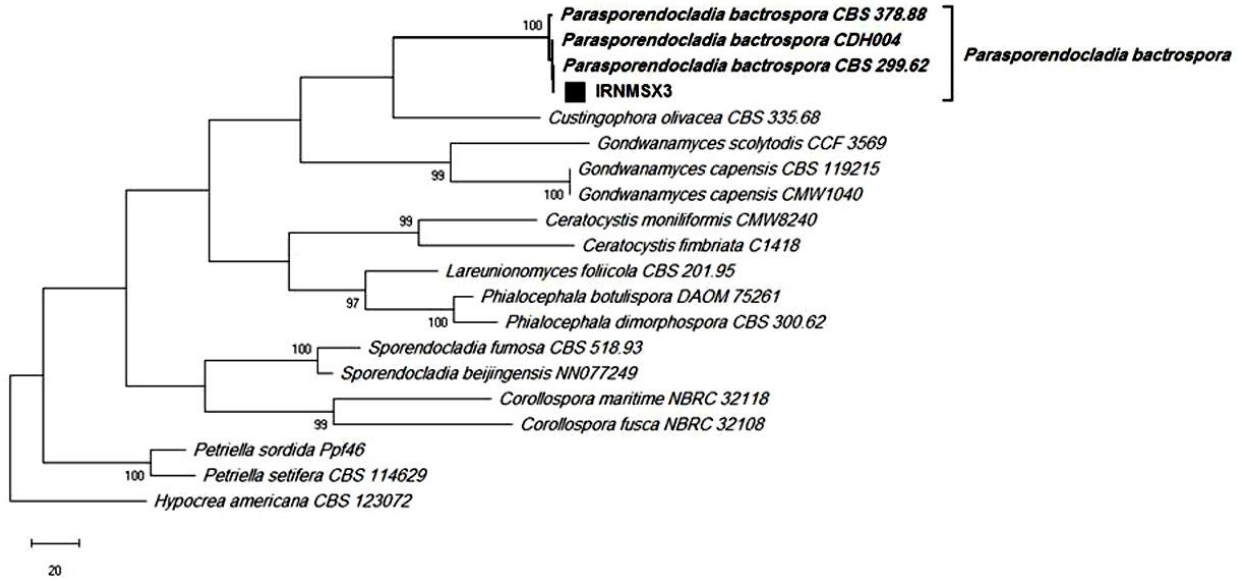

Fig. 3. One of the most parsimonious trees obtained from ITS-rDNA sequence data of *Parasporendocladia bactrospora*. MP bootstrap support (1000 replicates) above 70 % are shown at the nodes. *Hypocrea americana* (CBS123072) was used as an out-group and an Iranian isolate (IRNMSX3) was obtained in this study and isolates of *Parasporendocladia bactrospora* retrieved from GenBank are shown in bold type. The bar represents 20 changes.

Fig. 4. Pathogenicity test of *Parasporendocladia bactrospora* (IRNMSX3 isolate) on detached shoots of walnut trees, 40 days after inoculation: (a) Wood lesion caused by *Parasporendocladia bactrospora*, (b) Control (the arrow shows the point of inoculation), (c) Internal wood lesions caused by *Parasporendocladia bactrospora* in cross-section of a walnut shoot.

Based on the pathogenicity results, *P. bactrospora* caused wood on inoculated branches of walnut and was shown to be pathogenic on this host plant. Our results are consistent with previous pathogenicity studies conducted in Spain, in which this fungus was shown to be pathogenic on stems of *Q. suber* (Luque et al. 2000). Roux et al. (2014) also showed that *P. bactrospora* to be pathogenic on *P. tremula*, and *Betula pubescens* Ehrh. trees in Norway and Sweden (Roux et al. 2014). Considering the fungus-host association, this work is the first report of *P. bactrospora* occurring on necrotic wood tissues of walnut trees in the world. In addition, this study is the first report of *P. bactrospora* for the mycobiota of Iran.

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REFERENCES

Abbasi KH, Abbasi S, Fotouhifar KHB. 2012. Identification of *Cytospora* species from walnut trees in Iran. Plant Protection (Scientific Journal of Agriculture) 35: 59–69.

Abdollahzadeh J, Zare R, Phillips AJL. 2013. Phylogeny and taxonomy of *Botryosphaeria* and *Neofusicoccum* species in Iran, with description of *Botryosphaeria scharifii* sp. nov. Mycologia 105: 210–220.

Barbosa FR, Maia LC, Gusmao FLP. 2009. Novos registros de Hyphomycetes decompositores para o Estado da Bahia, Brasil. Acta Botanica Brasilica 23: 323–329.

Barreto GG, Gusmão LFP, Dianese JC. 2022. Checklist of ascomycetes recorded on eucalypts in Brazil (1976-2022). Asian Journal of Mycology 5: 107–129.

Chen SF, Morgan DP, Hasey JK, Anderson K, Michailides TJ. 2014. Phylogeny, morphology, distribution, and pathogenicity of *Botryosphaeriaceae* and *Diaporthaceae* from English walnut in California. Plant Disease 98: 636–652.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Eichmeier A, Pecenka J, Necas T, Ondrasek I, Armengol J, Leon M, Berlanas C, Gramaje D. 2020. Fungal trunk pathogens associated with *Juglans regia* in the Czech Republic. Plant Disease 104: 761-771.

Fan X, Hyde KD, Liu M, Liang Y, Tian C. 2015. *Cytospora* species associated with walnut canker disease in China, with description of a new species *C. gigalocus*. Fungal Biology 119: 310–319.

FAOSTAT 2021. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/es/#dat

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Grünig CR, Sieber TN, Rogers SO, Holdenrieder O. 2002. Genetic variability among strains of *Phialocephala fortinii* and phylogenetic analysis of the genus *Phialocephala* based on rDNA ITS sequence comparisons. Canadian Journal of Botany 80: 1239–1249.

Gusella G, Giambra S, Conigliaro G, Burruano S, Polizzi G. 2021. Botryosphaeriaceae species causing canker and dieback of English walnut (*Juglans regia*) in Italy. Forest Pathology 51:e12661.

Jacobs A, Coetzee MPA, Wingfield BD, Jacobs K, Wingfield MJ. 2003. Phylogenetic relationships among *Phialocephala* species and other ascomycetes. Mycologia 95: 637–645.

Kendrick WB. 1961. The *Leptographium* complex. *Phialocephala* gen. nov. Canadian Journal of Botany 39: 1079–1085.

Kendrick WB. 1963. The *Leptographium* complex: two new species of *Phialocephala*. Canadian Journal of Botany 41: 1015–1023.

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

Lee DH, Seo ST, Lee SH, Lee SK, Lee SK. 2017. Occurrence of *Sporendocladia bactrospora* on *Quercus variabilis* in Korea. Korean Journal of Mycology 45: 394–398.

Li GQ, Liu FF, Li JQ, Liu QL, Chen SF. 2016. Characterization of *Botryosphaeria dothidea* and *Lasiodiplodia pseudotheobromae* from English walnut in China. Journal of Phytopathology 164: 348–353.

Luque J, Parlade J, Pera J. 2000. Pathogenicity of fungi isolated from *Quercus suber* in Catalonia (NE Spain). Forest Pathology 30: 247–263.

Meng L, Yu C, Li G. 2018. First report of *Diaporthe amygdali* causing walnut twig canker in Shandong province of China. Plant Disease 102: 1859.

Michailides TJ, Chen SF, Coates B, Morgan D, Puckett R, Hasey J, Anderson K, Buchner R, DeBuse C, Fichtner E, Bentley W. 2012. Managing anthracnose blight and Botryosphaeria and Phomopsis cankers of walnut. Part1: *Botryosphaeria* and *Phomopsis* of walnut. In Walnut Research Reports (pp. 367–388). California Walnut Board.

Mohammadi H, Ezati Gh, Alaei H. 2013. *Diplodia seriata* and *Phaeoacremonium* species associated with walnut trees showing dieback symptoms in Ardabil province. 1st Iranian Mycological Congress, Rasht, Iran, 102p.

Rana JC, Singh D, Yadav SK, Verma MK, Kumar K, Predheep K. 2007. Genetic diversity collected and observed in Persian walnut (*Juglas regia* L.) in the

western himalayan region of India. Plant Genetic Resources 151: 68–73.

Raoufi F, Banihashemi Z, Mirtalebi M. 2016. First report of walnut canker caused by *Cryptosphaeria pullmanensis* in Iran. 22nd Iran Plant Protection Congress, 27–30 August.

Roux J, Solheim H, Kamgan N, kuekam G, Wingfield MJ. 2014. *Sporendocladia bactrospora* associated with wounds on native broadleaved trees in Norway and Sweden. Forest Pathology 44: 124–130.

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.

Sohrabi M, Mohammadi H. 2020. First report of *Graphium carbonarium* associated with walnut dieback in Iran. Mycologia Iranica 7: 15–19.

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, Gibson TJ. 1994. Clustral W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

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, p. 315–322.

Wingfield MJ, van Wyk PS, Wingfield BD. 1987. Reclassification of *Phialocephala* based on conidial development. Transactions of the British Mycological Society 89: 509–520.

Wu W, Diao Y. 2022. Anamorphic *Chaetosphaeriaceous* Fungi from China. Fungal Diversity116: 1–546.

Zhao SF, Guo KF, Yiming A. 2018. First report of *Cytospora nivea* causing *Cytospora* canker on walnut (*Juglans regia* L.) in the Tianshan mountains region of Xinjiang, China. Plant Disease 102: 2640.

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Parasporendocladia bactrospora همراه با شانکر و سرخشکیدگی درختان گردو (*Juglans regia* L.) در ایران

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چکیده: درخت گردو (*Juglans regia* L.) یکی از مهم ترین درختان میوه در ایران به شمار می رود. بیماری زوال درختان گردو یکی از مشکلات بزرگی است که در طول ۱۰ سال اخیر در مناطق مختلف استان کرمان (جنوب شرقی ایران) مورد توجه قرار گرفته است. بنابراین جهت شناسایی گونه های قارچی مرتبط با بیماری های شاخه و تنه درختان گردو، مطالعه ای بین خرداد ماه ۱۳۹۴ تا شهریور ماه ۱۳۹۵ انجام شد. نمونه های جمع آوری شده بعد برش زدن و ضدعفونی سطحی با محلول هیپوکلریت سدیم روی محیط کشت عصاره سیب زمینی، دکستروز-آگار (PDA) کشت داده شدند. در این مطالعه ۲۱ جدایه از یک قارچ از درختان بیمار با نشانه های شانکر و سرخشکیدگی و تغییر رنگ داخلی بافت چوب در منطقه بیدخون استان کرمان. به دست آمد. جدایه های به دست آمده ابتدا بر اساس مهم ترین ویژگی های ریخت شناسی شناسایی و گروه بندی شدند. کل DNA ژنومی استخراج و شناسایی جدایه ها با تجزیه و تحلیل توالی ناحیه ITS با استفاده از آغازگرهای ITS1 و ITS4 مورد تایید قرار گرفت. بر اساس مهم ترین ویژگی های ریخت شناسی و داده های مولکولی، این جدایه ها به عنوان *Parasporendocladia bactrospora* شناسایی شدند. این مطالعه اولین گزارش از همراهی این قارچ با نشانه های شانکر و سرخشکیدگی درختان گردو در دنیا و همچنین اولین گزارش از این گونه برای قارچ های ایران است.

کلمات کلیدی: سرخشکیدگی، شانکر، ITS، *Juglandaceae*، استان کرمان، بیماری زایی

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