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Alternaria telliensis, a new causal agent of cabbage leaf spot in Iran

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Abstract: In order to study of cabbage leaf spot disease in the Damavand region, Tehran province, Iran, symptomatic cabbage leaves (*Brassica oleracea* var. *capitata*) were collected during the late summer and fall of 2017. Twenty-one isolates with the main characteristics of the genus *Alternaria* were isolated from lesions on the cabbage leaves. Based on morphological characteristics and phylogenetic analysis using multi-gene sequences, they were identified as *Alternaria telliensis*. Pathogenicity tests were conducted on cabbage leaves under greenhouse conditions and characteristic lesions were formed on inoculated leaves. Re-isolation of the inoculated fungus from the treated leaves confirmed Koch's postulates. Based on the available information, this is the first occurrence of *A. telliensis* as a new species and pathogen to cabbage plants in Iran.

Keywords: Alternaria leaf spot, Brassicaceae, Pleosporaceae, phylogenetic analysis, pathogenicity

INTRODUCTION

Cabbage or headed cabbage (*Brassica oleracea* var. *capitata*), a member of Brassicaceae family, is an economically important leafy biennial vegetable crop grown worldwide as an annual crop for head harvest. Cabbages are used fresh in salads, as pickles, stir-fried and a fermented product (Wouters et al. 2013, Šamec et al. 2017) with antioxidant, antibacterial, anti-inflammatory, anti-obesity, anti-cancer, gastrointestinal and health promoting effects (Podşedek et al. 2006, Singh et al. 2006, Rokayya et al. 2013, Park et al. 2014, Lee et al. 2018). In 2017, global production of cabbages and other related brassicas was 71.2 million tonnes (FAO 2018). In Iran, cabbage is cultivated on 6979 ha with a total annual production of 306781 tonnes (FAO 2018). Tehran province is one of the main cabbages-growing areas in Iran (Koocheki et al. 2013).

Cabbages and other cruciferous crops are severely affected by different pathogens, causing economic losses in yield and crop quality. Leaf spot diseases caused by *Alternaria* spp. are the most prevalent and destructive diseases of these plants worldwide as well as in Iran (Humpherson-Jones 1992, Nowicki et al. 2012, Kumar et al. 2014, Rahimloo & Ghosta 2015). These diseases are characterized by small, dark specks that enlarge into relatively large, circular, brown to black lesions with concentric rings and are sometimes surrounded by chlorotic halos (Koike et al. 2007, Siciliano et al. 2017). Historically, *Alternaria brassicicola* and *A. brassicae* have been considered as the main causative agents of dark leaf spot disease in cruciferous plants (Maude & Humpherson-Jones 1980, Rotem 1994, Pedras et al. 2009, Michereff et al. 2012). Seeds, seedlings and pods are also damaged by these pathogens (Köhl et al. 2010). In a monographic study on *Alternaria* diseases of crucifers, four species, i.e., *A. alternata*, *A. brassicae*, *A. brassicicola* and *A. japonica* (as *A. raphani*) were reported to cause heavy losses in the crops (Verma & Saharan 1994). All four *Alternaria* species have been reported to be seed-borne and were detected from all parts of infected seeds (Saharan et al. 2016).

In a study on *Alternaria* species associated with cabbage leaf spot disease in Urmia, Iran, eight species were identified based only on morphological characteristics (Rahimloo & Ghosta 2015). In the most recent study on the incidence and diversity of *Alternaria* species associated with leaf spot disease of *Brassica napus*, 12 species were identified, indicating high diversity of *Alternaria* species associated with the disease (Al-Lami et al. 2019). In the present study, a set of *Alternaria* isolates was isolated from cabbage plants representing leaf spot symptoms. Based on the combination of morphological characteristics and molecular data obtained from four genomic loci (ITS-rDNA, glyceraldehyde-3- phosphate dehydrogenase, the second largest subunit of RNA Polymerase II and

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translation elongation factor 1-alpha), the isolates grouped well in *Alternaria* section *Japonicae* in close affinity with *A. telliensis* N. Bessadat, Ayad & P. Simoneau. Pathogenicity tests confirmed the pathogenic nature of the isolates on cabbage leaves. To our knowledge, this is the first occurrence of *A. telliensis* and its pathogenicity on cabbage plants in Iran.

MATERIALS AND METHODS

Sample collection and fungal isolation

Cabbage (*Brassica oleracea* var. *capitata*) leaves with necrotic leaf spot symptoms were collected randomly from different cabbage growing fields in Damavand city, Tehran province, Iran during the late summer and fall of 2017. Leaf samples were placed in new separate paper bags, transferred to the laboratory and processed immediately. Symptomatic leaf samples were washed gently under running tap water and surface disinfested by submerging in 70% ethanol for 2 min and washed again with sterile distilled water. Small pieces at the interfaces of healthy and necrotic areas were excised and plated directly onto potato dextrose agar (PDA, Merck, 39 g/L) medium. Also, some of the leaves were kept in moist chambers. The samples were examined at 48 h intervals for fungal growth, and the growing fungi were transferred onto new PDA Petri dishes. Purification of the fungi was done based on the single spore method. Pure cultures were grown on potato carrot agar slants (PCA, 20g carrot, 20g white potato, 20g agar in 1L distilled water) supplemented with twice-autoclaved pieces of filter papers and kept at 4°C.

Morphological characterization

Morphological characteristics were examined according to Simmons (2007). Briefly, recovered isolates were cultured on PCA medium and incubated at 24 ± 1 °C with 8/16 h light/dark photoperiod under cool white fluorescent light without humidity control. Sporulation pattern was observed after 5–7 days at 64× magnification using a stereo-microscope. Microscopic slides were prepared using clear transparent tape and 25% lactic acid solution as mounting fluid (Schubert et al. 2007). Micro-morphological characteristics including dimensions, septation, color and surface ornamentation were recorded for 50 conidiophores and conidia. Colony characteristics were observed on PCA, PDA, hay decoction agar (HA) and V8 juice agar (V8-A) culture media after 7 days (Simmons 2007). The living cultures of examined isolates were deposited in the fungal culture collection of the Agricultural Biotechnology Research Institute of Iran (ABRIICC) and additional isolates were kept in the fungal culture collection of Urmia University (FCCUU).

DNA extraction, amplification and sequencing

Freshly harvested mycelium from 5-day-old cultures grown in potato dextrose broth (PDB) was used for DNA isolation. Total genomic DNA was extracted using the Exgene™ Cell SV mini kit (GeneAll Biotechnology Co., Korea) following the manufacturer's instruction. Polymerase chain reaction amplification of ITS-rDNA region and parts of *TEF1-α*, *RPB2* and *GAPDH* gene regions were performed using primer pairs ITS1/ITS4 (White et al. 1990) for ITS-rDNA region, EF1-728F/EF1-986R (Carbone and Kohn 1999) for *TEF1-α*, fRPB2-5F/fRPB2-7cR (Liu et al. 1999; Sung et al. 2007) for *RPB2* and gpd1/gpd2 (Berbee et al. 1999) for *GAPDH*. The thermal conditions for PCR amplification and the reaction mixtures were the same as Hashemlou et al. (2020). PCR products were cleaned up and sequenced by Microsynth DNA company (Balgach, Switzerland).

Phylogenetic analysis

Multiple sequence alignments were produced using the type or ex-type strains sequences retrieved from GenBank according to Woudenberg et al. (2013, 2015), Lawrence et al. (2014), Deng et al. (2018), Poursafar et al. (2018, 2019) and Bessadat et al. (2020) (Table 1), and aligned with MAFFT online service (https://mafft.cbrc.jp/alignment/server/index.html). Concatenated sequence dataset (ITS-rDNA + *GAPDH* + *RPB2* + *TEF1-α*) was produced and proofed in Mesquite v. 2.74 (Maddison and Maddison 2010). Maximum likelihood analysis (ML) was performed on a concatenated dataset in RAxML-HPC BlackBox v. 8.2.12 (Stamatakis, 2014) through the CIPRES Science Gateway v 3.3 (Miller et al. 2010) using the GTRGAMMA+I as substitution model. The resultant phylogenetic tree was observed in FigTree v. 1.4.4 (Rambaut 2019). Sequences of *Stemphylium vesicarium* (formerly as *S. herbarum*) CBS 191.86 and *S. botryosum* strain CBS 714.68 served as the outgroup taxa.

Pathogenicity test

The healthy plants of white cabbage cv. Glory of Enkhuizen without any leaf symptoms were chosen for fungal inoculation. Cabbage leaves were firstly cleaned with moist cotton balls, then were sprayed with 70% ethanol and cleaned again with sterile distilled water. Mycelial plugs (5 mm diameter) containing fungal spores were taken from the edges of actively growing colonies (5-day-old) on PDA plates and placed upside down on cabbage leaves, both with and without small wounding caused by sterile needle. In the controls, only sterile agar plugs (without fungal mycelia or spores) were used. In addition, pathogenicity tests were done using the spore suspension (10^6 spores/mL) prepared from PCA cultures. The inoculated plants were covered with plastic bags for 48 h in order to maintain high humidity. Disease symptoms were evaluated 10 days after inoculation. Four replicates were used for each isolate and all the experiments were repeated once. Re-isolation of the inoculated fungi was made from the necrotic lesions formed on the inoculated leaves after surface disinfestation and morphologically compared to the inoculated isolates to fulfill Koch's postulates.

Table 1. Strains used for phylogenetic analysis in this study. Newly generated sequences are shown in bold.

Species	Collection numbers	Section	Host/ substrate	GenBank accession numbers				Reference
				ITS	*GAPDH*	*RPB2*	*TEF1-α*	
Alternaria anigozanthi	CBS 121920	*Eureka*	*Anigozanthus* sp.	KC584180	KC584097	KC584376	KC584635	Woudenberg et al. 2013
A. armoraciae	CBS 118702	*Chalastospora*	*Armoracia rusticana*	KC584182	KC584099	KC584379	KC584638	Woudenberg et al. 2013
A. avenicola	CBS 121459	*Panax*	*Avena* sp.	KC584183	KC584100	KC584380	KC584639	Woudenberg et al. 2013
A. botryospora	CBS 478.90	*Embellisioides*	*Leptinella dioica*	AY278844	AY278831	KC584461	KC584720	Woudenberg et al. 2013
A. brassicae	CBS 116528	-	*Brassica oleracea*	KC584185	KC584102	KC584382	KC584641	Woudenberg et al. 2013
A. brassicae-pekinensis	CBS 121493	*Ulocladioides*	*Brassica pekinensis*	KC584244	KC584170	KC584478	KC584738	Woudenberg et al. 2013
A. brassicicola	CBS 118699	*Brassicicola*	*Brassica oleracea*	JX499031	KC584103	KC584383	KC584642	Woudenberg et al. 2013
A. ershadii	ABRIICC 10179	*Pseudoalternaria*	*Triticum aestivum*	MK829646	MK829644	-	-	Poursafar et al. 2019
A. ershadii	IRAN3275C	*Pseudoalternaria*	*Triticum aestivum*	MK829647	MK829645	-	-	Poursafar et al. 2019
A. kordkuyana	IRAN 2764C	*Pseudoalternaria*	*Triticum aestivum*	MF033843	MF033826	-	-	Poursafar et al. 2018
A. rosae	EGS 41-30	*Pseudoalternaria*	*Rosa rubiginosa*	JQ693639	JQ646279	-	-	Lawrence et al. 2014
A. brassicinae (*A. alternata*)	CBS 118811	*Alternaria*	*Brassica oleracea*	KP124356	KP124210	KP124824	KP125132	Woudenberg et al. 2015
A. broccoli-italicae	EGS 40-134	*Infectoriae*	*Br. oleracea* var. *italica*	KM821536	KM821538	-	-	Deng et al. 2018
A. burnsii	CBS 107.38	*Alternaria*	*Cuminum cyminum*	KP124420	JQ646305	KP124889	KP125198	Woudenberg et al. 2015
A. caricis	CBS 480.90	*Nimbya*	*Carex hoodii*	AY278839	AY278826	KC584467	KC584726	Woudenberg et al. 2013
A. cetera	CBS 121340	*Chalastospora*	*Elymus scabrus*	JN383482	AY562398	KC584441	KC584699	Woudenberg et al. 2013
A. cheiranthi	CBS 109384	*Cheiranthus*	*Cheiranthus cheiri*	AF229457	KC584107	KC584387	KC584646	Woudenberg et al. 2013
A. chlamydospora	CBS 491.72	*Phragmosporae*	Soil	KC584189	KC584108	KC584388	KC584647	Woudenberg et al. 2013
A. consortialis	CBS 104.31	*Ulocladioides*	-	KC584247	KC584173	KC584482	KC584742	Woudenberg et al. 2013
A. dianthicola	CBS 116491	*Dianthicola*	*Dianthus* × *allwoodii*	KC584194	KC584113	KC584394	KC584653	Woudenberg et al. 2013
A. ethzedia	CBS 197.86	*Infectoriae*	*Brassica napus*	AF392987	AY278795	KC584398	KC584657	Woudenberg et al. 2013
A. japonica	CBS 118390	*Japonicae*	*Brassica chinensis*	KC584201	KC584121	KC584405	KC584663	Woudenberg et al. 2013
A. leucanthemi	CBS 421.65	*Teretispora*	*Chrysanthemum maximum*	KC584240	KC584164	KC584472	KC584732	Woudenberg et al. 2013
A. mimicula	CBS 118696	*Brassicicola*	*Lycopersicon esculentum*	FJ266477	AY562415	KC584411	KC584669	Woudenberg et al. 2013
A. mouchaccae	CBS 119671	*Phragmosporae*	Soil	KC584206	AY562399	KC584413	KC584671	Woudenberg et al. 2013
A. nepalensis	CBS 118700	*Japonicae*	*Brassica* sp.	KC584207	KC584126	KC584414	KC584672	Woudenberg et al. 2013
A. panax	CBS 482.81	*Panax*	*Aralia racemosa*	KC584209	KC584128	KC584417	KC584675	Woudenberg et al. 2013
A. telliensis	DA44	*Japonicae*	*Solanum tuberosum*	MT013034	MK904522	MK904537	MK904549	Bessadat et al. 2020
A. telliensis	NB319	*Japonicae*	*Lycopersicum esculentum*	MT013033	MK904521	MK904535	MK904548	Bessadat et al. 2020
A. telliensis	NB667	*Japonicae*	*Lycopersicum esculentum*	MT013035	MK904523	MK904536	MK904550	Bessadat et al. 2020
A. telliensis	**ABRIICC 10148**	***Japonicae***	***Br. oleracea var. capitata***	**MK660798**	**MK660796**	**MK660800**	**MK660802**	**This study**
A. telliensis	**ABRIICC 10150**	***Japonicae***	***B. oleracea var. capitata***	**MK660799**	**MK660797**	**MK660801**	**MK660803**	**This study**
A. petroselini	CBS 112.41	*Radicina*	*Petroselinum sativum*	KC584211	KC584130	KC584419	KC584677	Woudenberg et al. 2013
A. proteae	CBS 475.90	*Embellisioides*	*Protea* sp.	AY278842	KC584161	KC584464	KC584723	Woudenberg et al. 2013
A. radicina	CBS 245.67	*Radicina*	*Daucus carota*	KC584213	KC584133	KC584423	KC584681	Woudenberg et al. 2013
A. resedae (*Alternaria* sp.)	CBS 115.44	*Cheiranthus*	*Reseda odorata*	KC584214	KC584134	KC584424	KC584682	Woudenberg et al. 2013
A. scirpicola	CBS 481.90	*Nimbya*	*Scirpus* sp.	KC584237	KC584163	KC584469	KC584728	Woudenberg et al. 2013
A. subcucurbitae	CBS 121491	*Ulocladioides*	*Chenopodium glaucum*	KC584249	EU855803	KC584489	KC584749	Woudenberg et al. 2013
A. triglochinicola	CBS 119676	*Eureka*	*Triglochin procera*	KC584222	KC584145	KC584437	KC584695	Woudenberg et al. 2013
Stemphylium botryosum	CBS 714.68	-	*Medicago sativa*	KC584238	AF443881	AF107804	KC584729	Woudenberg et al. 2013
S. vesicarium	CBS 191.86	-	*Medicago sativa*	KC584239	AF443884	KC584471	KC584731	Woudenberg et al. 2013

RESULTS

Morphological characterization

Twenty-one *Alternaria* isolates with similar morphological characteristics were recovered from collected samples from different fields in Damavand city, Tehran province. Based on morphological characteristics, they were identified as *A. telliensis*.

Phylogenetic analysis

PCR amplification produced band sizes approximately 480 bp for ITS-rDNA, 531 bp for *GAPDH*, 909 bp for *RPB2* and 224 bp for *TEF-1α*. Sequence combination of four loci for a total of 41 fungal strains, including ingroup and outgroup taxa, contained 1919-characters. The best scoring RaxML tree with the final ML optimization likelihood value of -11504.616823 (ln) is selected to demonstrate the phylogenetic relationships among the studied strains (Fig. 1). The results of phylogenetic analysis revealed that the two studied Iranian *Alternaria* strains were closely related to the members of *Alternaria* section *Japonicae* with the nodal bootstrap support of 100% and clustered well in the subclade along with the strains of *A. telliensis* (Fig. 1), confirmed morphological identification.

Alternaria telliensis **N. Bessadat, D. Ayad & P. Simoneau, Phytotaxa 440 (2): 92. 2020**

Colonies on PCA reached 60 mm diam after 7 days, greenish olivaceous at the center and olivaceous grey at margins, with four well-defined concentric rings of growth and sporulation; 64 mm diam on PDA, smoke gray to whitish-grey; 54 mm diam on HA, hazel to brown and 65 mm diam on V-8 agar, greyish sepia to olivaceous. White aerial hyphae are present at the colony center of all media (Fig. 2a–c). Sporulation abundant after 4 days on PCA, HA and V8-A. On PCA, conidiophores arise directly from hyphae growing on agar surface or from aerial hyphae, mostly simple with an apical conidiogenous locus, sometimes with 2–3 geniculations, 20–90 × 3.5–5 µm; conidia mostly solitary, sometimes in chains of 2–3(–4) spores. Juvenile conidia are ovoid to ellipsoid, pale brown to brown, distinctly constricted at transverse septa, beakless, with 1–3 transverse septa and 0–1(–2) longitudinal septa, 17–40 × 10–17.5 µm. Mature conidia are long ovoid to cylindrical, brown to dark brown, outer wall punctate to verrucose, distinctly constricted at the transverse septa, 3–4(–5) transverse septa, 1–3 oblique septa and 1–3 longitudinal septa in the broadest transverse divisions, 40–65 × 18–43 µm, septa darker black-brown, 3–5 µm wide. In some of the conidia, body cells are enlarged and deformed the spore body. The apical secondary conidiophores that initiate chain formation are as single cells, slightly paler than that of the spore body, 5–8 × 5 µm, or a well-

differentiated apical outgrowth up to *ca* 17–48 × 5 µm. A distinct feature of the studied isolates is the transformation of all cells of some of the conidiophores into chlamydospore-like structures, 8.5–12.5 × 5.5–10 µm (Fig. 3a–z). The sexual form was not formed.

Specimens examined. IRAN, Tehran Province, Damavand City, on cabbage leaf (*Brassica oleracea* var. *capitata*) with leaf spot symptoms, 6 Oct. 2017. A. Poursafar, ABRIICC 10148, GenBank accession numbers : MK660798, ITS ; MK660796, *GAPDH* ; MK660800, *RPB2* ; MK660802, *TEF1-α* ; 25 Sept. 2017. A. Poursafar, ABRIICC 10150, GenBank accession numbers : MK660799, ITS ; MK660797, *GAPDH* ; MK660801, *RPB2* ; MK660803, *TEF1-α*.

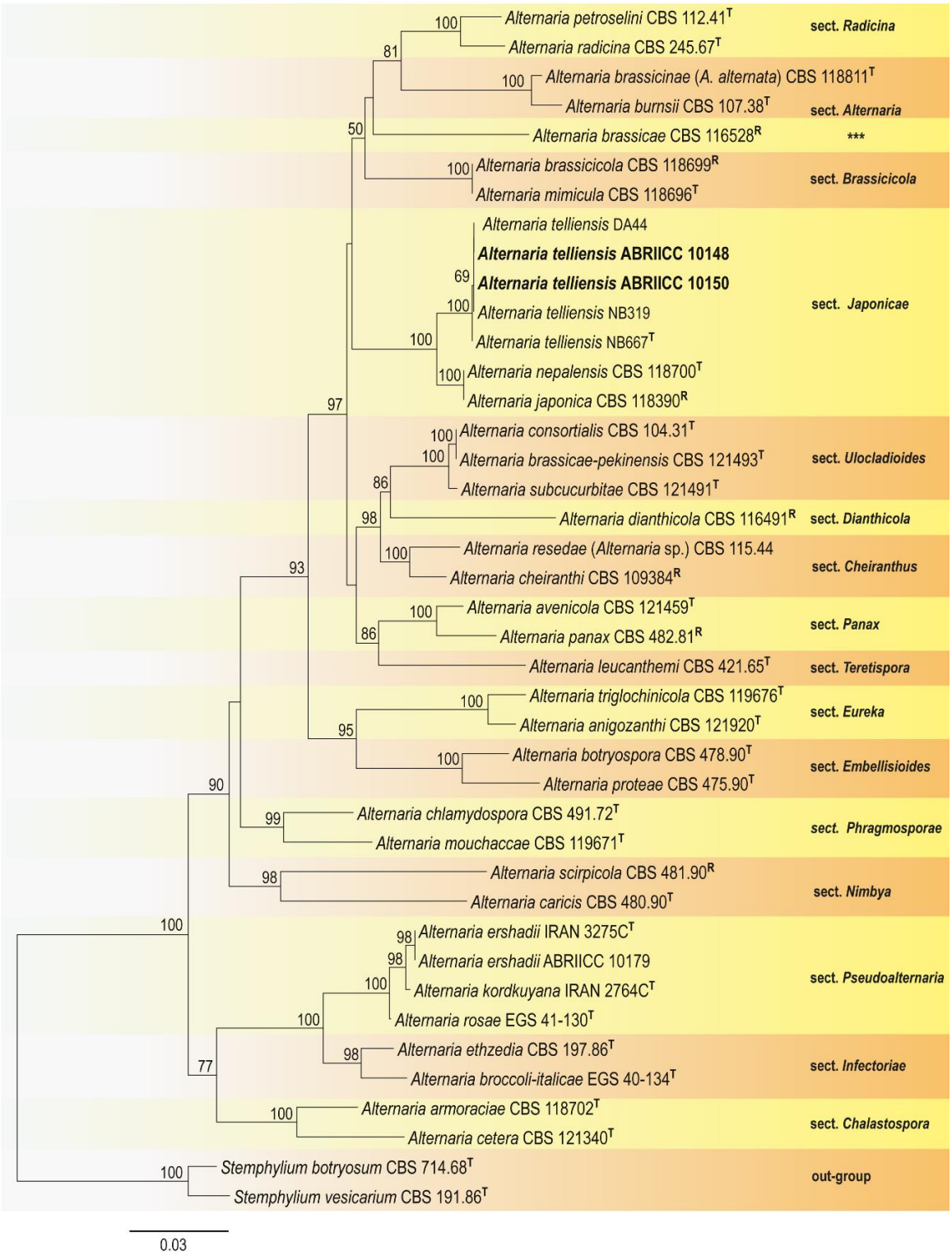

Fig. 1. Phylogenetic tree generated from maximum likelihood (ML) analysis based on the combined dataset of ITS, *TEF1-α*, *GAPDH* and *RPB2* sequences of 39 *Alternaria* strains. The RAxML maximum likelihood bootstrap values (>50%) are given at the nodes. The tree was rooted in *Stemphylium vesicarium* strain CBS 191.86 and *S. botryosum* strain CBS 714.68. The bar indicates the number of substitutions per position. T: Ex-type strain. R: Representative strain

Note. *Alternaria telliensis* is phylogenetically closely related to *A. japonica* and *A. nepalensis* in the *Alternaria* section *Japonicae*, but in a well-supported and distinct subclade. *Alternaria japonica* can easily be differentiated from *A. telliensis* by the production of distinctive chains of dark, thick-walled and often ornamented cells (micro-chlamydospores) in surface and subsurface hyphae and conidia with a smooth outer wall (Simmons 2007, Bessadat et al. 2020). Also, *A. nepalensis* can be differentiated from *A. telliensis* based on conidia with a smooth outer wall and with no formation of chlamydospores (Simmons 2007, Bessadat et al. 2020).

Pathogenicity test

All *Alternaria* isolates were used in pathogenicity experiments, and they produced conspicuous lesions on white cabbage leaves similar to those of naturally occurred under field conditions (Fig. 4a–e). No symptoms were formed on the control plants (Fig. 4f). Re-isolation of the inoculated fungus confirmed Koch's postulates.

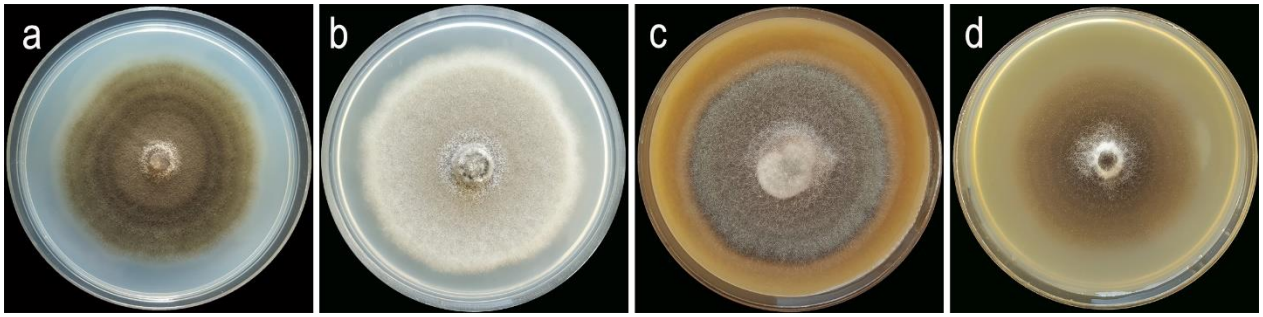

Fig. 2. *Alternaria telliensis* (ABRIICC 10148). Colony after 7 d on: a. PCA; b. PDA; c. V8-A; d. HA.

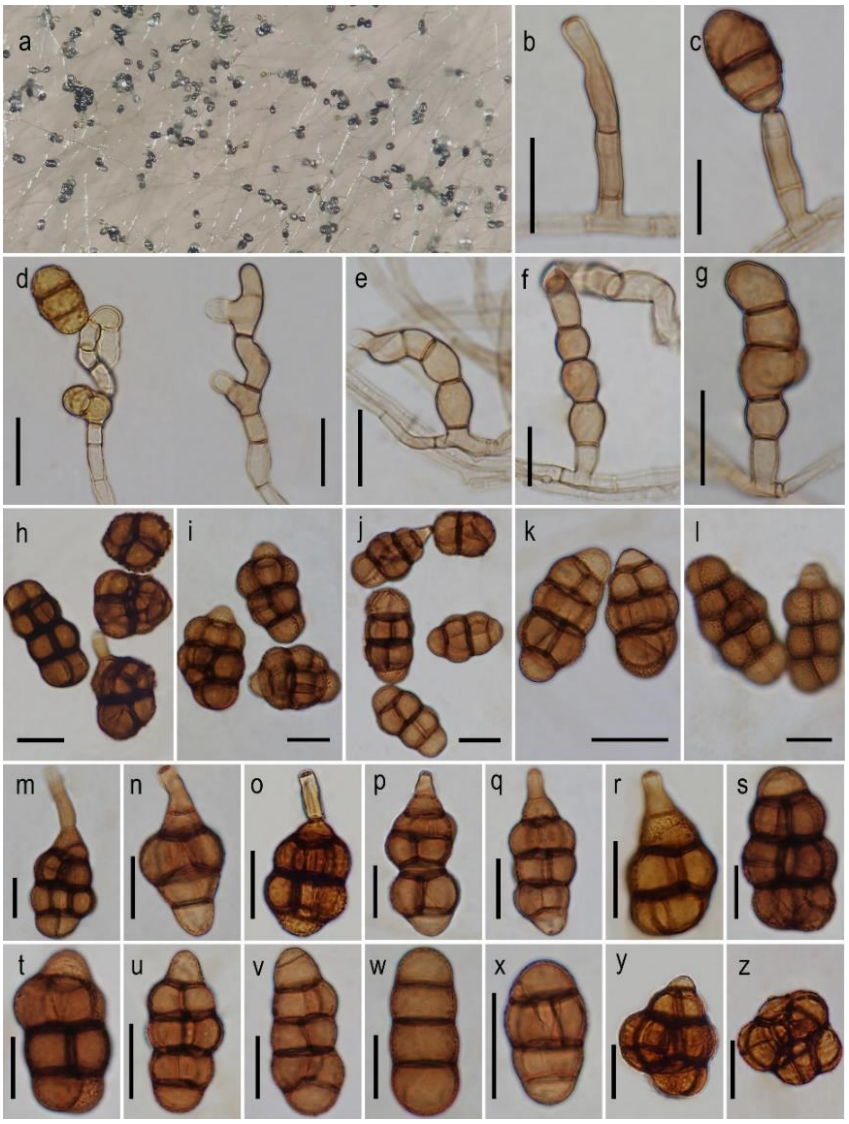

Fig. 3. *Alternaria telliensis* (ABRIICC 10148). a. Sporulation pattern on PCA; b–c. Primary conidiophores; d–g. Chlamydospore-like structures formed from the transformation of primary conidiophore cells; h–z. Conidia. — Scale bars = 20 µm.

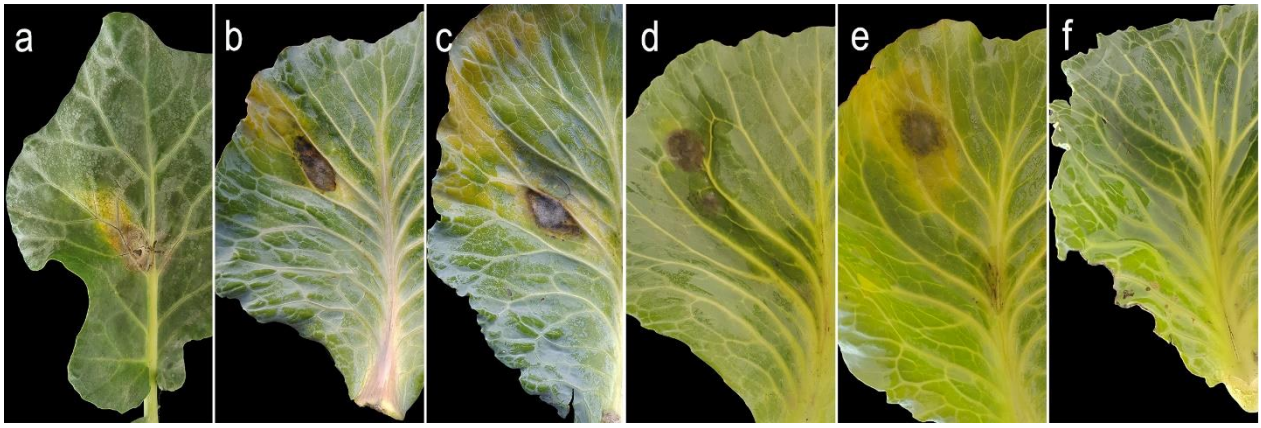

Fig. 4. a-e. Symptoms formed on cabbage leaves 10 days post-inoculation with agar plugs containing fungal mycelia and spores in greenhouse conditions; f. Control treatment.

DISCUSSION

Cabbage, because of its high nutritional value and high levels of anthocyanins and flavonoids and antimicrobial, antioxidant, anticancer and anti-inflammatory properties is one of the most commonly grown vegetables all around the world. It has been widely used as herbal medicine to treat different disorders (Rokkaya et al. 2013, Sarandy et al. 2015, Lee et al. 2018). Different biotic and abiotic stresses negatively affect plant growth and yield. Leaf spot diseases caused by different species of *Alternaria*, are among the most common and destructive diseases of cabbage and other cruciferous crops (Maude & Humpherson-Jones 1980, Yu et al. 1991, Cucuzza et al. 1994; Reis & Boiteux 2010). Although different species of *Alternaria* have been reported as causal agents of the disease (Verma & Saharan 1994, Simmons 2007, Rahimloo & Ghosta 2015, Siciliano et al. 2017), *A. brassicicola* and *A. brassicae* have commonly been recognized as the most prevalent species (Pedras et al. 2009, Köhl et al. 2010).

A review of available literature reveals the report of 17 *Alternaria* morpho-species associated with leaf spot disease of cabbage and other cruciferous plants around the world (Peruch et al. 2006, Aneja et al. 2014, Rahimloo & Ghosta 2015, Al-Lami et al. 2019). *Alternaria alternata*, *A. arborescens*, *A. destruens*, *A. malvae*, *A. perangusta*, *A. tenuissima*, *A. turkisafria* and *A. vaccini*, which were originally classified as small-spored *Alternaria*, are characterized by the production of relatively long to long, simple or branched chains of conidia and short to relatively long primary and secondary conidiophores (Simmons 2007).

In a recent comprehensive study using molecular and morphological characters, all these species (except for *A. arborescens* and *A. arbusti*) were synonymized under the single name *A. alternata* and placed in *Alternaria* section *Alternaria*. *Alternaria arborescens* and *A. arbusti* still exist as valid names within the section *Alternaria* (Woudenberg et al. 2015). *Alternaria telliensis* can easily be differentiated from species in section *Alternaria* based on its three-dimensional pattern of conidial chains as well as their morphologies. *Alternaria brassicicola* (*Alternaria* section *Brassicicola*), another important species reported from cabbage and other cruciferous plants, is characterized by the production of long branched chains of small, narrow conidia (30–60 × 6–17 µm) with few longitudinal septa (0–2) and formation of loose tufts of 50–60 or more conidia. *Alternaria* species reported on cabbage plants from *Alternaria* section *Infectoriae* such as *A. ethzedia* and *A. broccoli-italicae*, are characterized by the production of branched chains of small conidia, with more prominent secondary conidiophores and tufts of more than 50 conidia (Simmons 2007).

Alternaria brassicae (a monotypic lineage which is not placed in any section yet) has been classified as large-spored *Alternaria* (spore length >100 µm) (Simmons 2007), is characterized by the production of solitary or, more frequently short chains of conidia (2–3), long secondary conidiophores and relatively large conidia [60–200(–250) × 13–35(–40) µm] with 3–12 transverse septa. A large percentage of conidia have no longitudinal septa, but in some, 3–8 longitudinal septa are present. Currently, three species *viz. A. japonica* and *A. nepalensis* and *A. telliensis* are placed in *Alternaria* section *Japonicae*. Our morphological and multi-gene phylogenetic analysis strongly confirms the placement of the newly recovered isolates within this section. Due to the widespread occurrence of the disease symptoms under field conditions in the studied area and the results of pathogenicity tests, it can be claimed that *A. telliensis* is a potential leaf spot pathogen in cabbage plantations. *Alternaris telliensis* was originally isolated and described from the two solanaceous plants, *Lycopersicum esculentum* and *Solanum tuberosum*, showing leaf spot symptoms (Bessadat et al. 2020). Although in their pathogenicity tests, the isolates of this species were weakly pathogenic on their natural hosts, they were identified as highly virulent on cabbage (*Brassica oleracea*), radish (*Raphanus sativus*) and turnip (*Brassica rapa*) plants in laboratory conditions.

Here we report cabbage as a natural host of *A. telliensis*. Previous studies indicated that *Alternaria*

species infecting cabbage plants are all transmitted via infected seeds (Saharan et al. 2016), so additional studies are needed to explore seed transmissibility of this species and its pathogenicity on different cruciferous crops and weeds. Furthermore, since cabbage and other cruciferous plants are grown annually in different regions in Iran with diverse climatic conditions, more studies should be done in different locations to determine species diversity and richness for carefully planning efficient disease management programs.

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Alternaria telliensis، عامل جدید لکه‌برگی کلم در ایران

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چکیده: به منظور مطالعه بیماری لکه‌برگی کلم در شهرستان دماوند واقع در استان تهران، برگ‌های کلم (*Brassica oleracea* var. *capitata*) دارای نشانه‌های بیماری در اواخر تابستان و پاییز سال ۲۰۱۷ جمع‌آوری شدند. تعداد ۲۱ جدایه با مشخصات ریخت‌شناختی شاخص جنس *Alternaria* از لکه‌های برگ‌ها جداسازی گردید. بر اساس ویژگی‌های ریخت‌شناختی و نیز تجزیه و تحلیل تبارشناختی با استفاده از ترادف‌های چندژنی، جدایه‌ها به عنوان گونه *Alternaria telliensis* شناسایی شدند. آزمون بیماریزایی روی برگ‌های کلم در شرایط گلخانه‌ای انجام گرفت و لکه‌های مشخص مشابه با لکه‌های موجود روی نمونه‌های مزرعه‌ای در برگ‌های مایه‌زنی شده تشکیل شد. جداسازی مجدد قارچ مایه‌زنی شده از برگ‌های تیمار شده، اصول کخ را اثبات نمود. بر اساس منابع موجود، این اولین گزارش از وجود *A. telliensis* به عنوان گونه جدید و بیمارگر گیاهان کلم در ایران است.

کلمات کلیدی: لکه برگی آلترناریایی، Brassicaceae، Pleosporaceae، تجزیه و تحلیل تبارشناختی، بیماریزایی

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