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ORIGINAL PAPER

Lasiodiplodia pseudotheobromae causes stem canker of Chinese hackberry in China

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Abstract Chinese hackberry (*Celtis sinensis* Pers.) is an adaptable species widely growing in southern China. The symptoms of canker on stems of seedlings were discovered mid-July 2017 in Shuyang, Jiangsu Province. The diseased portions of the stems were dark brown due to discoloured xylem. Some seedlings showed symptoms of wilting, leaf fall, twig dieback, and tissue discolouration. The outbreak period was concentrated in July and August, suggesting that the disease spread during summer months. Possible fungal causal agents were isolated from naturally infected canker tissue and discoloured xylem. The isolate from xylem tissue with a high frequency (> 50%) was named Ls7 type. Pathogenicity tests were carried out on 4-year-old seedlings. The symptoms of canker began to develop 20 days after inoculation with Ls7 isolate and by day 35, there were dark, enlarged longitudinal lesions. A phylogenetic tree of the isolate was developed using the internal transcribed spacer, elongation factor-1α (*tef1-α*), *β-tubulin* gene (*TUB*) and RNA polymerase II subunit primer genes (*RPB2*). Based on morphological features and phylogenetic information, the pathogen was identified as *Lasiodiplodia pseudotheobromae*. This is the first report of *L. pseudotheobromae* causing canker on Chinese hackberry stems in China.

Keywords Botryosphaeriaceae · Plant pathogen · Tree disease

Introduction

Chinese hackberry (*Celtis sinensis* Pers.) is an important and widely planted ornamental tree species in China, Japan and Korea (Lee et al. 2019) and is tolerant of dry and nutrient-poor soils. The tender leaf, roots and bark of hackberry can reduce swelling, relieve pain, clearing away heat and toxic materials. It is also used as industrial materials and various furniture. Its rhizome fibers are strong and used in paper making, making ropes, artificial cotton and artificial fiber. The species has significant value in medicine and industry (Cao 2017). However, with increased planting, Chinese hackberry diseases are increasing and becoming more and more serious (Cacciola 2000; Berbegal et al. 2012; Luongo et al. 2015). Common diseases of Chinese hackberry are powdery mildew, stem and root rot, twig blight and leaf spot.

The Botryosphaeriaceae is a fungal family containing pathogens that cause canker disease of trees (Pavlic 2004). *Lasiodiplodia* species are members of the Botryosphaeriaceae and are widely found in tropical and subtropical regions (Phillips et al. 2013). *Lasiodiplodia* species causing disease of Chinese hackberry have not been found in China.

In the summer of 2017 in Shuyang, Jiangsu Province, a severe outbreak of Chinese hackberry disease appeared. The slightly sunken grey–black lesions enlarged longitudinally and the associated weakening of tree vigor affected the ornamental and commercial value of the species.

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The objectives of this study were to: (1) discover the epidemiology of canker occurring on Chinese hackberry; (2) prove the pathogenicity of the fungal pathogen using Koch's Postulates Test; and (3) identify the causal agent using morphological and molecular techniques.

Materials and methods

Sampling and fungal isolations

In mid-July 2017, symptoms of stem canker were found in commercial nurseries located in Shuyang County, Suqian City, Jiangsu Province (118°36′48″E; 34°15′25″N). The entire infected 4-year-old seedlings were brought to the laboratory for isolation of the pathogen.

Two ends of the cankers with spots in the middle were cut to form a small segment of 50 mm, and treated under aseptic conditions. The samples were sterilized in 75% alcohol for 3 min, dried quickly under a flame and rinsed three times with aseptic water for 30 s. Aseptic filter paper was used to absorb the water on the surface. The tissues were cut into 4 × 4 mm pieces between symptomatic and healthy tissues and cultured on fresh potato dextrose agar (PDA) medium containing 100 μg mL^{-1} ampicillin (Dai et al. 2007; Correia et al. 2016).

Samples were cultured at 25 °C in a dark incubator. Colony and spore morphologies were recorded every 24 h using a Nikon DS-Ri1 camera, Stereo Discovery, V20, Zeiss. The length and width of conidia were measured and the means, standard deviations and 95% reliable intervals computed from 50 samples.

Pathogenicity tests

Sixty fungal samples were divided into different groups according to colony morphology and ITS (internal transcribed spacer) sequences. All the isolate groups were tested for pathogenicity on the seedling stems. Each group was cultured for 7 days at 25 °C in darkness. Ten healthy Chinese hackberry seedlings (from 'Yanguan chunxiao farm'; Jiaxing City, Zhejiang Province 30°27′21″N, 120°32′40″E) were inoculated with each type of fungal isolates, respectively. Each plant was stabbed by placing a 4-mm-diameter mycelium plug of PDA placed face-down on each puncture wound. An additional ten seedlings were inoculated with sterilized PDA agar blocks as controls. All inoculated seedlings were kept in a greenhouse at 25 °C under ambient conditions of 70–80% humidity, with 24 h light every day. The experiment was carried out during the summer of 2018.

DNA extraction and sequence amplification

Fungal isolates were cultured in PDA medium for 5 days at 25 °C. Genomic DNA was extracted from mature mycelia according to Zhang et al. (2008). The procedure was: (1) mycelium grown for 3–4 days was placed in 2 mL test tubes with 500 μL of 2 × CTAB (containing 1% β-mercaptoethanol), rapidly cooled for 30 s in liquid nitrogen, and then heated immediately to 65 °C for 30 s. This was repeated three times; (2) a 1/2 volume of glass beads was added to each test tube before placing on a vortex shaker for 4–5 min, followed by heating to 65 °C for 20 min and shaking once every 10 min. Equal amounts of phenol: chloroform: isoamyl alcohol (25:24:1, v:v:v) were added and the tubes centrifuged at 12,000/min for 10 min; (3) two volumes of pre-cooled absolute ethanol was added to the supernatant, mixed well and the tubes placed in stand still for 20–30 min at 20 °C followed by centrifuging for 10 min at 12,000 r/min. The supernatant was poured out and 200 μL of 70% alcohol was added to wash the DNA pellet, which was then dried at room temperature. One hundred μL TE was added to completely dissolve the DNA; and, (4) 100 μL of RNase A (10 mg/mL) was attached to each tube and dried at 65 °C for 30 min. Step 3 was repeated and 30 μL of ddH_2O was added to obtain a solution of the DNA.

The polymerase chain reaction amplification system (PCR; 50 μL) contains 25 μL Etaq pre-Mix, 4 μL DNA template (approximately 200 ng), 2 μL forward primer, 2 μL reverse primer, and 17 μL ddH_2O.

Four pairs of primers were selected to amplify the target gene: ITS-1 and ITS-4 (ITS) (White et al. 1994); EF1-688F and EF1-1251R (*tef1-α*) (Alves et al. 2008); Bt2a and Bt2b (*TUB*) (Glass and Donaldson 1995); and, RPB2-LasF and RPB2-LasR (*RPB2*) (Cruywagen et al. 2016).

The PCR reaction procedure had the following steps: (1) initial denaturation at 95 °C for 5 min; (2) denaturation at 95 °C for 30 s, (3) annealing at 55 °C for 30 s and extending at 72 °C for 33 cycles; and (4) extending at 72 °C for 10 min. However, the annealing temperature of *TUB* and *RPB2* was 52 °C (Alves et al. 2004).

DNA amplification and sequencing were carried out with a GStorm Gradient PCR (Mastercycler nexus GSX1) using a program according to Zhang et al. (2008). The PCR products were sequenced by GenScript Biotechnology in Nanjing, China. To avoid errors in sequencing, DNA strands were spliced using forward and reverse primers (Two-way measurement: Sequencing Complete Sequences from Two Directions).

Phylogenetic analyses

The resulting ITS, *tef1-α*, *TUB* and *RPB2* gene sequences were blasted. Other sequences needed were obtained from

the GenBank database of NCBI (https://www.ncbi.nlm.nih.gov). These sequences were aligned using MEGA version 5.0. Ambiguously aligned regions were excluded and gaps were treated as missing data. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) approach (Tamura et al. 2011), which was validated using bootstrap analyses with 1000 repetitions (Saitou and Nei 1987). The phylogenetic tree was based on the concatenated sequences of ITS, *tef1-α*, *TUB* and *RPB2*.

Results

Incidence of the disease and symptoms

In the summer of 2016, a small amount of Chinese hackberry canker disease occurred but did not attract attention in Suqian City. However, by mid-July 2017, the incidence of Chinese hackberry canker was high with symptoms of the disease in over 21% of the 6000 m^2 of plantations of the species. The canker disease reappeared again in late July and August as well as spread rapidly.

The initial sites of infection appeared as watery reddish brown spots (Fig. 1a), and the top young leaves on twigs were wrinkled and brittle due to dehydration (Fig. 1b). With the development of the disease, slightly concave canker spots extended and gradually turned black–brown (Fig. 1c).

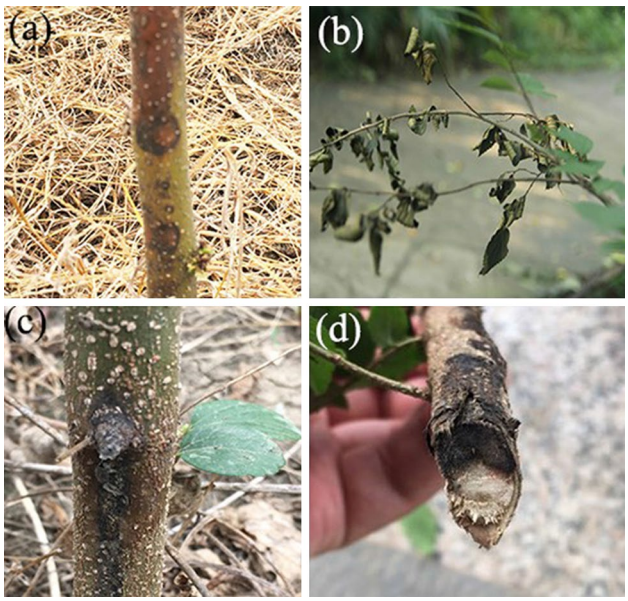

Fig. 1 Disease symptoms observed on Chinese hackberry in Shuyang, Jiangsu. **a** Stem blotch and canker in the beginning. **b** The leaves of the diseased plant showed dieback. **c** Canker spots extended longitudinally in the later stages of the canker disease. **d** Lesions in the inner stem of a diseased plant

Transverse sections of xylem tissue were also black–brown (Fig. 1d). Eventually, the twigs wilted.

Fungal isolations and pathogenicity tests

Sixty fungal samples were divided into seven types numbered Ls1 to Ls7 type according to colony morphology. Among these, more than 50% were classified as Ls7 type and found to be similar to *Lasiodiplodia pseudotheobromae*. According to the ITS sequence, Ls1–Ls7 types were identified as *Fusarium tricinctum* (2%), *Diaporthe* spp. (3%), *Clonostachys rosea* (8%), *Alternaria alternaria* (11%), *Fusarium equiseti* (12%), *Rhizoctonia solani* (14%) and *L. pseudotheobromae* (50%).

In mid-June 2018, each of the seven types of fungi was inoculated to 4-year-old seedlings with 10 replicates. After 20 days, eight seedlings inoculated Ls7 isolate showed disease symptoms similar to those that appeared in the field, including local, spindle-shaped necrosis and slightly concave red-brown spots (approximately 25 mm × 5 mm) (Fig. 2b). Two seedlings inoculated with Ls7 isolate developed canker spots on days 23 and 27 after inoculation, respectively. After 35 days, the lesions on these seedlings spread longitudinally on the stem, gradually changed shape from round to fusiform and became slightly concave. The lesions were approximately 98 mm × 6 mm (Fig. 2c, d). These dark brown lesions contained pycnidia densely packed with conidia, and leaves on the twigs began to wilt (Fig. 2c). The xylem was also dark brown (Fig. 2e). Seedlings inoculated with sterile PDA showed no symptoms of canker (Fig. 2a) and resembled the Ls1–Ls6 isolates.

The fungus was recovered from the symptomatic inoculated tissues and the culture's characteristics and morphology were similar to the Ls7 isolate. Therefore, the Ls7 isolate was determined to be the pathogen, although the seedlings infected with Ls7 isolate did not die.

Morphological characteristics of pathogen

Colonies of the Ls7 isolate grew rapidly, and after 3 days of culturing at 25 °C, were grayish-white with uneven edges (Fig. 3a). The colonies turned dark gray after 5 days (Fig. 3b), at which stage aerial hyphae were thick and fluffy. Following 2 weeks of treatment under UV light, the colonies produced pycnidia (Fig. 3c, d). There were many short conidiophores on the inner walls and conidia on the conidiophores. The pycnidia were nearly spherical, approximately 220–240 µm diameter, and formed scattered, unilocular, dark-brown to black areas on the stem and host epidermis (Fig. 4a). Conidiogenous cells were hyaline, cylindrical and holoblastic (Fig. 4c). Conidiophores were inflated at their base with one diaphragm which reduced to conidiogenous cells (Fig. 4d, e). Conidia were oval, both ends round and

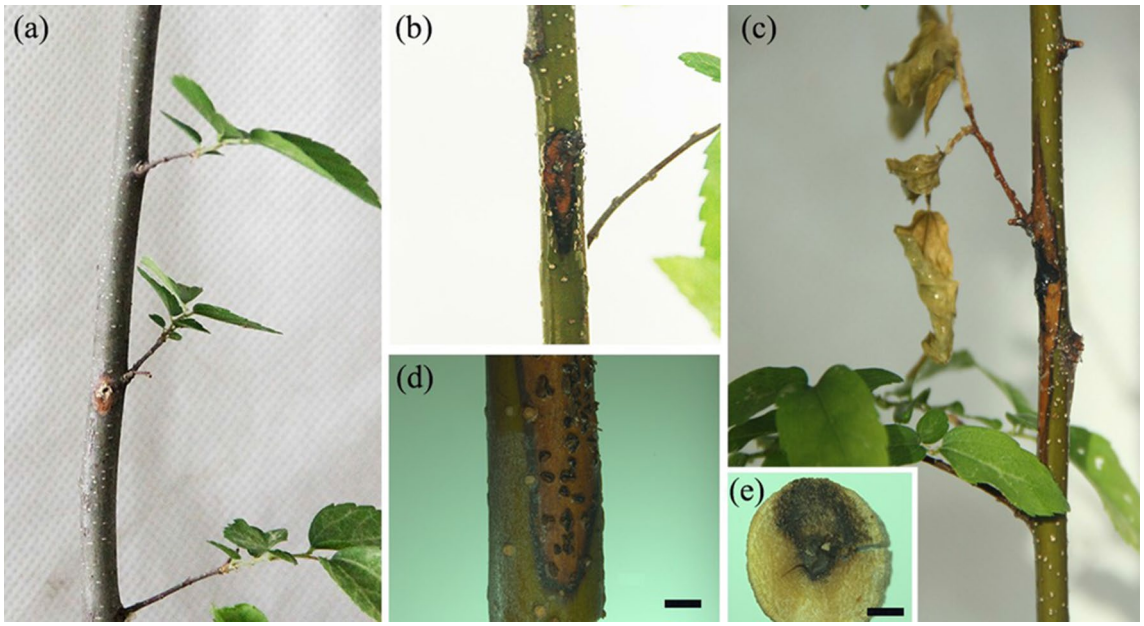

Fig. 2 Results of the pathogenicity trial. **a** Control stem (not inoculated with *L. pseudothebromae*). **b** Symptoms at the early stage, reddish brown spots and canker developing around the inoculation sites. **c** Symptoms at a later stage; typical canker symptoms of Chinese hackberry 35 days after stem inoculation. **d** Mature conidiomata on the disease site in this disease. **e** Xylem discoloration (Bars = 500 μm)

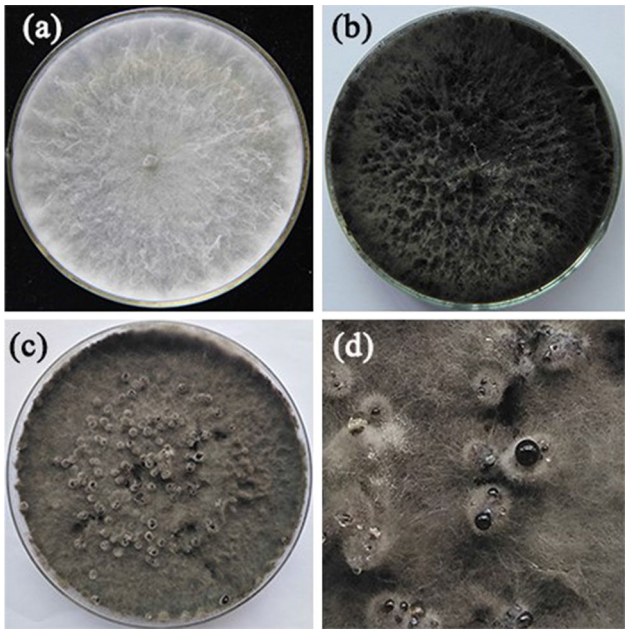

Fig. 3 Colony characteristics for *L. pseudotheobromae* isolate Ls7. **a** 4-day-old white to light-cream colony. **b** 7-day-old dark gray colonies, fluffy aerial mycelium. **c** Pycnidia produced under an ultraviolet lamp. **d** Liquid secretions may mark the open position of the conidia

blunt, wider in the middle, initially transparent and aseptate or not partitioned (Fig. 4f). These had one septum and became puce after maturity; melanin appeared in the conidia and the surface was striped (Fig. 4g, h). [A condium accompany with partial germ tube (Fig. 4i)]. The sizes of conidia were 22.2–28.6 × 12.1–17.4 μm (95% confidence limits = 25.2 ± 2.6 × 14.4 ± 2.4) and the average conidia length–width ratio was approximately 1.7 (Table 1). The paraphyses were hyaline and upward cylindrical. (Fig. 4b). In addition, the Ls7 isolate grew at 10 °C and produced dark pink colonies at 35 °C (Fig. 5). The morphological characteristics of the Ls7 isolate fit those of *L. pseudotheobromae* (Alves et al. 2008) and therefore confirmed as *L. pseudotheobromae* causing Chinese hackberry canker.

Molecular identification of pathogen

After PCR amplification, the Ls7 isolate with primers ITS1/ITS4, EF1-1251r/EF1-688f, BT2a/BT2b and rpb2-LasR/rpb2-LasF, segment sizes of 544, 315, 462 and 532 bp were acquired, respectively. The sequence accession numbers in GenBank were registered as MH454038.1, MH746713, MK041547 and MK069598, respectively. The ITS sequence of the test pathogen revealed 100% homology with *L. pseudotheobromae* in GenBank of BLAST search.

Before the establishment of phylogenetic trees with four groups of gene links, two tree-building methods were used to sequence and analyze individual gene sequences. There were no obvious conflicts in a single gene phylogeny and the ITS, *tef1-α*, *TUB* and *rpb2* datasets can be amalgamated. Sequences downloaded from NCBI (National Center for Biotechnology Information) were then used to carry out

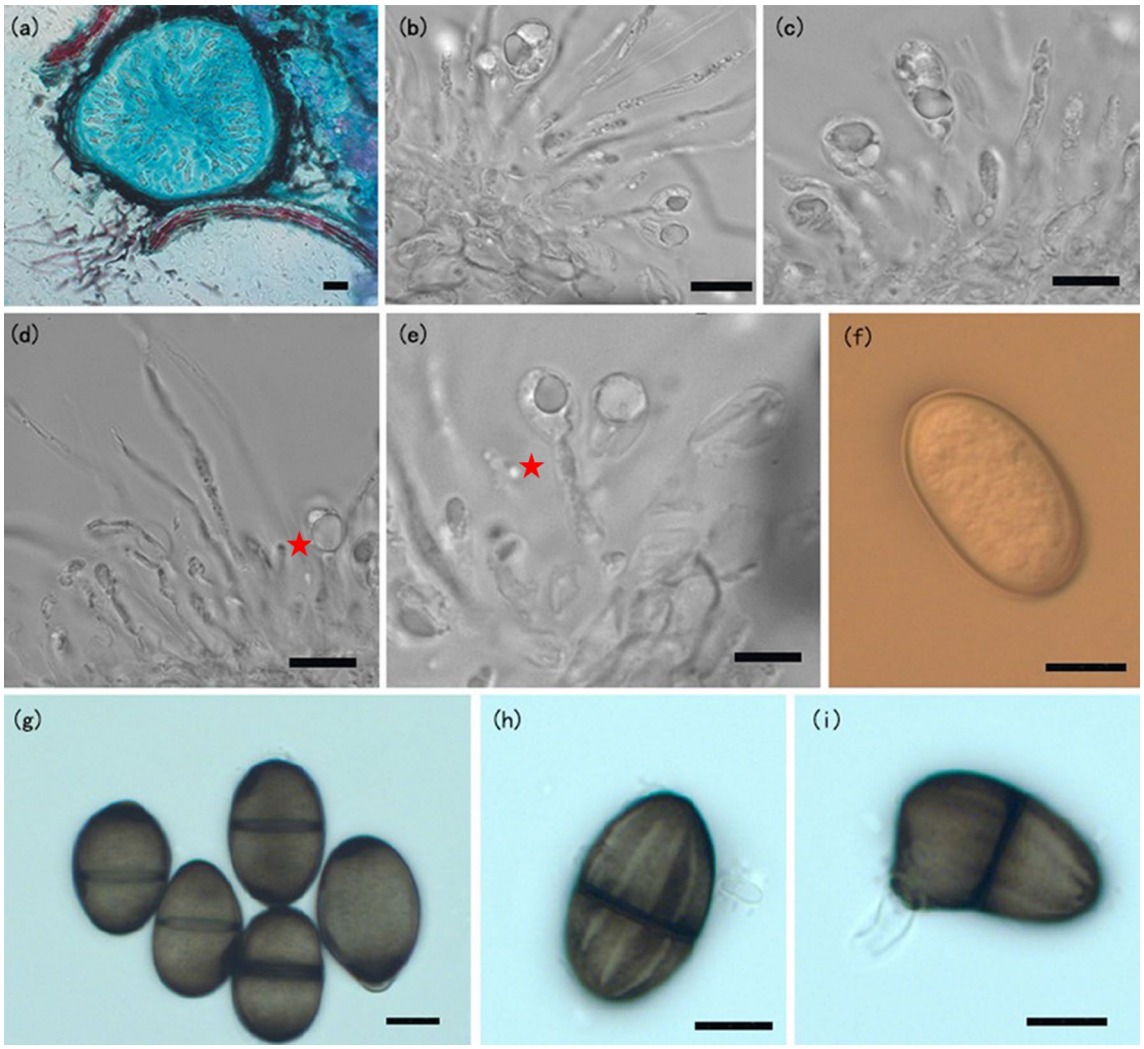

Fig. 4 Conidial morphological characteristics for *L. pseudotheobromae* isolate Ls7 seen under the light microscope. **a** Pycnidia. **b** Paraphyses. **c**, **d** Conidiogenous cell. **d**, **e** Conidiophore (red star). **f** Hyaline, aseptic conidia. **f**–**h** Septate, melanin deposits on the inner surface of the wall arranged longitudinally giving a striate appearance to the conidia. **i** A condium with partial germ tube (Bars = 10 µm)

Table 1 Conidia measurement of *Lasiodiplodia pseudotheobromae* isolates from Chinese hackberry and comparison with previous studies

Isolate	Host	Mean ± SD (µm)	L/W	Source
Ls7	Chinese hackberry	25.2 ± 2.6 × 14.4 ± 2.4	1.7	This study
HMQAU 140073	Blueberry	25.7 ± 1.6 × 14.2 ± 1.4	1.7	Wang et al. (2016)
Type	*Gmelina arborea*	28 ± 2.5 × 16 ± 1.2	1.7 ± 0.2	Alves et al. (2008)

cluster analysis (Dou et al. 2017). The *Diplodia mutila* isolate CMW7060 was selected as an out-group (Table 2).

A phylogenetic tree based on ITS, *tef1-α*, *TUB* and *RPB2* sequences was constructed by cluster analysis of the gene sequences of the test pathogen and other *Lasiodiplodia* species complex strains (number of sites = 410, bootstrap replicates = 1000). The results show that isolates of *Lasiodiplodia* sp. were clearly divided into several clades or groups with a common ancestor representing different species within *Lasiodiplodia*. The test isolate showed high homology with *L. pseudotheobromae* (Fig. 6). Therefore, the isolate was confirmed to be *L. pseudotheobromae* on the grounds of molecular identification.

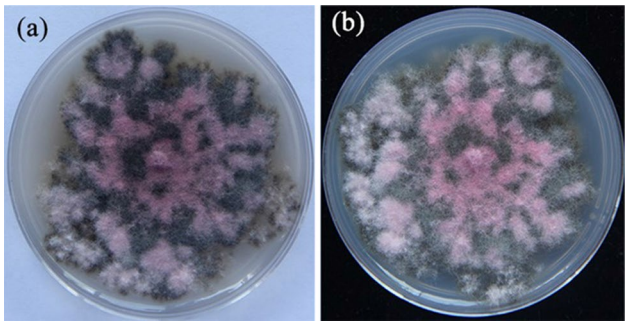

Fig. 5 Fungus isolate Ls7 a unique dark pink colony under incubation at 35 °C. **a** Colony on white background. **b** Colony on black background

Discussion

Based on available knowledge, this is the first report of Chinese hackberry stem canker disease caused by *L. pseudotheobromae* in China. The twigs and stems of all infected seedlings became cankerous and turned black, which was devastating to Chinese hackberry plantations.

Lasiodiplodia pseudotheobromae is important in agriculture and forestry as it causes cankers, stem-end rot, dieback and fruit rot (Sakalidis et al. 2011; Ismail et al. 2012; Marques et al. 2013). These diseases lead to bark discolouration, browning of medullary tissue, a decline in growth, and drying of leaves on upper branches. In severe cases, they may even lead to plant death (Alves et al. 2004), resulting in considerable economic losses. The pathogen overwinters in the soil, and in the summer of the following year, conidia and conidiomata are spread by wind and rain (Yee et al. 2019).

Worldwide, this species has a wide range. *L. pseudotheobromae* is an aggressive species in Australia, Cameroon, Egypt, Brazil, Mexico, China and other countries (Begoude et al. 2010; Sakalidis et al. 2011; Ismail et al. 2012; Maricarmen et al. 2013; Coutinho et al. 2016). In China, it is mainly distributed in central and eastern portions of the country, including Beijing, Jiangsu, Hubei, Sichuan, and Shandong (Li and Li 2015).

At the same time, it has a wide range of hosts. It causes stem-end rot or dieback in branches of mango (Kwon et al. 2017), papaya stem-end rot (Netto et al. 2014), English walnut stem canker (Li et al. 2016), blueberry dieback (Wang et al. 2016), pedicel and peduncle discoloration of grapes (Dissanayake et al. 2015), and *Eucalyptus* cankers (Chen et al. 2011) in China. In addition, it also can infect *Acacia confusa* Merr (Dou et al. 2017), *Anacardium occidentale* L., *Citrus*, *Coffea* and *Gmelina* species (Phillips et al. 2008; Abdollahzadeh et al. 2010; Perez et al. 2010; Sakalidis et al. 2011; Slippers et al. 2014; Trakunyingcharoen et al. 2015; Coutinho et al. 2016), *Albizia falcataria* (L.), *Paulownia* spp., *Mangifera sylvatica*, *Eucalyptus* spp. and grapevine etc. (Alves et al. 2008; Zhao et al. 2010; Dissanayake et al. 2015).

This study focused on a Chinese hackberry canker disease outbreak between July and August. The maximum temperature tolerated by *L. pseudotheobromae* was 54 °C this resistance to high temperatures enables it to survive during the summer months. The disease spreads rapidly and is most severe during summers when temperatures reaches 30 °C and above (Zhang 2012). In other seasons the disease is less aggressive (Wu et al. 2018).

Conidia exist all year-round but are rarely detected in winter (Xu et al. 2014). The pathogen overwinters in the diseased twigs or soil and becomes the source of infection the following year, with spores travelling by means of wind, rain and insects (Wu et al. 2001). The pycnidia or fruiting body of the fungus is produced near the canker before spreading to neighboring hosts. Pathogens commonly enter the plant through wounds left by pruning of twigs and this may be the main reason for the spread of the disease. The presence of the disease cannot be ignored as it leads to significant economic losses in forestry and agriculture (Wang 2016). Therefore, control measures should be carried out in the early stage of discovery of the disease.

In the genus *Lasiodiplodia*, *L. pseudotheobromae* is phylogenetically close to *L. theobromae* (Li et al. 2018). Although they are closely related, the size and shape of *L. pseudotheobromae* conidia differ from those of *L. theobromae* which are larger and have orbicular ends (Alves et al 2008). In addition, *L. pseudotheobromae* can also grow at 10 °C and form deep-pink colonies at 35 °C but *L. theobromae* does not (Marques et al. 2013; Netto et al. 2014). The fungal samples categorized as Ls7 type in this study showed similar features with *L. pseudotheobromae* and was consistent with these characteristics.

Based on the phylogenetic relationship of *β-tubulin*, Cheng (2012) concluded that the sexual stage of *L. pseudotheobromae* may be *Botryosphaeria rhodina*. In 2016, Tennakoon et al. (2016) found the sexual morph of *L. pseudotheobromae* collected from dead leaves of *Plukenetia volubilis* L. (*Euphorbiaceae*) in Yunnan Province, China. Using phylogenetic analyses based on ITS and *tef1-α* sequence data, they authenticated the sexual-asexual connection in *L. pseudotheobromae*. It was the first evidence in molecular genetics of a sexual morph for this species. However, the sexual morph of the Ls7 isolate was not found in this study.

Table 2 GenBank accession numbers for ITS, *tef1-α*, *TUB* and *RPB2* sequence data for *Lasiodiplodia* species used in this study

Species	Cultures	GenBank				Locality
		ITS	*tef1-α*	*TUB*	*RPB2*	
L. avicenniae	**CMW41467**	**KP860835**	**KP860680**	**KP860758**	**KU587878**	South Africa
avicenniae	LAS199	KU587957	KU587947	KU587868	KU587880	South Africa
L. bruguierae	**CMW41470**	**KP860833**	**KP860678**	**KP860756**	**KU587875**	South Africa
L. bruguierae	CMW42480	KP860832	KP860677	KP860755	KU587876	South Africa
L. chinensis	**CGMCC3.18061**	**KX499889**	**KX499927**	**KX500002**	**KX499965**	China
L. citricola	**IRAN1522C**	**GU945354**	**GU945340**	**KU887505**	**KU696351**	Iran
L. citricola	IRAN1521C	GU945353	GU945339	KU887504	KU696350	Iran
L. crassispora	**WAC12533**	**DQ103550**	**DQ103557**	**KU887506**	**KU696353**	Australia
L. crassispora	CMW13488	DQ103552	DQ103559	KU887507	KU696352	Venezuela
L. gilanensis	**IRAN1523C**	**GU945351**	**GU945342**	**KU887511**	**KU696357**	Iran
L. gilanensis	IRAN1501C	GU945352	GU945341	KU887510	KU696356	Iran
L. gonubiensis	**CMW14077**	**AY639595**	**DQ103566**	**DQ458860**	**KU696359**	South Africa
L. gonubiensis	CMW14078	AY639594	DQ103567	EU673126	KU696358	South Africa
L. hormozganensis	**IRAN1500C**	**GU945355**	**GU945343**	**KU887515**	**KU696361**	Iran
L. hormozganensis	IRAN1498C	GU945356	GU945344	KU887514	KU696360	Iran
L. mahajangana	**CMW27801**	**FJ900595**	**FJ900641**	**FJ900630**	**KU696365**	Madagascar
L. mahajangana	CMW27818	FJ900596	FJ900642	FJ900631	KU696366	Madagascar
L. iraniensis	**IRAN1520C**	**GU945348**	**GU945336**	**KU887516**	**KU696363**	Iran
L. iraniensis	IRAN1502C	GU945347	GU945335	KU887517	KU696362	Iran
L. mediterranea	**CBS 137,783**	**KJ638312**	**KJ638331**	**KU887521**	**KU696368**	Italy
L. mediterranea	CBS 137,784	KJ638311	KJ638330	KU887522	KU696369	Italy
L. missouriana	**UCD2193MO**	**HQ288225**	**HQ288267**	**HQ288304**	**KU696370**	USA
L. missouriana	UCD2199MO	HQ288226	HQ288268	HQ288305	KU696371	USA
L. parva	**CBS 456.78**	**EF622083**	**EF622063**	**KU887523**	**KU696372**	Colombia
L. parva	CBS 494.78	EF622084	EF622064	EU673114	KU696373	Colombia
L. plurivora	**STE-U 5803**	**EF445362**	**EF445395**	**KU887524**	**KU696374**	South Africa
L. plurivora	STE-U 4583	AY343482	EF445396	KU887525	KU696375	South Africa
L. pseudotheobromae	**CBS 116,459**	**EF622077**	**EF622057**	**EU673111**	**KU696376**	Costa Rica
L. pseudotheobromae	UFRPE CFS 048	MG870583	MG870575	MG870603	MG870613	Pernambuco/Paraiba
L. pseudotheobromae	CGMCC 3.18047	KX499876	KX499914	KX499989	KX499952	China
L. pseudotheobromae	Ls7 (this study)	MH454038	MH746713	MK041547	MK069598	China
L. pyriformis	**CBS121770**	**EU101307**	**EU101352**	**KU887527**	**KU696378**	Namibia
L. rubropurpurea	**WAC12535**	**DQ103553**	**DQ103571**	**EU673136**	**KU696380**	Australia
L. rubropurpurea	WAC12536	DQ103554	DQ103572	KU887530	KU696381	Australia
L. theobromae	**CBS 164.96**	**AY640255**	**AY640258**	**KU887532**	**KU696383**	Papua New Guinea
L. theobromae	CBS 111,530	EF622074	EF622054	KU887531	KU696382	Unknown
L. venezuelensis	**WAC12539**	**DQ103547**	**DQ103568**	**KU887533**	**KU696384**	Venezuela
L. viticola	**UCD 2553AR**	**HQ288227**	**HQ288269**	**HQ288306**	**KU696385**	USA
L. viticola	UCD 2604MO	HQ288228	HQ288270	HQ288307	KU696386	USA
L. vitis	**CBS 124,060**	**KX464148**	**KX464642**	**KX464917**	**KX463994**	Italy
Diplodia mutila	CMW 7060	AY236955	AY236904	AY236933	EU339574	Netherlands

Extype isolates were in bold (Dou et al. 2017)

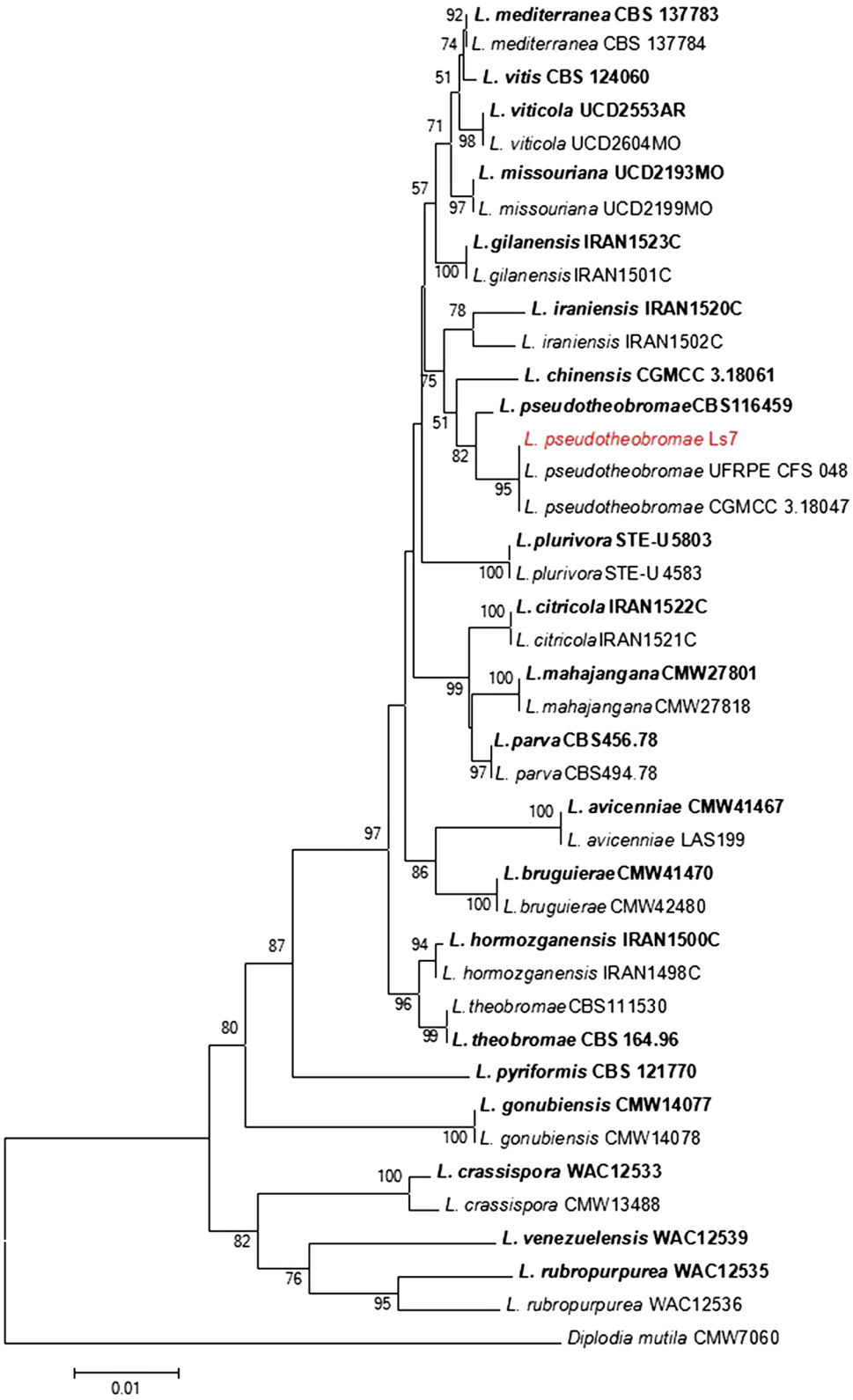

Fig. 6 Phylogenetic tree based on combined rDNA-ITS, *tef1-α*, *TUB* and *RPB2* gene sequences of Chinese hackberry *Lasiodiplodia* stem canker pathogen and related fungi. Bootstrap support values from 1000 bootstrap replications are showed. Outgroup: *Diplodia mutila*

Conclusions

This is the first report of hackberry canker in China and Chinese hackberry (*C. sinensis*) is a new host of *L. pseudotheobromae*. The occurrence of the disease should be closely monitored and preventative measures put in place to avoid the spread of the disease. This study may provide reference value for the prevention of Chinese hackberry canker.

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