Brief Report

Occurrence of Anthracnose Caused by Colletotrichum gloeosporioides on Lucky Bamboo in China

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Occurrence of Anthracnose Caused by *Colletotrichum gloeosporioides* on Lucky Bamboo in China

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Abstract: Lucky bamboo (*Dracaena sanderiana* hort. ex Mast. = *Dracaena braunii*) is a domestic species widely cultivated for ornamental purposes in China. In March 2022, a severe occurrence of anthracnose disease was observed on the stems of lucky bamboo plants in a greenery retail store located in Nanjing, Jiangsu Province, China. The morphological characteristics of isolates obtained from diseased stem tissues were identical with those described for *Colletotrichum* species. Based on multilocus phylogenetic analysis with the internal transcribed spacer (ITS), *actin* (*ACT*) gene, and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene, the pathogen was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Pathogenicity was verified by mycelial plug-inoculating stem cuttings of one-year-old lucky bamboo plants and spray-inoculating whole one-year-old lucky bamboo plants, respectively. Koch's postulates were fulfilled by the re-isolation of *C. gloeosporioides* from symptomatic tissues. To the best of our knowledge, this report describes the first time that *C. gloeosporioides* was observed to cause anthracnose on lucky bamboo in China.

Keywords: *Dracaena braunii*; *Colletotrichum gloeosporioides*; internal transcribed spacer (ITS); *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*); molecular characterization; pathogenicity test

1. Introduction

Lucky bamboo (*Dracaena sanderiana* hort. ex Mast. = *Dracaena braunii*) is a domestic species that is one of the most popular ornamental houseplants in China [1,2]. Lucky bamboo belongs to an entirely different taxonomic order from true bamboos, although the stems have a similar appearance [3]. The history of the lucky bamboo plant goes back to Chinese culture for more than 4000 years [4], because they are believed to bring good luck and fortune into life. While the lucky bamboo plants are easy to care for and grows well under diverse indoor conditions, they still inevitably encounter several diseases worldwide, including leaf spots caused by *Alternaria alternata* in Egypt [5], leaf blight caused by *Phytophthora nicotianae* in Brazil [6], stem rot caused by *Aspergillus niger* [7] and *Fusarium solani* [8] in Iran, stem and root rot caused by *Fusarium proliferatum* in Iraq [9], leaf chlorosis caused by pepper mild mottle virus (PMMoV) in Korea [10], and antharacnoses caused by *Colletotrichum dracaenophilum* in Bulgaria [11], Iran [12], USA [13], Egypt [3], and Brazil [14], respectively, as well as *Colletotrichum karstii* [15] and *Colletotrichum truncatum* [16] in China.

In March 2022, typical anthracnose symptoms were observed on *D. sanderiana* plants grown in a greenery retail store located in Nanjing, China (32°6′27.281″N,118°50′24.169″E). About 40% of the lucky bamboo plants for sale were affected. Ornamentals are expected to have an impeccable appearance, and anthracnose poses a threat to the ornamental value of lucky bamboo. Therefore, the present study aims to (i) isolate the pathogen causing anthracnose, (ii) identify the causal agent using morphological characterization and molecular analysis, and (iii) assess its pathogenicity.

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2. Results

2.1. Isolation and Morphological Characteristics of Isolates

Disease symptoms on stems initially appeared as large, sunken, black lesions, covered with numerous black acervuli. Orange-to-pink spore masses were visible within the senescent and dead plants. Symptomatic stem tissues gradually became necrotic and turned soft, and several leaves wilted and eventually abscised from the plants (Figure 1).

Figure 1. Natural anthracnose symptoms of lucky bamboo plants. A close-up of anthracnose lesions crowded with acervuli and pink spores is indicated with a red box.

Thirty-two fungal isolates with a notable colony morphology for the *Colletotrichum* taxon were obtained from five distinct symptomatic lucky bamboo plants. Follwing that, pure cultures of the 32 isolates were obtained by single-spore isolation. Colonies grown on V8 agar plates in the dark initially produced white, thin, and flat mycelia and eventually became pale gray in the center with concentric rings (Figure 2a,b). Colonies on potato dextrose agar (PDA) had fluffy mycelia, pale white margins with concentric rings, and an olive-green center, which gradually turned dark green with age and extended to the margins after seven days of incubation in the dark. The reverse side of the colonies completely turned dark green, and the production of crystals in PDA agar was observed when the colonies were incubated at 25°C with a 12-hour photoperiod for nine days (Figure 2c,d). Microscopically, conidia were hyaline, unicellular, and cylindrical with rounded ends, measuring 10.8 to 18.5 × 2.8 to 6.9 µm (Figure 2*e*). Acervuli were circular to elliptical with no setae (Figure 2g). Appressoria were formed at the tip of the germ tube and were brown and ovate to obovate, measuring 6 to 10 × 4.5 to 7.5 µm (Figure 2f).

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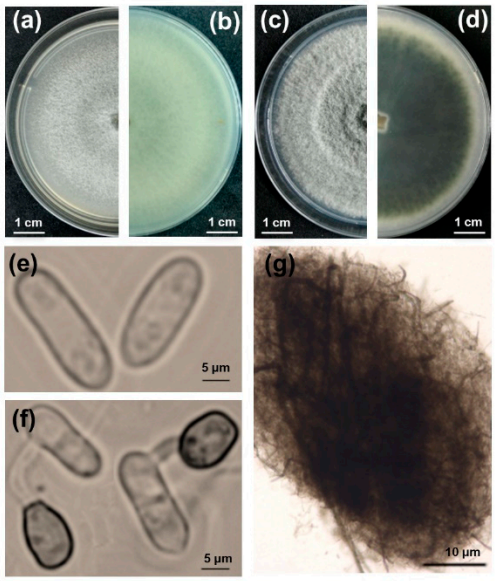

Figure 2. Morphological characteristics, colony morphology, and microscopic examination of fungi isolated from lucky bamboo. (**a-d**), Nine-day-old colonies grown on V8 (**a, b**) and potato dextrose agar (**c, d**) viewed from the top and bottom, respectively; (e), conidia; (f), appressorium; (g), acervuli. Scale bars: a-d = 1 cm, e-f = 5 µm, g = 10 µm.

2.2. Molecular characterization

Fragments of the 573 bp *internal transcribed spacer* (*ITS*) region, 282 bp actin (*ACT*), and 277 bp *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) were respectively amplified from the genomic DNA of all 32 single-spore isolates. Multiple sequence alignments by ClustalW revealed that the *ITS*, *ACT* and *GAPDH* sequences of 32 isolates shared 100% sequence similarity with each other. One representative single-spore isolate, named FGZ-1, was deposited in the China Center of Industrial Culture Collection (CICC) under number CICC 41715. The Basic Local Alignment Search Tool (BLAST) search in GenBank revealed that the obtained sequences of FGZ-1 (GenBank accession nos. MH752444.1-*ITS*, MH757114.1-*ACT*, and MH757113.1-*GAPDH*) shared 99-100% similarity with related sequences of *C. gloeosporioides* in Genbank (strain Sour8-accession no. KX227593.1, strain BWH1-accession no. KF712382.1, and strain TAX-1-accession no. HM575314.1, respectively).

2.3. Phylogenetic analysis

In the phylogenetic tree established on the basis of the concatenated *ITS*, *ACT* and *GAPDH* sequences, isolate FGZ-1 was placed within a monophyletic clade that comprised three reference isolates of *Colletotrichum gloeosporioides* with a robust bootstrap and formed a separate clade distinct from other *Colletotrichum* species (Figure 3). Isolate FGZ-1 was separated from *C. dracaenophilum*, which was reported to commonly cause anthracnose on lucky bamboo in Bulgaria, Iran, USA, Egypt, and Brazil [3,11–14]. In addition to *C. dracaenophilum*, there have been new reports of another three species of *Colletotrichum* associated with anthracnose of lucky bamboo, namely, *C. petchii* [17], *C. karstii* [15], and *C. truncatum* [16], which were also clearly separated from *C. gloeosporioides* isolate FGZ-1 (Figure 3).

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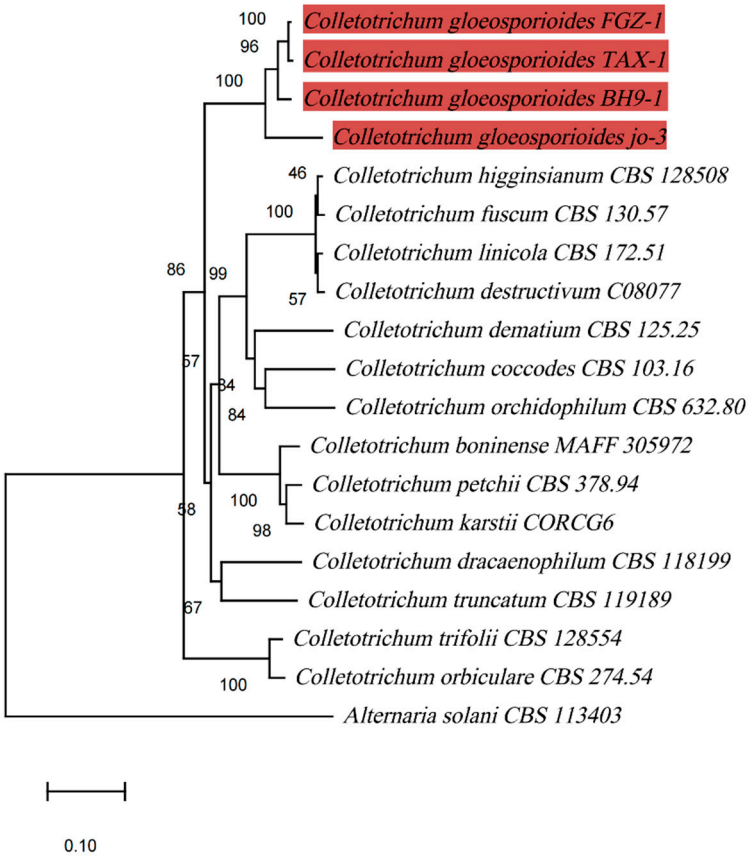

Figure 3. Phylogenetic maximum-likelihood tree computed from the concatenated sequences of *ITS*, *ACT* and *GAPDH* of 19 fungal strains using MEGA software (version X). *Alternaria solani* CBS 113403 was chosen as an outgroup. Bootstrap support values higher than 50% are shown above the branches. The scale bar indicates the number of nucleotide substitutions per site.

2.4. Pathogenicity Tests

Symptoms of black spots and stem rot on lucky bamboo segments were observed three weeks after being artificially inoculated with mycelial plugs of isolate FGZ-1, and the disease severity reached 100%, while the controls with pure agar plugs remained symptomless (Figure 4a). Similar disease symptoms were observed on the whole one-year-old plants after eight weeks of inoculation (Figure 4b). All of the inoculated stem segments and whole plants showed typical anthracnose symptoms similar to those of naturally diseased lucky bamboo plants (Figure 1). Koch's postulates were fulfilled by the reisolation of fungal colonies from all symptomatic stems and plants but not from controls, and these isolates had the same colony morphology, microscopic morphology, and molecular characteristics as described above. Similar results were obtained from the three repetitions of the experiment.

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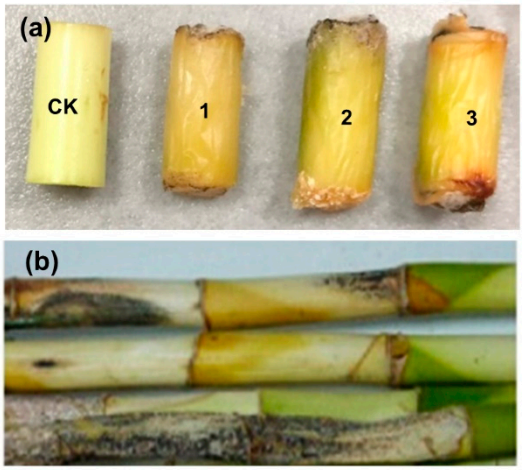

Figure 4. Symptoms of *Colletotrichum gloeosporioides* FGZ-1 inoculated on lucky bamboo segments (**a**) and live plants (**b**). CK, inoculated with pure PDA agar plugs as controls; 1-3, inoculated with *C. gloeosporioides* FGZ-1 mycelial plugs.

3. Discussion

Based on the above morphological characterization, multilocus phylogenetic analysis, and pathogenicity tests, isolates were identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. [18] and found to be the causal agent of anthracnose of lucky bamboo in China. On the other hand, *C. gloeosporioides* is one of the most common *Colletotrichum* fungal pathogens, infecting many economically important plants throughout the world, including citrus, yam, papaya, avocado, coffee, eggplant, loquat, sweet pepper, and tomato [19]. So far, *C. dracaenophilum* is the most widely reported *Colletotrichum* species that can cause anthracnose disease on *D. sanderiana* plants [3,11–14]. In the United States, Sharma, et al. [13] previously reported that an isolate of the *C. gloeosporioides* species complex caused anthracnose disease in lucky bamboo but was less pathogenic than isolates of *C. dracaenophilum*. Moreover, in China, Liu, et al. [16] and Li, et al. [15], respectively, provided the first reports that lucky bamboo anthracnose is caused by *C. truncatum* and *C. karstii*. Nevertheless, to the best of our knowledge, the occurrence of anthracnose symptoms caused by *C. gloeosporioides* was observed for the first time on *D. sanderiana* in China. The report of *C. gloeosporioides* on lucky bamboo in China widens the range of *Colletotrichum* species involved in the anthracnose disease of this popular ornamental houseplant. The occurrence and spread of *C. gloeosporioides* could be a new potential threat to lucky bamboo production in China. Further investigation is needed on the development of molecular diagnostic techniques and control measures for the anthracnose disease.

4. Materials and Methods

4.1. Sampling and Isolation

Infected stem samples displaying typical anthracnose symptoms were collected from five independent lucky bamboo plants. Stem tissues containing acervuli and spore masses were surface sterilized in a 1% sodium hypochlorite solution for 2 minutes, and rinsed three to four times with sterilized distilled water. The stem tissues were then aseptically cut into small pieces (approximately 0.5 cm long), plated onto potato dextrose agar (PDA) containing rifampicin (100 μg/ml) and streptomycin sulfate (100 μg/ml), and incubated at 25 °C in the dark. After 3 days, the emerging fungal colonies that grew from the stem tissues were sub-cultured on PDA and incubated at 25 °C in the dark. Pure cultures of the isolates were obtained using a single-spore isolation method described by Fei, et al. [20].

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4.2. DNA Extraction, PCR Amplification and Sequencing

Mycelia of isolates were obtained by inoculating the mycelial plugs into 100 ml of liquid potato dextrose broth (PDB) in a 250-ml baffle flask, culturing them at 25°C with shaking for at least 3 days, and harvesting them by filtration [21]. Total genomic DNA was extracted from the mycelium using the DNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's protocols. The amount and purity of extracted DNA were determined by a NanoDrop ND-3300 fluorospectrometer (Thermo-Fisher Scientific, USA). DNA integrity was visualized by electrophoresis on 1% (w/v) agarose gels that were stained with ethidium bromide (0.1 mg/l) and viewed under transmitted ultraviolet light.

The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified using universal primers ITS1 and ITS4 [22]. The *actin* (*ACT*) gene was amplified using primer pairs ACT-512F and ACT-783R [23]. The *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene was amplified using primers GDF1 and GDR1 [24]. PCR amplification was carried out in 50-µl reaction mixtures containing 25 µl of 2×PrimeSTAR Max Premix (Takara, Japan), 0.3 µM each primer (GenScript Corporation, China), and 20 ng of genomic DNA. All reactions were performed under the following thermal conditions: 30 cycles of 98°C for 10 s, 55°C for 5 s, and 72°C for 5 s using a PTC2000 PCR instrument (MJ Research, USA). Following PCR amplification, the quality and concentration of PCR products were verified on 1% agarose gels stained with ethidium bromide (0.1 mg/l), viewed with transmitted ultraviolet light, and the sizes of PCR products were determined against Marker II (Tiangen, China). The bands of expected size were excised from the agarose gel, purified by the Agarose Gel DNA Extraction Kit from Takara, and subsequently ligated into the pMDTM19-T vector (Takara, Japan) at 4 °C overnight. Ligated DNA was transformed into *Escherichia coli* DH5α cells (Tiangen, China) as described previously [25]. Positive clones were selected by PCR amplification of inserts using the M13 forward primer and the reverse primers of the corresponding targets. The colony PCRs were carried out directly with a suspension of transformed bacteria in ultrapure water. Clones containing the bands of interest were selected according to the expected PCR product sizes. Plasmid DNA was isolated from the positive clones using the plasmid DNA purification kit (Promega Corporation, USA) according to the manufacturer's instructions. The inserts were sequenced bi-directionally using universal primers M13-F/R. Sequencing was performed by the GenScript Corporation (Nanjing, China).

4.3. Sequence Alignment and Phylogenetic Analysis

Comparative analysis of the inserted fragments (*ITS*, *ACT*, and *GAPDH*) of all the isolates was performed with ClustalW in BioEdit version 7.0.90 (www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequences of selected isolates were subsequently deposited in GenBank with accession numbers. The *ITS*, *ACT*, and *GAPDH* sequences obtained in this study, together with the sequences of 16 strains representing closely related species of *Colletotrichum* retrieved from GenBank (Table 1), were aligned using MAFFT version 7 [26] (http://mafft.cbrc.jp/alignment/server/). For each strain, the sequences of *ITS*, *ACT*, and *GAPDH* were optimized to the same length and concatenated in the SATé-II v.2.2.7 (Simultaneous Alignment and Tree Estimation) high-throughput alignment platform (http://phylo.bio.ku.edu/software/sate/sate.html) [27]. Phylogenetic analyses of the concatenated sequence data of *ITS*, *ACT*, and *GAPDH* were performed by the Maximum Composite Likelihood (ML) method using the substitution model of the Tamura-Nei model [28], with sequence distances calculated in MEGA version X [29]. *Alternaria solani* CBS 113403 was included as an outgroup in the phylogenetic analyses. Confidence intervals were estimated using bootstrap analysis with 1,000 replications. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

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Table 1. Sources of the strains and GenBank accession numbers used in this study.

Species	Strain no.[1]	GenBank accession number (*ITS*, *ACT* and *GAPDH*)[2]		
		ITS	*ACT*	*GAPDH*
C. gloeosporioides	FGZ-1	MH752444.1	MH757114.1	MH757113.1
	Tax-1	KR075160.1	KX185669.1	KX378683.1
	BH9-1	AB632351.1	JN184702.1	JN211078.1
	jo-3	MN913584.1	MN919196.1	MN919195.1
C. dracaenophilum	CBS 118199	JX519222.1	JX519238.1	JX546707.1
C. boninense	MAFF 305972	AB051400.1	HM582001.1	GQ221769.1
C. petchii	CBS 378.94	JQ005223.1	JQ005571.1	JQ005310.1
C. fuscum	CBS 130.57	JQ005762.1	JQ005825.1	KM105530.1
C. higginsianum	CBS 128508	KM105190.1	KM105400.1	KM105543.1
C. linicola	CBS 172.51	JQ005765.1	JQ005828.1	KM105581.1
C. destructivum	C08077	GU935874.1	GU935795.1	GU935854.1
C. dematium	CBS 125.25	MH854810.1	GU227917.1	GU228211.1
C. coccodes	CBS 103.16	JX546820.1	JX546628.1	JX546724.1
C. orbiculare	CBS 274.54	KF178462.1	KF178559.1	KF178486.1
C. trifolii	CBS 128554	MH865019.1	KF178573.1	KF178500.1
C. truncatum	CBS 119189	GU227863.1	GU227961.1	GU228255.1
C. orchidophilum	CBS 632.80	JQ948151.1	JQ949472.1	Q948481.1
C. karstii	CORCG6	HM585409.1	HM581995.1	HM585391.1
*Alternaria solani**	CBS 113403	KJ718243.1	JQ646233.1	JQ646333.1

[1] *CBS* Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; *MAFF* NIAS GeneBank, Microorganism Section, Tsukuba, Japan; [2] *ITS* nuclear ribosomal internal transcribed spacer; *ACT* actin; *GAPDH* glyceraldehyde-3-phosphate dehydrogenase; *indicates the outgroup isolate.

4.4. Morphology Assay

Pure culture FGZ-1 was grown on potato dextrose agar (PDA) (200 g of potato, 20 g of dextrose, 15 g of agar powder, and 1,000 ml of distilled water) or V8 juice medium (100 ml of V8 tomato juice, 1 g of $CaCO_3$, and 15 g of agar per 1000 ml of distilled water) [30] and incubated at 25±2°C with a 12-h photoperiod for analyzing the colony morphology [31]. The colony morphology and color were recorded on day nine. The surface of the PDA cultures was flooded with sterilized distilled water and gently scraped to prepare spore suspension. The conidial suspension was then filtered through two layers of Miracloth (Calbiochem, San Diego, CA) to remove mycelia and then placed at 25°C in darkness on the hydrophobic surface of a GelBond membrane (Lonza) to allow germination and appressoria formation. The detailed fungal structures (conidia, germ tube, appressorium, and acervuli) of the obtained isolates were examined under a Zeiss Axio Scope. A1 microscope (equipped with a high-resolution Carl Zeiss AxioCam MRc5x microscopy camera), and at least 30 units per structure were measured.

4.5. Pathogenicity assay

One fungal isolate (FGZ-1) was used as inoculum. For pathogenicity assessment, healthy one-year-old lucky bamboo plants were bought from a flower market in Nanjing, China, planted individually in containers with water, and placed in a greenhouse at day/night temperatures of 26°/23°C under a 16-h light/8-h dark photoperiod for five days. The stems were cut into cylindrical segments (approximately 0.5 cm in width × 2 cm in height) and surface sterilized with 70% ethanol, and the top and bottom bases were inoculated with mycelial plugs (5 mm diameter from 7-day-old PDA cultures). Sterile PDA plugs were inoculated as a control. Inoculation sites were subsequently covered with Parafilm strips to prevent dehydration and to hold the mycelial plugs in position. In each inoculation procedure, there were three stem segments inoculated per treatment, and the pathogenicity test was repeated three times. All treated stem segments were incubated at 25°C in a moist chamber (relative humidity >90%) with a 12-h photoperiod and observed for symptom

appearance every three days after inoculation. For the pathogenicity assay on live plants, the conidial suspension was prepared as mentioned above and adjusted to 1×10^6 conidia/ml using a hemocytometer. Four plants per treatment were spray- inoculated with 10 ml of conidial suspension, sealed with plastic wrap to prevent desiccation following the method described by Xiong, et al. [30], and placed back in a greenhouse. Simultaneously, sterile distilled water was used as a control. All experiments were repeated three times. Fungi were reisolated from any resulting lesions, and the morphological and cultural characteristics of the isolated fungi were compared with those of the original fungus.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abdel-Rahman, T.F.; Abdel-Megeed, A.; Salem, M.Z. Characterization and control of *Rhizoctonia solani* affecting lucky bamboo (*Dracaena sanderiana* hort. ex. Mast.) using some bioagents. *Scientific Reports* **2023**, *13*, 6691, doi:10.1038/s41598-023-33628-8.
2. Junaid, A.; Mujib, A.; Sharma, M. Cell and tissue culture of *Dracaena sanderiana* Sander ex Mast: a review. *Hamdard Medicus* **2009**, *52*, 31-35.
3. Morsy, A.A.; Elshahawy, I.E. Anthracnose of lucky bamboo *Dracaena sanderiana* caused by the fungus *Colletotrichum dracaenophilum* in Egypt. *Journal of Advanced Research* **2016**, *7*, 327-335, doi:10.1016/j.jare.2016.01.002.
4. Chongtham, N.; Bisht, M.S. *Bamboo shoot: superfood for nutrition, health and medicine*; CRC Press: 2020.
5. Hilal, A.; El-Argawy, E.; Korany, A.E.; Fekry, T. Chemical and biological control of *Dracaena marginata* leaf spots in northern Egypt. *International Journal of Agriculture & Biology* **2016**, *18*, 1201-1212.
6. Santos, M.V.O.D.; Luz, E.D.M.N.; de Souza, J.T. First record of *Phytophthora nicotianae* causing leaf blight on *Dracaena sanderiana*. *New Disease Reports* **2011**, *24*, 28-28, doi:10.5197/j.2044-0588.2011.024.028.
7. Abbasi, M.; Aliabadi, F. First report of stem rot of *Dracaena* caused by *Aspergillus niger* in Iran. *Plant Health Progress* **2008**, *9*, 48, doi:10.1094/php-2008-0212-01-br.
8. Abedi-Tizaki, M.; Zafari, D.; Sadeghi, J. First report of *Fusarium solani* causing stem rot of *Dracaena* in Iran. *Journal of Plant Protection Research* **2016**, *56*, 100-103, doi:10.1515/jppr-2016-0013.
9. Lahuf, A.A. First report of causing stem and root rot on lucky bamboo (*Dracaena braunii*) in Iraq. *Hellenic Plant Protection Journal* **2019**, *12*, 1-5, doi:10.2478/hppj-2019-0001.
10. Kim, S.-W.; Jeong, Y.; Yang, K.-Y.; Jeong, R.-D. First report of natural infection of *Dracaena braunii* by pepper mild mottle virus in Korea. *Journal of Plant Pathology* **2022**, *104*, 1579-1579, doi:10.1007/s42161-022-01205-z.
11. Bobev, S.G.; Castlebury, L.A.; Rossman, A.Y. First report of *Colletotrichum dracaenophilum* on *Dracaena sanderiana* in Bulgaria. *Plant Disease* **2008**, *92*, 173-173, doi:10.1094/pdis-92-1-0173a.
12. Komaki, A.M.; Aghapour, B.; Aghajani, M.A. First report of *Colletotrichum dracaenophilum* on *Dracaena sanderiana*. *Rostaniha* **2012**, *13*, 111-112, doi:10.22092/botany.2012.101401.
13. Sharma, K.; Merritt, J.L.; Palmateer, A.; Goss, E.; Smith, M.; Schubert, T.; Johnson, R.S.; van Bruggen, A.H.C. Isolation, characterization, and management of *Colletotrichum* spp. causing anthracnose on lucky bamboo (*Dracaena sanderiana*). *HortScience horts* **2014**, *49*, 453-459, doi:10.21273/hortsci.49.4.453.
14. Macedo, D.M.; Barreto, R.W. *Colletotrichum dracaenophilum* causes anthracnose on *Dracaena braunii* in Brazil. *Australasian Plant Disease Notes* **2016**, *11*, 5, doi:10.1007/s13314-016-0192-7.
15. Li, Y.L.; Yan, Z.B.; Wang, Y.H.; Lin, Q.K.; Wang, S.B.; Zhou, Z. First report of *Colletotrichum karstii* causing anthracnose on lotus bamboo (*Dracaena sanderiana*) in China. *Plant Disease* **2018**, *102*, 2641-2641, doi:10.1094/pdis-04-18-0579-pdn.

16. Liu, Y.L.; Lu, J.N.; Zhou, Y.H. First report of *Colletotrichum truncatum* causing anthracnose of lucky bamboo in Zhanjiang, China. *Plant Disease* **2019**, *103*, 2947, doi:10.1094/pdis-05-19-1122-pdn.
17. Damm, U.; Sato, T.; Alizadeh, A.; Groenewald, J.Z.; Crous, P.W. The *Colletotrichum dracaenophilum*, *C. magnum* and *C. orchidearum* species complexes. *Studies in mycology* **2019**, *92*, 1-46.
18. Agostini, J.P.; Timmer, L.W.; Mitchell, D. Morphological and pathological characteristics of strains of *Colletotrichum gloeosporioides* from citrus. *Phytopathology* **1992**, *82*, 1377-1382.
19. Farr, D.; Rossman, A. Fungal Databases, US National Fungus Collections, ARS, USDA. Retrieved January 16, 2018. **2018**.
20. Fei, L.; Lu, W.; Xu, X.; Yan, F.; Zhang, L.; Liu, J.; Bai, Y.; Li, Z.; Zhao, W.; Yang, J.; et al. A rapid approach for isolating a single fungal spore from rice blast diseased leaves. *Journal of Integrative Agriculture* **2019**, *18*, 1415-1418, doi:10.1016/S2095-3119(19)62581-5.
21. Xiong, Q.; Xu, J.; Zhao, Y.; Wang, K. *CtPMK1*, a mitogen-activated-protein kinase gene, is required for conidiation, appressorium formation, and pathogenicity of *Colletotrichum truncatum* on soybean. *Annals of Applied Biology* **2015**, *167*, 63-74, doi:10.1111/aab.12209.
22. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **1990**, *18*, 315-322.
23. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **1999**, 553-556.
24. Guerber, J.C.; Liu, B.; Correll, J.C.; Johnston, P.R. Characterization of diversity in *Colletotrichum acutatum* sensu lato by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. *Mycologia* **2003**, *95*, 872-895.
25. Sambrook, J.; Fritsch, E.F.; Maniatis, T. *Molecular cloning*; Cold spring harbor laboratory press New York: 1989; Volume 2.
26. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics* **2017**, doi: 10.1093/bib/bbx108.
27. Liu, K.; Warnow, T.J.; Holder, M.T.; Nelesen, S.M.; Yu, J.; Stamatakis, A.P.; Linder, C.R. SATé-II: very fast and accurate simultaneous estimation of multiple sequence alignments and phylogenetic trees. *Systematic Biology* **2012**, *61*, 90-90, doi: 10.1093/sysbio/syr095.
28. Tamura, K.; Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution* **1993**, *10*, 512-526.
29. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **2018**, *35*, 1547-1549, doi: 10.1093/molbev/msy096.
30. Xiong, Q.; Zhang, L.; Waletich, J.; Zhang, L.; Zhang, C.; Zheng, X.; Qian, Y.; Zhang, Z.; Wang, Y.; Cheng, Q. Characterization of the papain-like protease p29 of the Hypovirus CHV1-CN280 in its natural host fungus *Cryphonectria parasitica* and nonhost fungus *Magnaporthe oryzae*. *Phytopathology®* **2019**, *109*, 736-747, doi:10.1094/phyto-08-18-0318-r.
31. Yang, Y.; Liu, Z.; Cai, L.; Hyde, K.; Yu, Z.; McKenzie, E. *Colletotrichum* anthracnose of *Amaryllidaceae*. *Fungal Diversity* **2009**, *39*, 123-146.