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Rapid communication

First report of *Fusarium solani* causing stem rot of *Dracaena* in Iran

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Abstract: In July 2013, symptoms of stem rot were observed in the *Dracaena sanderiana* cuttings in greenhouses of Mahallat County, Markazi Province, Iran. The symptoms first appeared as severe wilting. Later, leaves became brown and necrotic. Symptoms on the cuttings were observed as rotted areas on the middle of the stems. The cortical tissues of the plants showed a distinct brown discoloration. Eventually, the infected plants died. The pathogen was isolated from *Dracaena* stems and identified as *F. solani* by a fragment of the translation elongation factor 1-alpha (*EF-1α*) gene. *Fusarium solani* was confirmed by a pathogenicity test, and the causal agent was re-isolated from infected *D. sanderiana* plants. To the best of our knowledge, this is the first report of stem rot caused by *F. solani* on the cuttings of *D. sanderiana.*

Key words: *Dracaena*, *Fusarium solani*, stem rot

Introduction

Dracaena sanderiana Sander ex Mast., common name Lucky Bamboo, is a tropical, tender, Sander ex Mast. evergreen perennial, which is native to Cameroon in tropical West Africa. Lucky Bamboo is a vertical, woody, evergreen shrubby species with slender stems and flexible strap-shaped leaves. It grows as understory plants in rainforests (Grewal *et al*. 1999). As a popular houseplant, it can survive under various indoor conditions because it grows very well in indirect lighting. It has become widely popular due to its ability to interweave eastern mysticism with western new age culture. This plant is usually propagated by stem cuttings.

Many fungi species have already been reported as the causal agents of stem rot and leaf spot from *Dracaena* plants. Abbasi and Aliabadi (2008) observed symptoms of stem rot in the *D. sanderiana* cuttings at a local market in Tehran, Iran. Among fungal pathogens on *Dracaena* plants, only stem rot caused by *Aspergillus niger* has been recorded in Iran (Abbasi and Aliabadi 2008). Also, *Colletotrichum dracaenophilum* as a causal agent of stem rot in *D. sanderiana*, has been reported from Bulgaria (Bobev *et al*. 2008). So far, many *Fusarium* species including *F. equiseti, F. oxysporum, F. proliferatum, F. phyllophitum, F. semitectum, F. solani,* and *F. subglutinans* have been reported to occur on the genus *Dracaena,* mainly causing leaf spots (Choi *et al*. 2008; Thongkantha *et al*. 2008). It was reported by Choi *et al*. (2008) that *F. solani*, *F. oxysporum,* and *F. moniliforme* are causal agents of stem rot in *Dracaena marginata* Lam. in Korea. Baka and Krzywinski (1996) found that among fungi isolated from *Dracaena ombet* Heuglin ex Kotschy & Peyr only *Cladosporium dracaenatum* and *Alternaria alternata* were pathogenic on the leaves (leaf spot). Mahallat is one of the most important areas in Iran, where many ornamental plants such as Lucky Bamboo are produced. Contamination of ornamental plants by various pathogenic agents could irreparably damage the ornamentals. This is why identifying these agents is essential to their control. The present study aimed to identify the pathogen causing stem rot of *D. sanderiana*.

Materials and Methods

Isolation and morphological identification of fungi causing stem rot

A causal pathogen of stem rot was isolated from infected *D. sanderiana* cuttings. Causal agents were isolated from the rotted and discolored stem tissues. The infected stem tissues were sterilised with a 1% bleach solution for 30 s and washed five times with distilled water. Then, the tissues were placed on Potato Dextrose Agar (PDA) amended with 0.5 g · l^{-1} streptomycin sulfate, and Carnation Leaf Agar (CLA) medium, for 14 days, under a 12-h alternating cycle of light and dark, at 25°C. Fungal isolates were identified according to *Fusarium* key by Nelson *et al*. (1983).

Molecular identification and phylogenetic analysis

For molecular analysis, DNA was extracted from the mycelia by centrifugation at 180 rpm for 48 h at 25°C, using

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the modified cetyl trimethylammonium bromide (CTAB) protocol (Nicholson *et al*. 1997). The molecular identity of the fungus was confirmed by amplifying a fragment of the translation elongation factor 1-alpha (*EF1-α*) gene using the primers EF-1/EF-2 (ATGGGTAAGGARGACAAGAC/GGARGTACCAGTSATCATGTT), as described by Geiser *et al.* (2004). To amplify this region, the reaction mixtures were prepared in a total volume of 25 μl with a final concentration of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.2 mM of each dNTP, and 1.5 mM $MgCl_2$. For each reaction, 1.5 U of *Taq* polymerase, 15 pmol of each primer, and approximately 25 ng of fungal template DNA were used. Polymerase chain reaction (PCR) conditions were as follows: denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 50 s, annealing at 55°C for 50 s, extension at 72°C for 50 s, and final extension at 72°C for 7 min. Polymerase chain reaction products were purified using a Wizard PCR prep kit (Promega, Madison, WI, USA) and sequenced using a commercial sequencing service provider (Macrogen, Seoul, Korea), and deposited (Accession No. KR021390) in GenBank. The sequence was compared with sequences in the GenBank database using the NCBI BLAST search program. A pair-wise alignment of all sequences was completed using the ClustalW program. Phylogenetic analysis was conducted by neighbor-joining methods using MEGA version 5.0.

Pathogenicity test

For the pathogenicity test, *F. solani* was incubated for 2 weeks on PDA at 25°C. Conidial suspension was separated from the mycelium using 10 ml sterile water and cheesecloth. The conidial suspension was adjusted to 1×10^6 spores · ml^{-1}, and then 0.1 ml of conidial suspension was injected into the cortex region under the epidermis in the stem of healthy *Dracaena* plants, between the nodes. Sterilised water was used as the control. All *Dracaena* plants were placed in a growth chamber with a night temperature of 20°C, a day temperature of 25°C, and a 12-h photoperiod. The *Dracaena* plants were re-examined one month later. At this time, the pathogen was constantly re-isolated from artificially developed symptoms.

Results

Morphological identification and pathogenicity tests

In July 2013, symptoms of stem rot were observed in the *D. sanderiana* cuttings in greenhouses in Mahallat County, Markazi Province, Iran. The symptoms first appeared as severe wilting. Later, the leaves became brown and necrotic. Symptoms on the cuttings were observed as rotted areas on the middle of the stems. Cortical tissues of plant showed a distinct brown discoloration. Eventually, the infected plants died (Fig. 1A). The isolated fungus was morphologically identified as *F. solani* on CLA and PDA media according to Nelson *et al*. (1983). Fungal colonies on PDA medium were cream or white and in rare cases, the lower surface was light violet. Ring-shaped sporodochia, with a cream or sometimes blue color were observed (Fig. 1B). Macroconidia, and microconidia as micromorphological features of this fungus, were observed in CLA medium. The fungus produced two types of spores on CLA: microconidia which were thin-walled, hyaline, fusiform to ovoid, generally 1- or 2-celled (3.2–9.1 × 1.5–2.5 μm) (Fig. 1C), and macroconidia which were slightly curved with blunt and rounded apical cell, and rounded or foot-shaped basal cells, mostly 3- to 4-celled (14.2–34.2 × 2.3–3.5 μm) (Fig. 1C). Conidiogenous cells were observed as monophialides (quite long) (Fig. 1D).

Pathogenicity was tested twice. Development of typical symptoms on leaves (wilting and necrosis) started after 15 days (Fig. 1E). Symptoms observed on inoculated plants were similar to those in the greenhouses, including leaf chlorosis, necrosis, and internal brown discoloration of the stem (Fig. 1F). In the end, all of the inoculated plants died, while the control plants showed no symptoms. Koch's postulates were fulfilled and *F. solani* was successfully re-isolated from artificially developed symptoms. The fungus re-isolated from infected stem tissues showed the same characteristics as described above, and was totally identical in appearance to the isolates used to inoculate the plants.

Phylogenetic analysis

Molecular characterisation of the pathogen was conducted by amplifying a fragment of the translation elongation factor 1-alpha (*EF-1α*) gene, using the primers EF-1 and EF-2. A 690 base pair long sequence that showed 100% similarity to the sequences of several *F. solani* strains, was obtained. The NCBI Accession Nos. DQ247354.1, DQ247593.1, DQ247604.1, KF255995.1, and KC820964.1 indicated the causal fungus as FBM (Fig. 2).

Discussion

Previously, *A. niger* and *C. dracaenophilum* as stem rot, have been reported from *D. sanderiana* in Iran and Bulgaria, respectively (Abbasi and Aliabadi 2008; Bobev *et al*. 2008). The *Dracaena* genus has several species, such as *D. marginata, D. braunii,* and *D. americana. Thielaviopsis paradoxa* causing stem rot of *D. marginata* has been reported from Brazil (Santos *et al*. 2012). It was found by Zaher *et al*. (2005), that all isolates of *Corynespora cassiicola* were pathogenic to *D. marginata* in Egypt.

To the best of our knowledge, this is the first report of stem rot caused by *F. solani* on the cuttings of *D. sanderiana*. According to the results, *F. solani* is a new fungal pathogenic agent for the *D. sanderiana* plant in Iran. Lucky Bamboo (*D. sanderiana*) is one of the most important cut-flower crops grown worldwide on a commercial scale. It is the main production of Mahallat, one of the most important ornamental plants production centers of Iran. So, occurrence of the fungus in this area is expected to have a significant economic impact on *D. sanderiana* plants. For this reason, appropriate measures must be done to control this agent.

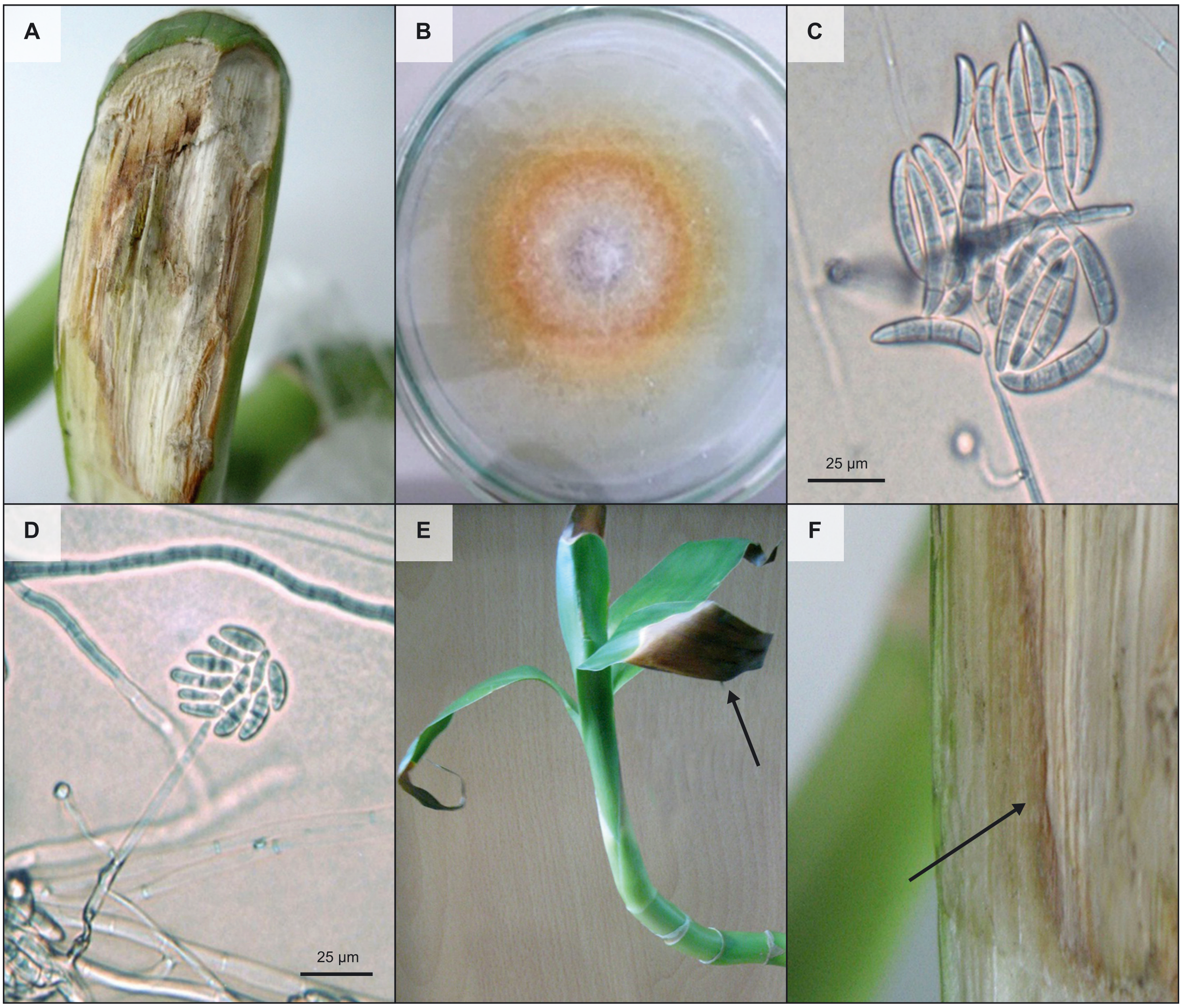

Fig. 1. Symptoms of stem rot disease in *Dracaena sanderiana* caused by *Fusarium solani*: A – brown discoloration (rotting) on the middle of the stem; B – colony on PDA medium; C – macroconidia and microconidia; D – phialides; E – pathogenicity test, inoculated *D. sanderiana* plant (leaf necrosis); F – internal brown discoloration of the stem after pathogenicity test

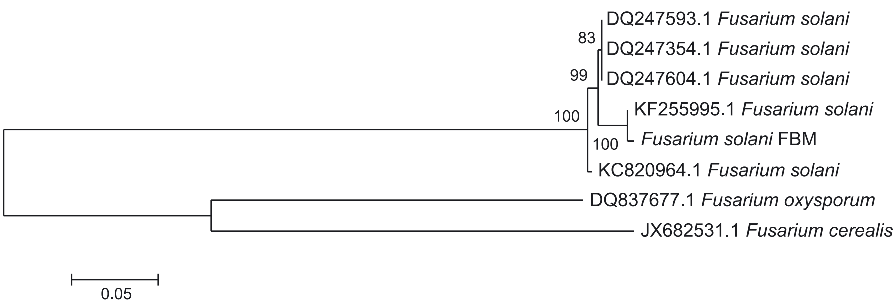

Fig. 2. Phylogenetic relationships of *Fusarium solani* (FBM) on the basis of the translation elongation factor 1-alpha (*EF1-α*) gene. A phylogenetic tree was constructed using the MAGA 5 program, and phylogenetic distances were calculated using the neighbor-joining method

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