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SHORT COMMUNICATION

First report of *Fusarium proliferatum* causing stem and root rot on lucky bamboo (*Dracaena braunii*) in Iraq

A.A. Lahuf

Summary Lucky bamboo (*Dracaena braunii*) is a popular ornamental plant in Iraq. Individuals of this plant showing stem and root rot symptoms were observed during a survey conducted from November 2015 to February 2016 in several nurseries in Kerbala province, Iraq. Based on morphological characteristics and sequence analyses of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA), the pathogen was identified as *Fusarium proliferatum*. This is the first report of stem and root rot caused by *F. proliferatum* on lucky bamboo (*D. braunii*) in Iraq.

Additional keywords: molecular identification, morphological characterization, pathogenicity

Lucky bamboo [*Dracaena braunii* (= *D. sanderiana*)] is an evergreen perennial ornamental plant of the Asparagaceae family, native to Cameroon in West Africa (Macedo and Barreto, 2016). Recently, it has become a popular ornamental houseplant in Iraq because of its beautiful appearance, low cost, its ability to grow under diverse indoor conditions and no experience required to take care of it.

During a survey conducted between November 2015 and February 2016 in ornamental nurseries in Kerbala province, Iraq, *D. braunii* plants showing stem and root rot symptoms were observed (Fig. 1A-D). Symptoms initially appeared on roots as water-soaked, red-brown lesions, becoming dark brown with time (Fig. 1B, D). Eventually, affected roots became completely rotten. On the lower part of the stem, a yellow discoloration was observed, tissues were soft and as the rot progressed, the diseased plants died Fig. 1A, C). The disease resulted in a significant loss of *D. braunii* plants in most of the nurseries examined. However, the pathogen causing this disease has not been previously investigated in Iraq. Thus, this study aims to isolate and identify the pathogen and assess its pathogenicity.

The symptomatic tissues of roots and stems were surface disinfected in 1% sodium hypochlorite for 2 min, rinsed three times with sterilized distilled water and dried with sterilized filter paper. Then the tissues were aseptically cut (0.5-1 cm long), placed onto 2% water agar (WA) medium and incubated in the dark at 25 ± 1°C for 3 days. Subsequently, a hyphal tip of each emerging fungal colony was sub-cultured on potato dextrose agar (PDA) medium supplemented with streptomycin sulphate (200 mg/l) and incubated in the dark at 25 ± 1°C for 7 days (Watanabe, 2010). Fungal colonies grew rapidly producing white aerial mycelia, occasionally with a violet pigmentation (Fig. 1E). The reverse colony color was pink to dark violet (Fig. 1F). Macroconidia were colourless and slightly curved with 3-5 septa and average size 33.4 × 3.2 µm. Microconidia were more than macroconidia, colourless, non-curved, occasionally in chains, with 0-1 septa and average size 8.2 × 3.1 µm. No chlamydospores were observed (Fig. 1G). These morphological features agree with the description of Leslie and Summerell (2006), except for the septation of the microconidia (0-septate according to Leslie and Summerell, 2006). However, the number of septa found in the present study are in line with

Plant Protection Department, Agriculture College, University of Kerbala, Kerbala, Iraq.
E-mail: adnan.lahuf@yahoo.com

the description of microconidia provided by Ichikawa, and Aoki (2000), Zhang *et. al.* (2013) and Kim *et. al.* (2016). Based on these morphological characteristics, the fungus was putatively identified as *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg. To fulfil Koch's postulates, the pathogenicity of the isolated fungus was tested on 20 healthy lucky bamboo plants growing in 0.5 L containers filled with the commercial nutrition solution (AgroFiro®, Aljoud Company, Iraq). Fifteen plants were inoculated by adding directly to the nutrient solution five mycelium plugs (each 0.5 cm in diameter) cut from a 7-day old colony of *F. proliferatum* grown on PDA medium. The same number of plugs of un-inoculated PDA was added to the nutrient solution of the remaining five lucky bamboo plants, which were used as controls. All plants were incubated in a growth cabinet at 25 ± 2°C with 12-h photoperiod and 70% humidity. After 21 days, stem and root rot symptoms identical to those observed in the nurseries appeared on 13 out of the 15 inoculated plants. The control plants were symptomless. The fungal pathogen was re-isolated from the symptomatic plant tissues and showed the same morphological characteristics as described above.

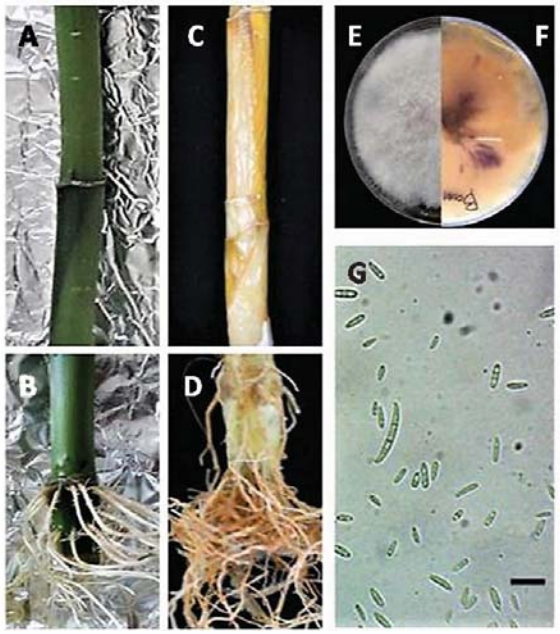

Figure 1. Symptoms of stem and root rot on *Dracaena braunii* plants, and cultural and morphological characteristics of the causal agent, *Fusarium proliferatum*. Stem (A) and roots (B) of a healthy *D. braunii* plant; rot symptoms on stem (C) and roots (D) of *D. braunii* plant infected by *F. proliferatum*; (E)-(F): colony of *F. proliferatum* on PDA medium (E: top surface and F: lower surface); (G): micro- and macroconidia of *F. proliferatum*; bar in (G) = 10 µm.

To confirm the initial morphological identification, the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) from the isolated fungus was sequenced. Genomic DNA of *F. proliferatum* was extracted from pure cultures using a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. The universal primer pair ITS1/ITS4 was used to amplify the entire ITS region by PCR (White *et al*., 1990). The 679 bp amplicon was sequenced (Macrogen, Korea; http://www.macrogen.com/en/main/index.php) using the same primers used for the PCR amplification. The sequence was deposited into the GenBank database and was identified with the accession number MF099864.1. Subsequently, BLAST analysis of the isolate sequence showed >99% identity with several known sequences of *F. proliferatum* species. Phylogenetic analysis was performed using MEGA 7, utilizing the neighbor-joining technique (Tamura *et al*., 2013). This analysis showed that the ITS sequence of the isolate MF099864.1 was grouped in a clade comprising reference isolates of *F. proliferatum*. The out-group isolates were those of *Fusarium oxysporum* (accession No: EU326203.1), *F. camptoceras* (accession No: KU055634.1) and *F. solani* (accession No: L36632.1, L36634.1, AY097316.1, AY097317.1 and AY097318.1) (Fig. 2). Thus, these results support the preliminary morphological identification of the fungus as *F. proliferatum* (Leslie and Summerell, 2006; Zhang *et. al*., 2013; Aoki *et al*., 2014).

Numerous fungal pathogens are known to affect *Dracaena* spp. worldwide. For example, *Colletotrichum dracaenophilum* was reported to cause stem rot on *D. braunii* (syn. *D. sanderiana*) in Bulgaria, USA, Egypt and Brazil (Bobev *et al.,* 2008; Sharma *et al.,* 2014; Macedo and Barreto, 2016; Morsy and Elshahawy, 2016). In Iran, *Fusarium solani* was

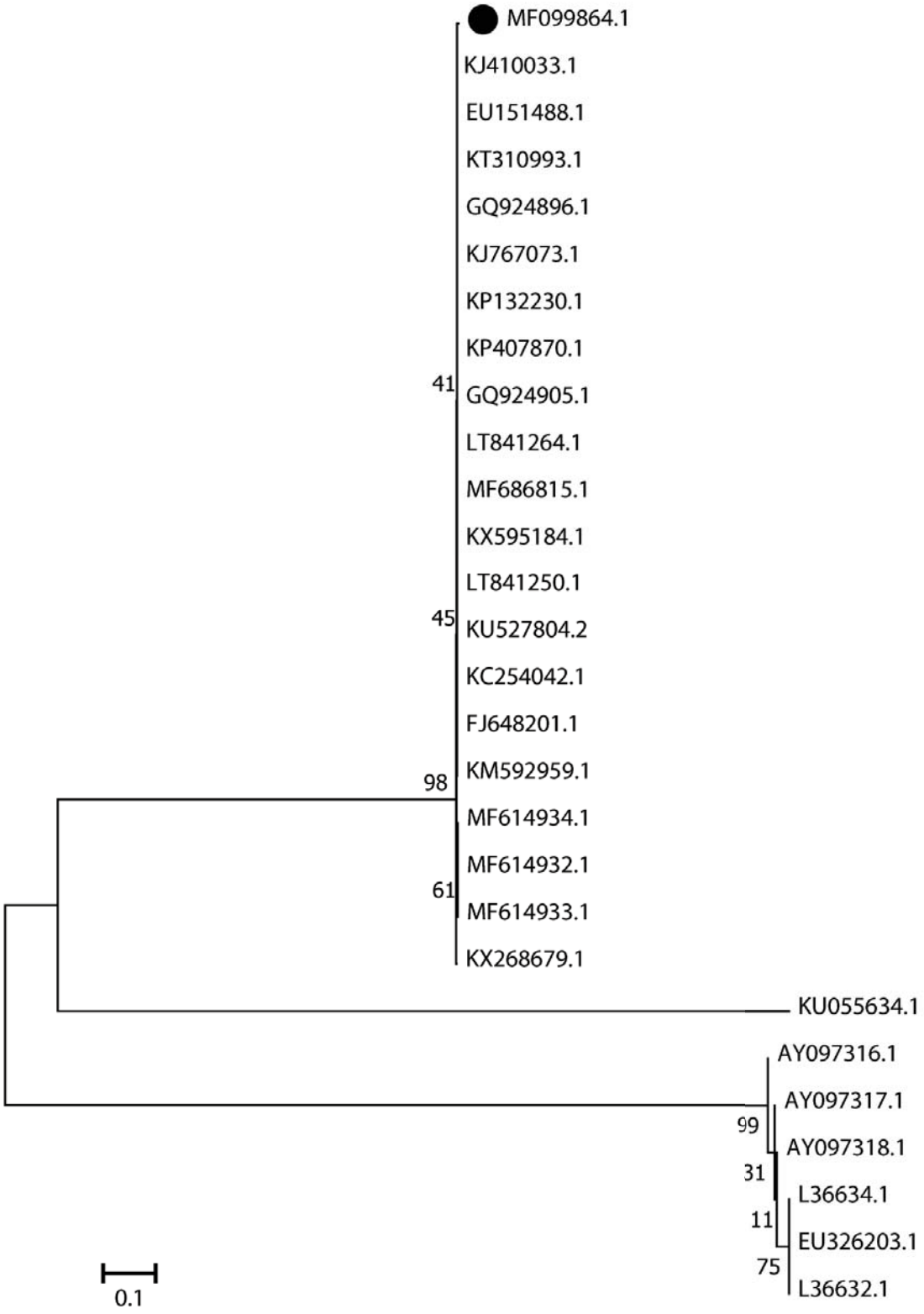

Figure 2. Phylogenetic tree constructed using ITS-rDNA sequences, presenting 21 known *Fusarium proliferatum* strains obtained from GenBank database, including that isolated in the present study from *Dracaena braunii* plants (MF099864.1; indicated with a black dot). Phylogenetic distances were calculated using the neighbor-joining method. Numbers above the branches refer to bootstrap values. *Fusarium oxysporum* (EU326203.1), *F. camptoceras* (KU055634.1) and *F. solani* (L36632.1, L36634.1, AY097316.1, AY097317.1 and AY097318.1) were the out-group species.

identified as causing stem rot disease on *D. sanderiana* (Abedi-Tizaki *et al.,* 2016). On the other hand, *F. proliferatum* is a devastating pathogen infecting a wide range of plant species throughout the world causing stem, crown and root rot as well as leaf proliferosis. In the USA and Canada, *F. proliferatum* was identified to cause root rot on *Glycine max* (soybean) (Arias *et al*., 2011; Chang *et al*., 2015). It was also reported on *Asparagus officinalis* (asparagus) causing crown and root rot in the USA and Turkey (Elmer, 1990; Özer *et al.,* 2011). In Argentina, *F. proliferatum* is described as a new pathogen causing root rot on *Vaccinium corymbosum* (blueberry) (Pérez *et al.,* 2011). In Malaysia, it was found associated with a stem rot disease of *Hylocereus polyrhizus* (Hawa *et al.,* 2013). In China, it was recorded causing root rot of *Medicago sativa* (alfalfa) and *Codonopsis lanceolata* (Cong *et al*., 2016; Gao *et al*., 2017). In Egypt, *F. proliferatum* var. *minus* was identified as the causal agent of leaf proliferosis disease of *D. sanderiana* (Wagih *et al*., 1989). To the best of my knowledge, this is the first report of *F. proliferatum* affecting *D. braunii* in Iraq.

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Literature cited

Abedi-Tizaki, M., Zafari, D. and Sadeghi, J. 2016. First report of *Fusarium solani* causing stem rot of *Dracaena* in Iran. *Journal of Plant Protection Research*, 56: 100-103.

Aoki, T., O'Donnell, K. and Geiser, D.M. 2014. Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. *Journal of General Plant Pathology*, 80: 189–201.

Arias, M.M.D., Munkvold, G.P. and Leandro. L.F. 2011. First report of *Fusarium proliferatum* causing root rot on soybean (*Glycine max*) in the United States. *Plant Disease*, 95(10): 316.

Bobev, S.G., Castlebury, L.A., Rossman, A.Y. 2008. First report of *Colletotrichum dracaenophilum* on *Dracaena sanderiana* in Bulgaria. *Plant Disease*, 92: 173–173.

Chang, K.F., Hwang, S.F., Conner, R.L., Ahmed H.U., Zhou, Q., Turnbull, G.D., Strelkov, S.E., McLaren, D.L. and Gossen, B.D. 2015. First report of *Fusarium proliferatum* causing root rot in soybean (*Glycine max* L.) in Canada. *Crop Protection,* 67: 52-58.

Cong, L.L., Sun, Y., Kang, J.M., Li, M.N., Long, R.C., Zhang, T.J. and Yang, Q.C. 2016. First report of root rot disease caused by *Fusarium proliferatum* on alfalfa in China. *Plant Disease,* 100 (12): 2526.

Elmer, W.H. 1990. *Fusarium proliferatum*, as causal agent in Fusarium crown root rot of asparagus. *Plant Disease*, 74: 938.

Gao, J., Wang, J., Yang, C., Wang, Y., Lu, B.H. and Yang, L.N. 2017. *Fusarium proliferatum*, a new pathogen causing *Codonopsis lanceolata* root rot in China. *Plant Disease*, 101(9): 1679.

Hawa, M.M., Salleh, B. and Latiffah, Z. 2013. Characterization and pathogenicity of *Fusarium proliferatum* causing stem rot of *Hylocereus polyrhizus* in Malaysia. *Annals of Applied Biology*, 163(2): 269–280.

Ichikawa, K. and Aokl, T. 2000. New leaf spot disease of *Cymbidium* species caused by *Fusarium subglutinans* and *Fusarium proliferatum*. *Journal of General Pathology,* 66: 213-218.

Kim, S. G., Ko, H., Hur, O., Luitel, B. P., Rhee J. H. Yoon, M., Baek, H. Ryu, K. and Sung, J. S. 2016. First report of Fusarium wilt caused by *Fusarium proliferatum* on safflower. *Research of Plant Disease*, 22(2): 111-115

Leslie, J.F. and Summerell, B.A. 2006. *The Fusarium laboratory manual*. Wiley-Blackwell. 388 p.

Macedo, D.M. and Barreto, R.W. 2016. *Colletotrichum dracaenophilum* causes anthracnose on *Dracaena braunii* in Brazil. *Australasian Plant Disease Notes*, 11: 5.

Morsy, A.A. and Elshahawy, I.E. 2016. Anthracnose of lucky bamboo *Dracaena sanderiana* caused by the fungus *Colletotrichum dracaenophilum* in Egypt. *Journal of Advanced Research,* 7: 327-335.

Özer, G., Bayraktar, H. and Oksal, E. 2011. First report of *Fusarium proliferatum* causing crown and root rot of asparagus officinalis in Turkey. *Journal of Plant Pathology*, 93: S4.63-S4.89.

Pérez, B.A., Berretta, M.F., Carrión, E. and Wright, E.R. 2011. First report of root rot caused by *Fusarium proliferatum* on blueberry in Argentina. *Plant Disease,* 95(11): 1478

Sharma, K., Merrit, J.L., Palmateer, A., Goss, E., Smith, M., Schubert, T., Johnson, R.S. and van Bruggen A.H.C. 2014. Isolation, characterization, and management of *Colletotrichum* spp. causing anthracnose on lucky bamboo (*Dracaena sanderiana*). *HortScience,* 49(4): 453–459.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30: 2725–2729.

Watanabe, T. 2010. *Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species (3rd Ed.)*. CRC Press, Taylor and Francis Group. 426 p.

Wagih, E. E., Shehata, M. R. A., Farag, S. A. and Dawood, M. K. 1989. Dracaena Leaf Proliferosis, a Newly Recorded Disease Affecting *Dracaena sanderiana* in Egypt. *Journal of Phytopathology*, 126 (1):7-16.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: Innis, M.A. *et al.* (eds). *PCR protocols: a Guide to Methods and Applications*. New York: Academic Press, p. 315–322.

Zhang, J., Wu, X., Bi, Y., Wu, Y., lin, G., He, Y. and Mao, Z. 2013. First report of *Fusarium proliferatum* infecting carnation (*Dianthus caryophyllus* L.) in China. *Journal of Phytopathology*, 161: 850–854.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη αναφορά του μύκητα *Fusarium proliferatum* ως αιτίου της σήψης στελέχους και ριζών σε φυτά *Dracaena braunii* (lucky bamboo) στο Ιράκ

A.A. Lahuf

Περίληψη Το *Dracaena braunii* (lucky bamboo) είναι ένα δημοφιλές καλλωπιστικό φυτό στο Ιράκ. Φυτά του συγκεκριμένου είδους, που εμφάνιζαν συμπτώματα σήψης του στελέχους και των ριζών, εντοπίστηκαν κατά τη διάρκεια επισκόπησης που διενεργήθηκε την περίοδο Νοέμβριος 2015-Φεβρουάριος 2016 σε αρκετά φυτώρια της επαρχίας Kerbala του Ιράκ. Με βάση τα μορφολογικά χαρακτηριστικά και τις αναλύσεις αλληλουχίας της περιοχής του εσωτερικού μεταγραφόμενου διαχωριστή (Internal Transcribed Spacer, ITS) του ριβοσωμικού DNA (rDNA), το παθογόνο ταυτοποιήθηκε ως ο μύκητας *Fusarium proliferatum*. Αυτή είναι η πρώτη αναφορά σήψης στελέχους και ριζών φυτών *D. braunii* από το μύκητα *F. proliferatum* στο Ιράκ.

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