Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium* Wilt of *Coleus forskohlii* Caused by *Fusarium oxysporum* in China

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Coleus forskohlii (Wild) Briq. is an aromatic plant in the Lamiaceae family cultivated primarily in India, Sri Lanka, Nepal, and China (Yunnan Province). This herb is considered to have medicinal properties, and the whole plant can be used to treat asthma, cancer, and other diseases with remarkable efficacy. Due to the high medicinal and economic value of *C. forskohlii*, it has been introduced to Tongcheng (N29°18'12.24", E113°53'59.36"), Hubei Province, for cultivation. However, severe *Fusarium* wilt disease of *C. forskohlii* has been epidemic in Tongcheng since 2018 with a disease incidence of 5 to 30% in surveyed fields. This disease is characterized typically by root rot, vascular discoloration, and leaf wilting of *C. forskohlii*, resulting in progressive plant death. Ten diseased plants were collected from the fields, and the roots and stems were rinsed in 70% ethanol for 5 min. Samples at the junction of diseased and healthy tissues (0.5 × 0.5 cm) were cut and placed on potato dextrose agar (PDA) for fungal isolation in a dark chamber at 28°C. Eventually, 10 pure isolates were obtained from hyphal-tip followed by single-spore purification on PDA. Seven of the purified isolates showed white aerial mycelium initially and secreted orange-brown pigment 8 days after incubation. Macroconidia were falciform, hyaline, three to five septate, and 2.02 to 4.17 µm (mean 2.98 µm) × 10.05 to 21.90 µm (mean 12.04 µm) in size (*n* = 30). These morphological characteristics resembled *Fusarium oxysporum* (Leslie and Summerell 2006), and we selected one of them for molecular identification. Genome DNA was extracted from isolate (RS-4) using the CTAB method (Mahadevakumar et al. 2018). The translation elongation factor 1 alpha (*EF-1α*) DNA sequence was amplified using primers EF1/EF2

(Geiser et al. 2004), and the second largest subunit of RNA polymerase II (*RPB2*) DNA sequence was amplified using primers fRPB2-5F/fRPB2-7cR (Liu et al. 1999). The obtained *EF-1α* sequence of RS-4 (MW219142) showed 100% identity with that of *F. oxysporum* (FD_01376) (*FUSARIUM-ID* database). *RPB2* sequences of RS-4 (MW219143) showed 100% identity with *F. oxysporum* (FD_01679) (*FUSARIUM-ID* database). Moreover, a phylogenetic tree of the *EF-1α* gene sequence of RS-4 was constructed based on the neighbor-joining method in MEGA7 software (Tamura et al. 2013) and revealed that strain RS-4 was closest to *F. oxysporum*. To test the pathogenicity of RS-4, six healthy leaves of *C. forskohlii* were collected and inoculated either with the colonized PDA discs (diameter, 5 mm) of RS-4 or control PDA discs, in a moist chamber at 25 ± 2°C. Five days later, brown-black lesions were observed on all inoculated leaves. However, the noninoculated leaves remained asymptomatic. For in vivo pathogenicity testing, 20-day-old *C. forskohlii* plants (*n* = 3) were inoculated with 10⁶ spores/ml of the RS-4 at a position approximately 1 cm above the soil. Three seedlings treated with sterile water were used as controls. These inoculated and control seedlings were incubated in a moist chamber (25 ± 2°C, 85% RH). Three days later, typical *Fusarium* rot symptoms were observed on all inoculated seedlings with rotten stems and withering leaves. Fungal pathogens were reisolated from the inoculated sites of in vitro and in vivo inoculations by repeating the above isolating operation and were reconfirmed through morphological features. This is the first report of *F. oxysporum* causing root rot on *C. forskohlii* in China. *F. oxysporum* is one of the most economically important fungal pathogens causing vascular wilt on a wide range of plants worldwide (Dean et al. 2012). The identification of *F. oxysporum* as the causal agent of the observed *Fusarium* wilt on *C. forskohlii* is critical to the prevention and control of this disease in the future.

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