Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of *Neopestalotiopsis clavispora* Causing Leaf Spot on *Dendrobium officinale* in China

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Dendrobium officinale Kimura L., an endangered orchid, is a precious herb used in Chinese traditional medicine (Zheng et al. 2005). In August 2021, significant indications of an unknown leaf spot disease were observed on greenhouse-grown *D. officinale* in Yueqing, Wenzhou (28.39°N, 121.04°E), Zhejiang Province, China, the main production center of this orchid. Approximately 20% of plants surveyed showed typical infection symptoms. Initially, symptoms appeared as small, circular black spots. As the disease developed, the center of the lesions was sunken with a black border. To determine the causal agent, 10 symptomatic samples were collected and all pieces from symptomatic leaves were used for isolating the pathogen. Tissues between healthy and necrotic areas were cut into pieces (5 × 5 mm, $n = 10$), disinfected with 10% NaOCl for 1 min, rinsed three times with sterile water, and dried on sterile tissue. Single samples were placed on potato dextrose agar medium (PDA) plates and incubated at 25°C in a dark biochemical incubator. After 3 days, hyphal tips growing from the disinfected tissues were individually transferred to new PDA plates and incubated at 25°C in the dark. Twelve identical fungal isolates were obtained from all symptomatic leaf fragments; DDO11 was chosen as a representative for further study. Colonies had white aerial mycelium after 5 days of culture at 25°C on PDA. Black viscous acervuli were scattered on the colony surface after 8 to 12 days of culture. Conidia were spindle shaped, five cells, four septa, average $29.3 \times 8.5 \mu\text{m}$ ($n = 30$). Apical and basal cells were lighter in color, and most were hyaline. The middle three cells were darker in color, and mostly brown. There were two to four colorless, transparent unbranched accessory filaments at the top, $32.5 \mu\text{m}$ in average length, and basal cells had a small appendage, $9.2 \mu\text{m}$ in

average length, $n = 30$. For species identification, the internal transcribed spacer (ITS) region, β -tubulin gene (*TUB2*), and translation elongation factor-1 α (*TEF-1 α*) were amplified (Qiu et al. 2020). The ITS, *TUB2*, and *TEF-1 α* gene sequences of DDO11 were deposited in NCBI GenBank (OK631881, OK655895, and OK655896, respectively). BLASTn analysis showed, respectively, 100, 100, and 99.6% nucleotide sequence identity with *Neopestalotiopsis clavispora* strain accessions MG729690, MG740736, and MH423940, indicating that the pathogen belonged to *N. clavispora*. A maximum-likelihood phylogenetic analysis based on multilocus sequence (ITS, *TUB2*, and *TEF-1 α*) using MEGA X had a similar result (Kumar et al. 2018). Pathogenicity was tested on healthy, 1-year-old *D. officinale* ‘Yandang1’. Spores of DDO11 were produced on PDA for 7 days at 28°C and washed with sterile distilled water, and concentrations were adjusted to 10^6 spores/ml using a hemocytometer. Fifteen surface-disinfected healthy plants were inoculated by spraying the suspension (2 ml, 10^6 spores/ml) and covered with plastic bags for 24 h; 15 control plants were treated with sterile distilled water. The plants were placed in a chamber at >95% relative humidity and 25°C for 48 h and kept in a growth chamber (Kiangnan, China) at 25°C with 12-h day/night cycle for 8 days (Cao et al. 2019). All inoculated leaves showed symptoms identical to those observed in the field. No disease occurred on the controls. The *Neopestalotiopsis* isolate was reisolated from the symptomatic leaves, and the species was confirmed by the morphological and molecular methods described above. *N. clavispora* has been reported to cause diseases on various plants worldwide, such as strawberry (Gilardi et al. 2019), blueberry (Shi et al. 2022), *Syzygium cumini* (Banerjee and Rana 2020), and *Macadamia* (Qiu et al. 2020). To the best of our knowledge, this is the first report of *N. clavispora* causing leaf spot on *D. officinale* in China. This report will help to recognize the leaf spot disease of *D. officinale* and establish a basis for future studies on *N. clavispora* to develop management strategies.

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