Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease Caused by *Alternaria brassicae* on *Orychophragmus violaceus* in China

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Orychophragmus violaceus, belonging to the Brassicaceae family, is widely grown in many provinces of China as an ornamental plant and also as a green manure crop. In December 2019, field investigations showed that a leaf spot disease occurred on *O. violaceus* in three fields with 50 to 80% incidence (50 plants in each field were investigated) in Huize City, Yunnan Province of China. Infected leaves showed symptoms of small black point spots in the early stage of onset. The lesions distributed throughout the leaves and finally became 10 to 15 mm in diameter after 10 to 15 days of onset. At this time, the lesions are gray to black, and some have round patterns, and gray-white mildew layers can be seen on the front and back of the lesions in a humid environment. Leaves with typical lesion symptoms were sampled and photographed, and then subjected to isolation and characterization of the pathogen. Six pure cultures (HEYA2, HEYA4, HEYC6, HEYD7, HEYD8, and HEYD10) were obtained by single hyphal isolation. On acidulated potato carrot agar medium, a colony can reach 27 mm after 7 days at 25°C in darkness. Aerial hypha is cottony with white to pale gray color, and the colony reverse is fawn to dark. On V8 medium, conidiophore solitary or clustered, erect or knee-curved, occasionally branched, pale brown, separated, 82 to 130 × 5 to 9 µm. Conidia on V8 medium are solitary, straight or slightly curved, inverted rod-shaped, pale brown to brown, with 6 to 10 transverse septa, 0 to 5 oblique and longitudinal septa, columnar beak, conidial bodies (47.7 to 69.3 to 103.8 (to 119.6) × (11.2 to) 16.6 to 23.6 (to 27.8) µm. Beak septum, pale brown, (29.2 to) 34.4 to 72.4 (to 101.3) × (4.2 to) 6.6 to 9.5 (to 11.3) µm. Morphologically these isolates resembled species belonging to genus *Alternaria* (Simmons 2007). Genomic DNA of each culture was quickly extracted from mycelia using the quick and safe method (Chi et al. 2009). The internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate

dehydrogenase (GAPDH), RNA polymerase II second largest subunit (RPB2), and translation elongation factor-1α (TEF-1α) genes were amplified according to described procedures (Berbee et al. 1999; Carbone and Kohn 1999; Liu et al. 1999; Sung et al. 2007; White et al. 1990). The sequences obtained in this study were deposited in GenBank with accession numbers MW867245 to MW867250 and MW882913 to MW882930. Phylogenetic analysis was conducted with combined sequences of the four loci in the order of ITS-GAPDH-RBP2-TEF, using the maximum likelihood (ML) method and the maximum parsimony (MP) methods. In the phylogenetic tree, the six isolates and *Alternaria brassicae* (CBS 116528) clustered together with high bootstrap (BS) support values (MLBS = 100; MPBS = 100). Consistently, all the sequences of our isolates show 100% identity to those of CBS 116528, except the GAPDH gene of HEYA2 (MW882913), which shows 99.6% identity to that of CBS 116528. Based on both morphological characters and phylogenetic results, our isolates were identified as *A. brassicae*. Pathogenicity testing of isolate HEYA2 was carried out on detached leaves in a dark thermostat incubator at 25°C. Five pots per leaf were inoculated with mycelial plugs (5 mm in diameter), and another five pots were inoculated with pure agar plugs and used as the negative control. In addition, conidial suspension (10⁵ conidia/ml) of isolate HEYD8 was sprayed on 3-month-old healthy plants grown in a greenhouse at 22 to 28°C. Plants sprayed with sterilized water were used as negative controls. The test was conducted three times. After 5 to 7 days, the leaves inoculated with either the conidial suspension or the mycelium plugs showed brown necrotic lesions that were similar to the symptoms observed in the field, but the controls remained healthy. The pathogen was reisolated and confirmed to be *A. brassicae*. To our knowledge, this is the first report of leaf spot disease caused by *A. brassicae* on *O. violaceus* in China.

References:

- Berbee, M. L., et al. 1999. Mycologia 91:964.
- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Chi, M. H., et al. 2009. Plant Pathol. J. 25:108.
- Liu, Y. L., et al. 1999. Mol. Biol. Evol. 16:1799.
- Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Biodiversity Centre, Utrecht, The Netherlands.
- Sung, G. H., et al. 2007. Mol. Phylogenet. Evol. 44:1204.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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