Occurrence of anthracnose, caused by *Colletotrichum gloeosporioides*, on *Lycoris radiata* in Korea

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Received: 18 January 2015 / Accepted: 1 April 2015 / Published online: 17 April 2015
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Abstract Anthracnose symptoms were observed on leaves of red spider lilies in Korea. At the early stages of infection the symptoms appeared as small red spots on the leaves. In later stages, the infected leaves gradually turned yellow to brown and developed wilting symptoms. The pathogen isolated from the infected leaves of red spider lily was identified as *C. gloeosporioides* by morphological and molecular characteristics. *C. gloeosporioides* was confirmed as the pathogen responsible for the observed symptoms by performing a pathogenicity test.

Keywords *Amaryllidaceae* · Anthracnose · *Colletotrichum gloeosporioides* · *Lycoris radiata* · Red spider lily

The red spider lily (*Lycoris radiata*) belongs to the family *Amaryllidaceae* and is a fall-blooming perennial bulb native to China. The bulb of the red spider lily has been used as a medicinal plant in Asia and also as an emergency food item during ancient times (Ji and Meerow 2000; Kurita 1998). This plant is also used to prevent soil erosion and suppress weed growth in paddy fields (Itoh and Takahashi 1997; Takahashi and Itoh 1997; Takahashi et al. 1998). *Colletotrichum* spp. is one of the most destructive fungal genera, causing anthracnose symptoms in host plants that range broadly in type and include different 44 varieties of fruits, crops, and ornamental plants (Baily et al. 1992).

This genus is capable of infecting its host at the blossom, stem, fruit, root, leaf, and other parts of the plant. However, anthracnose symptoms in the red spider lily have not yet been associated with *C. gloeosporioides*. In this study, we identified this pathogen in a direct causal association with the anthracnose symptoms in *L. radiata*.

In October 2013, anthracnose symptoms were observed in seedlings of red spider lily located in Southern Forest Resource Research Center, Jinju, South Korea. The symptoms were red circles varying from 0.5 to 2 cm in size with a brownish color at the spot center, where the tissue appeared to be dehydrated (Fig. 1a). As the disease progressed, the small red spots that first appeared on the leaves gradually grew larger in size, and the infected leaves later changed in color from yellow to brown. While Liu et al. (2000) have previously reported observations of anthracnose diseases in the family of *Amaryllidaceae* plants, to the best of our knowledge, no instances of anthracnose symptoms in *L. radiata* have been previously reported. We isolated the pathogen causing anthracnose symptoms in the red spider lily by removing it from the leaves by using the surface sterilization method (1 % bleach solution for 1 min). The pathogen was grown on potato dextrose agar (PDA) for 5 days at 28 °C. The optimum conditions for mycelia growth were determined to be a pH of 5.5 at 27 °C on PDA (data not shown). The representative pathogen isolate was deposited in the Korean Agricultural Culture Collection Center (KACC#47837).

Mycelia on the PDA medium appeared pale gray in the inner regions and white at edges. The isolates produced pink spores in a mucilaginous matrix (Fig. 1b). The morphological characteristics of conidia can be described as hyaline in appearance, aseptate and ellipsoid in structure (13.6–16.4×5.1–7.2 μm; Fig. 1c). These morphological characteristics were in close

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Fig. 1 Symptoms and morphological characteristics of *Colletotrichum gloeosporioides* in *Lycoris radiata* **a**: Typical symptoms of anthracnose on red spider lily leaves in field condition. **b**: Colony morphology of the pathogen on PDA media. **c**: Conidiospores of *C. gloeosporioides*. **d**: Anthracnose symptom was artificially induced, by the inoculation of *Lycoris radiata* leaves with isolated *C. gloeosporioides*

correspondence with morphological records for *C. gloeosporioides* described by Bailey et al. (1992). To test pathogenicity, the isolate was incubated for 1 week on PDA at 28 °C to induce sporulation. To separate the spores from the mycelium, the petri dish

containing the pathogen was washed with 10 ml of sterilized water and then sieved through a cheesecloth twice. The inoculum suspension was adjusted to a concentration of 1×10^6 spores/ml, 10 μ l of which was inoculated onto healthy leaves that were wounded by a needle. The inoculated red spider lily was kept for 1 week in 90 % relative humidity at 28 °C. Mock inoculations (sterilized water) were also performed to serve as controls. Within 5 days of the artificial inoculation of the plant with the pathogen spore suspension, typical anthracnose symptoms appeared on the leaf surface: At 2 days after infection, small and brown spots were observed and after 5 days, the spots developed into large lesions (Fig. 1d). These symptoms were similar to those observed on the red spider lilies in the fields. The mock-inoculated plant did not develop any disease lesions and only exhibited signs of the needle wound. The pathogen was re-isolated from the disease lesions of inoculated leaves and analyzed for morphological characteristics. The characteristics of these re-isolated pathogens were indistinguishable from those in the original isolates.

Fungal mycelium was separated from the PDA medium by using an inoculating loop, and a sample was prepared for DNA extraction. Total genomic DNA of the fungus was extracted using a modified cetyltrimethylammonium bromide DNA extraction protocol (Graham et al. 2003). To identify

Fig. 2 Phylogenetic identification of *C. gloeosporioides* based on internal transcribed spacer (ITS) rRNA sequences. Numerical values on branches are bootstrap values calculated as a percentage of bootstrap replications from 1000 replicate analyses. *C. gloeosporioides* Funbio5 represented the isolate (KJ563297.1). A phylogenetic tree inference was conducted with the MAGA 5 program and phylogenetic distances were calculated using the neighbor-joining method. Bar=0.05 units of genetic distance between samples. *Rhizoctonia solani* and *Alternaria tenuissima* served as out-group references (Weir et al. 2012)

the fungus, a region within the internal transcribed space (ITS) of the rRNA was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The ITS region amplification was carried out in 20 µl of reaction mixture containing 50 ng of the template DNA, 10 pico moles of each of the ITS1 and ITS4 primers, 1 µl of *Taq* DNA polymerase, 1 µl of dNTP, 2 µl of 10× reaction buffer and topped to 20 µl with deionized distilled water. PCR conditions were as follows: pre-denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s in each cycle, annealing at 55 °C for 45 s, and extension at 72 °C for 40 s; and a final extension at 72 °C for 10 min. The sequence was deposited in GenBank (NCBI accession No. KJ563297.1) and compared with sequences in the GenBank database using BLAST. BLAST results for the ITS showed 100 % similarity to sequences belonging to *C. gloeosporioides* strains (such as its teleomorph: *Glomerella cingulate* (Stoneman) Spauld. & H. Schrenk). These results confirm our previous conclusions based on morphological similarities, that the fungus responsible for anthracnose in the *L. radiata* was *C. gloeosporioides* (Fig. 2).

Acknowledgments This research was performed with the support of the “Export strategy & technology development (114095-3)” from IPET, Korea.

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