New Disease Reports

First report of *Diaphorthe masirevicii* causing leaf blight of *Gloriosa superba* in India

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Gloriosa superba (Glory lily, Colchicaceae) is an endangered perennial climbing herb, grown in Africa and Asia for its medicinal properties (Jain & Suryavanshi, 2010). A leaf blight disease (Fig. 1) was observed during surveys performed in the region of Mysore, India. The first symptoms of infection were small (2-5 mm diameter), circular to oval, light brownish spots, surrounded by a yellow halo. Leaf spots occured on the leaf tips, margins and midribs of the leaves, enlarging to form spots with concentric rings. Embedded in the necrotic tissues were black fruiting bodies of a fungus. These symptoms also appeared later on the stem.

Infected leaves were surface sterilised with 70% ethanol followed by three washes with sterile distilled water and incubated for seven days on moist blotter discs. After incubation, colony development was examined and the fungus isolated and maintained on potato dextrose agar (PDA). The fungus was identified based on its colony type, morphology and production of pycnidial ooze (Fig. 2). Pycnidiospores were produced in slimy masses and were of two types, alpha and beta. Alpha conidia were fusoid to ellipsoidal and biguttulate, while beta conidia were filiform, slightly curved and rarely straight (Fig. 3). The morphological characters of the fungus were compared with the description of Thompson *et al*. (2015) and identified as *Diaphorthe* sp. Fungal DNA was extracted from mycelium following standard procedures (Saitho *et al*., 2006). The fungus was identified by amplifying and sequencing the internal transcribed spacer (ITS), β-tubulin and transition elongation factor (TEF) regions using specific primers (Table 1). BLAST analysis revealed identities of 99% for ITS, 99% for β-tubulin and 100% for TEF to *D. masirevicii* isolate 054 (GenBank Accession No. KR024727), *D. masirevicii* isolate BRIP 57892a (KJ197257) and *D. masirevicii* isolate BRIP 54120a (KJ197243), respectively. These sequences were submitted to GenBank (ITS: MF682435, β-tubulin: MF668289 and TEF: MF668290).

Pathogenicity tests were conducted using a detached leaf assay (n=25) and whole plant assay (n=10), under greenhouse conditions (22-28°C and under natural light). A suspension of alpha conidia was prepared by flooding 18 to 21-day-old PDA cultures with sterile distilled water and adjusting the spore concentration to 1 × 10^6 conidia/ml. Detached healthy and surface-sterilised leaves were wounded by pricking with a sterile needle, and 10 µl of conidial suspension placed onto the wound site. Wounded control leaves received only distilled water. The leaves were incubated in a moist chamber at 28 ±2°C for three-seven days. The appearance of water-soaked lesions and brown spots on inoculated leaves confirmed pathogenicity (Fig. 4). The whole plant assay was conducted using three-month-old plants. Plants were spray inoculated with either a suspension of 1 × 10^6 conidia/ml, or water as a control and observed for symptoms for the next thirty days. The pathogen was reisolated from infected leaves showing typical symptoms, thus fulfilling Koch postulates.

As per our knowledge and from a literature survey, this is the first report of *Diaphorthe masirevicii* causing leaf blight of *Gloriosa superba* in India.

References

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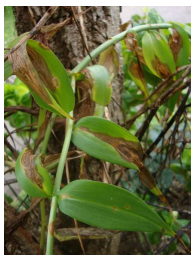

Figure 1

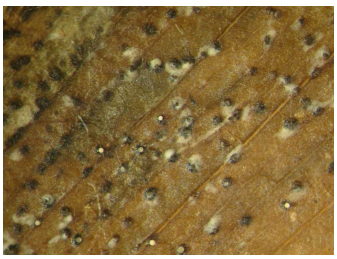

Figure 2

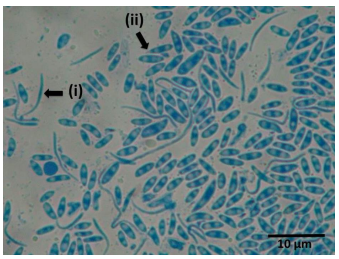

Figure 3

Figure 4

Table 1. Primers designed to detect the internal transcribed spacer, β-tubulin and transition elongation factor regions.

Region	Primer name	Sequence (5'-3')
Internal transcribed spacer	ITS1	TCCGTAGGTGAACCTGCGG
	ITS4	TCCTCCGCTTATTGATATGC
β-tubulin	Bt2a	GGTAACCAAATCGGTGCTGCTTTC
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC
Transition elongation factor	EF1-728F	CATCGAGAAGTTCGAGAAGG
	EF1-986R	TACTTGAAGGAACCCTTACC

Figure 5

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