Disease Note

FIRST REPORT OF LENTIL WILT CAUSED BY *FUSARIUM NYGAMAI* IN PAKISTAN

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During 2011-13, in the Layyah district of Punjab (Pakistan), lentil (*Lens culinaris* Medikus) plants displayed wilt symptoms with about 42.5% incidence. The wilted lentils also showed yellowing, stunting, tissue discoloration and eventual death. Small surface disinfected root pieces from wilted plant samples were placed on potato dextrose agar (PDA) and incubated at 25 ± 2°C. Purified fungal colonies produced white fluffy mycelia and violet pigmentation. Macro-conidia (7.5-17 × 2-3 µm) were 3-septate, slender and straight with curved apical cell and foot-shaped basal cell. Micro-conidia (3-5 × 1.25-2.5 µm) were non-septate and oval, and formed in short chains or false heads on monophialides. Chlamydospores (6-11 µm) were intercalary, singles, in short chains or clusters. Morphologically, the fungus was identified as *F. nygamai* Burgess & Trimboli (Leslie and Summerell, 2006). Sequences of 700 bp in the translation elongation factor-1α (TEF-1α) region, amplified using primers EF1/EF2 (Geiser *et al*., 2004), were deposited in GenBank (Accession Nos. KR061303 and KR061304). BLASTn analysis showed 99 to 100% identity with those from previously characterized *F. nygamai* strains (KP267250 in GenBank and AF160273 in Fusarium-ID). Roots of 15-days old lentil seedlings (susceptible line NARC-08-1) were dipped in conidial suspension (1×10^7 conidia/ml) before potting them and kept in greenhouse at 25 ± 2°C. Inoculated plants developed wilt symptoms similar to those observed in the field, exhibiting 100% incidence and 60.74% severity index, and *F. nygamai* was re-isolated consistently from inoculated plants. Control plants inoculated with sterile distilled water remained symptomless. To our knowledge, this is the first report of *F. nygamai* causing wilt disease on lentil in Pakistan.

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Disease Note

OCCURRENCE OF *CUCUMBER MOSAIC VIRUS* IN OLEANDER (*NERIUM OLEANDER*) IN HUNGARY

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In June 2016 mosaic symptoms characteristic of viral infection were observed in a ten year-old potted oleander (*Nerium oleander*) maintained in Velence (Hungary). Inoculation of test plants (*Chenopodium*, *Cucumis* and *Nicotiana* spp.) with extracts of diseased oleander leaves demonstrated the presence of a mechanically transmitted plant virus marked Nol. The Nol isolate caused local lesions on *Chenopodium quinoa*, mosaic on *Cucumis sativus* and local etched ring pattern followed by vein clearing and recovery on the top leaves of *Nicotiana tabacum* cv. Xanthi-nc. These symptoms suggested the presence of B pathotype (subgroup II strain) of *Cucumber mosaic virus* (CMV). CMV was identified in the oleander leaves using CMV specific lateral flow immunoassays (Bioreba AgriStrip).

To confirm the presence of CMV, total RNA was extracted from *N. clevelandii* infected with CMV-Nol and used in RT-PCR with subgroup I and II CMV coat protein (CP) gene specific primers, respectively (Salanki *et al*., 1997). The DNA fragment of the expected size (1460 bp) was amplified from tobacco leaves using subgroup II specific primers solely. The PCR product was cloned into pGEM-T Easy Vector (Promega, USA), sequenced (Biomi, Hungary) and the complete CP gene nucleotide sequence was deposited in GenBank (accession No. KX951465). The complete CP gene of CMV-Nol showed 99% nucleotide and 100% amino acid sequence identity with a Serbian CMV isolate (232Mrg, accession No. KP034963.1). This is the first record on the natural occurrence of CMV in oleander in Hungary.

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