Disease Note

FIRST REPORT OF LEAF SPOT CAUSED BY *BARTALINIA ROBILLARDOIDES* ON *PARTHENOCISSUS QUINQUEFOLIA* IN CHINA

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In late August 2013, leaf spot symptoms were first noticed on Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch], an ornamental cum medicinal plant of Vitaceae family, in the Forest Park, Luoyang City of Henan Province, China. Symptoms appeared as small circular brown dots on the leaves, gradually expanding into circular or irregularly-shaped yellow-brown to dark brown spots with raised margins and black dots in the center of the lesions. A fungus was carefully isolated from the leaf spot and cultured on potato dextrose agar, which produced dark brown colonies. Pycnidia were brown, ovoid, 13.3 µm in diameter. Conidiophores were cylindric and colorless, 8.1-10.1×3.8-4.0 µm. Conidia (n=50) were 18.1-20.8×2.5-4.2 µm, 2 to 4 septate, with apical cell hyaline, other cells pale brown. One eccentric pedicel, hyaline, 8-10.6×1.2 µm. Apical appendages 1 to 4, 13.3-16.0×1.2 µm, unbranched. Based on the isolate characteristics (Sutton, 1980; Barnett and Hunter, 1998), the fungus was subsequently confirmed as *Bartalinia robillardoides* by DNA sequence analysis of internal transcribed spacer, which was 100% identical to other known *B. robillardoides* isolates (GenBank Accession No. KF656706.1). To confirm pathogenicity of the fungus, ten surface disinfected healthy leaves of Virginia creeper were inoculated with a fresh conidial suspension (40 µl, 5×10^6 conidia/ml) of *B. robillardoides* at 26°C. Leaf necrosis symptoms appeared on the inoculated leaves within 7 days. The same fungus was successfully reisolated from the lesions, confirming Koch's postulates, whereas, control plants showed no symptoms. To our knowledge, this is the first report of *B. robillardoides* infecting *P. quinquefolia* in China.

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Disease Note

FIRST REPORT OF 16SrVI-A AND 16SrXII-A PHYTOPLASMAS ASSOCIATED WITH ALFALFA WITCHES' BROOM DISEASE IN IRAN

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In 2014-15 surveys, alfalfa witches' broom (AWB) disease was observed in a number of Iranian provinces. The main symptoms were crown proliferation, witches' broom, little leaf, flower virescence, phyllody, sterility and stunting. To investigate the phytoplasma presence, total DNAs were extracted from 132 symptomatic and 19 asymptomatic alfalfa plants and were tested by direct PCR using P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) and nested PCR using R16F2n/R16R2 (Gundersen and Lee, 1996) primers. Amplicons were obtained only from all the symptomatic alfalfa plants and RFLP analyses with *Kpn*I, *Alu*I, *Hae*III, *Hha*I, *Hpa*II, *Mse*I, *Rsa*I, *Taq*I and *Hpa*I enzymes showed the presence of two profiles. Mixed infections were never detected. From AWB samples collected in East Azarbayijan and Zanjan provinces, six amplicons were directly sequenced and showed 100% identity to each other (GenBank accession No. KU240021). The second profile was present in samples from Qom, Markazi, East Azarbayijan, Kordestan, Fars, Kermanshah, Kohgiluyeh and Boyer-Ahmad, Lorestan and Chaharamahal and Bakhtiari provinces; the 15 amplicons from Tabriz, Dehgolan, Arak, Qhorveh, and Shahrekian showed 100% identity in each area and formed 5 groups having 99% identity (GenBank accession Nos. KT763372, KT763371, KT781662, KT763373 and KT750060, respectively). The KU240021 strain sequence and its virtual RFLP indicated that it could be enclosed in the 16SrVI-A subgroup. The five-sequence group showed the highest homology with 16SrXII-A phytoplasma subgroup that was confirmed by virtual RFLP and phylogenetic analysis (MEGA 6.0). This is the first report of 16SrVI-A and 16SrXII-A phytoplasmas associated with AWB in Iran.

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