First Report of Leaf Blight Caused by *Nigrospora oryzae* on Poplar in China

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*Populus alba* L. × *P. berolinensis* Dipp. (a hybrid poplar, ‘PaPb poplar’) exhibits fast growth and beautiful tree shape with high drought and cold tolerance, and is widely planted in the cities of Northeast China because of its ornamental and ecological value (Wang et al. 2008). In October 2020, an unknown leaf blight symptom was observed on the seedlings of ‘PaPb poplar’ at Shenyang Agricultural University (41°49′N, 123°34′E) located in Shenyang City, Liaoning Province, China. The disease incidence was 50% in a survey of 200 seedlings on the campus. The typical symptoms were brown-to-black, irregular-shaped lesions (Fig. 1A). To investigate the disease, five symptomatic leaves were collected, and pieces were cut at the margin of diseased and healthy tissue. These pieces were surface sterilized with 2% sodium hypochlorite for 2 min, rinsed three times with sterile distilled water, air dried, placed on potato...
dextrose agar (PDA) and incubated at 28°C. After 5 days of incubation, three isolates with similar morphological characteristics were observed. Isolate N03 was chosen and used for pathogen identification. The fungal colonies were initially white in color, and later turned gray to black (Fig. 1D). Conidia were single-celled, black, spherical or oblate in shape measuring 9.19 to 12.78 μm × 12.61 to 14.81 μm in diameter (n=40) (Fig. 1E). These were borne on hyaline vesicles at the tip of a conidiophore. Morphologically, the isolate N03 was identified as *Nigrospora oryzae* (Berk. and Broome) Petch (Wang et al. 2017). The genomic DNA was extracted with a SP Fungal DNA Kit (D5542-01, OMEGA). The internal transcribed spacer (*ITS*) region, translation elongation factor 1-alpha (*TEF1-α*), and partial beta-tubulin (*TUB*) genes were amplified using the primers ITS5/ITS4 (White et al. 1990), EF1-728F/EF-2R (Carbone and Kohn, 1999; O’Donnell et al. 1998), and Bt-2a/Bt-2b (Glass and Donaldson, 1995) primer sets, respectively. The PCR products of *ITS*, *TEF1-α*, and *TUB* were amplified, sequenced, and deposited in GenBank with the following accession numbers MZ148528, MZ182080, and MZ182079, respectively. BLASTn analysis of the *ITS*, *TEF1-α*, and *TUB* sequences had 99.3%, 99.8%, and 99.27% nucleotide identities to MK131325, KY019328, and KY019559, respectively. A phylogenetic tree based on combined *ITS*, *TEF1-α*, and *TUB* sequences was constructed using a Maximum Likelihood method with 1000 bootstraps showing that N03 was grouped with other *N. oryzae* isolates (Fig. 2). The fungus was identified as *N. oryzae* based on morphological characteristics and molecular analyses. Koch’s postulates were completed to confirm the pathogenicity of *N. oryzae* on ‘PaPb poplar’. The N03 spore suspension (10^5 spores/mL) was used to inoculate detached leaves and field leaves in two experiments. The two experiments were repeated three times, respectively. In the detached leaf test, 10 healthy leaves collected from 1-year-old ‘PaPb poplar’ seedlings were inoculated with N03 by spraying with the spore suspension followed by incubation at 28°C on wet filter papers in a petri dish for 7 days. 10 leaves were sprayed with sterile water to save as the controls. For field leaf test, leaves of 5 plants were spray-inoculated with the spore suspension at the 4-week-old growth stage, and an additional 5 plants were sprayed with sterile water.
Seven days after inoculation, brown-to-black, irregular-shaped lesions on the margin of leaves were observed on inoculated leaves but not on the controls (Fig. 1B and C). All detached leaves inoculated with N03 were symptomatic. In the field tests, symptom appeared on 20 of the 30 inoculated leaves. *N. oryzae* was re-isolated from all the inoculated detached leaves and inoculated plants, but not from the controls. *N. oryzae* is a known pathogen of several hosts, such as *Costus speciosus* (Koen.) Sm. and *Mentha spicata* L., but has not been reported on any species of *Populus*. To our knowledge, this is the first report of leaf blight of ‘PaPb poplar’ caused by *N. oryzae* in China and the world. This disease could affect growth and development of ‘PaPb poplar’ seedlings, and may cause economic losses in the future. Appropriate strategies should be developed to manage this disease.

**References:**


Fig. 1 The Leaf blight symptom of ‘PaPb poplar’ and morphological characters of isolated Nigrospora oryzae N03.

(A) Leaf blight symptom of naturally infected poplar leaves (‘PaPb poplar’). (B) Pathogenicity of N. oryzae N03 on detached leaf of ‘PaPb poplar’ after 7 days post-inoculation. (C) Leaf of ‘PaPb poplar’ in the field inoculated with N. oryzae N03 developed leaf blight symptom after 7 days post-inoculation. (D) Colony morphology of N. oryzae N03 on PDA after 9 days at 28°C. (E) Conidial morphology; scale = 10 µm.
Fig. 2 Phylogenetic tree showing the relationship of N03 with other related taxa. Phylogenetic tree based on combined ITS, TEF1-α, and TUB sequences was constructed using Maximum-likelihood (ML) method with MEGA software version 6.0.