Leaf spot disease on seedlings of *Quercus acutissima* caused by *Tubakia dryina* in Korea

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Abstract

Severe attack by a leaf spot disease was found on seedlings of *Quercus acutissima* in Jeungpyeong, Korea in 2017. Based on the morphological characteristics of the fungus, it was identified as a member of *Tubakia*. Sequence comparisons based on the ITS and 28S rDNA regions confirmed the identity of the fungus as *Tubakia dryina*. This is the first report of *Tubakia* leaf spot caused by *T. dryina* on *Q. acutissima*.

Keywords Fagaceae · Melanconidaceae · Oak

Tubakia species have been reported as endophytes or causing leaf spots on *Castanea* spp., *Quercus* spp., and some other members of Fagaceae in Asia, Europe and North America (Yun and Rossman 2011; Harrington et al. 2012; Boroń and Grad 2017). *Tubakia dryina* is a known leaf spotting fungus which has been reported in many countries. Although a wide range of host species have been recorded for *T. dryina*, the fungus is most frequently found on oaks (Proffer 1990; Kowalski 2006), including at least 40 species according to Farr and Rossman (2017).

During a recent survey of plant diseases carried out to identify the fungal pathogens infecting oaks in 2017, a damaging leaf spot disease was consistently observed on seedlings of *Quercus acutissima* (sawtooth oak) (Fagaceae), in Jeungpyeong, Korea. *Quercus acutissima* is a deciduous broad-leaved tree, native to eastern Asia with a wide distribution and is one of the important forest components in hillsides of South Korea.

Leaf spots were brown necrotic, circular to irregular, 49–74 mm diam, occasionally coalescing to form large blotches (Fig. 1a–b). Fresh specimen were mounted in water and examined under the following microscopes: Olympus BX51 and Zeiss AX10 (equipped with a Carl Zeiss AxioCam MRc5 camera). At least 30 measurements

were made for each structure examined. Fungal morphology was as follows: Conidiomata (pycnothyria) scutelloid, convex, 40–75 µm in diam, membranaceous, consisting of brown to blackish, thick-walled cells radiating from a central point, blackish; conidiogenous cells, 5 to 7 × 9 to 13 µm, cylindrical to clavate, narrowing toward the neck, brown to dark brown, radiating from the center below the developing scutellum; conidia solitary, acrogenous, ellipsoidal, 5–7 × 15–18 µm, with a basal frill, pale to light brown, walls smooth; microconidia absent (Fig. 1d–f). Pure cultures were obtained by single conidia transfer onto 2% malt extract-agar (MEA; 20 g Bacto malt extract and 20 g Bacto Agar) plates (Fig. 1c). All isolates obtained in this study were deposited in the culture collection (CFPR) of the Forest Pathology Research Laboratory, National Institute of Forest Science, Seoul, South Korea (Accession Nos. CFPR-QA1TD and CFPR-QA2TD) as well as in the Korean Agricultural Culture Collection (KACC) of the National Academy of Agricultural Science in South Korea (Accession Nos. KACC48481 for CFPR-QA1TD and KACC48482 for CFPR-QA2TD).

In addition to the morphological identifications, DNA barcode sequences were compared with sequences deposited in GenBank to assure the correct identity of the fungus. DNA sequence analyses were carried out following the techniques described by Lee et al. (2015) for internal transcribed spacer (ITS) regions including the 5.8S rDNA gene region and large-subunit (LSU) 28S rDNA. A BLASTn search with the isolates obtained in this study further revealed >99% similarity with those of *T. dryina* isolates from Poland (KR362909 for ITS) and from Italy

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Fig. 1 Leaf spot on seedlings of *Quercus acutissima* caused by *Tubakia dryina*. **a** Symptoms on leaves. **b** Close-up view of symptoms and conidiomata. **c** Colony on MEA. **d** Top view of radiate scutella. **e** Scutellum and conidia in germination. **f** Conidia

(JF704187 for LSU). All sequence data obtained in this study were deposited in the NCBI database (accession numbers; MF579755 and MF579756 for ITS; MG798658 and MG793660 for LSU).

To construct a phylogenetic tree, sequences obtained were aligned with ten published sequences of *Tubakia* species retrieved from GenBank using the online version of MAFFT ver.7.215 (<http://mafft.cbrc.jp/alignment/server/>) (Kato et al. 2002). A Maximum likelihood (ML) analysis was performed using RAxML HPC BlackBox ver.8.1.11 (Stamatakis 2006; Stamatakis et al. 2008), by using the default option with the GTR substitution model implemented in the CIPRES cluster server (<https://www.phylo.org/>) at the San Diego Supercomputing Center. The ML analysis based on the ITS region resulted in a marginally supported placement of isolates obtained in this study (CFPR-QA1TD, CFPR-QA2TD) with authenticated isolates of *T. dryina* retrieved from GenBank (Fig. 2).

Pathogenicity of CFPR-QA1TD and CFPR-QA2TD to *Q. acutissima*, was tested through inoculations of healthy

detached leaves of *Q. acutissima*. Three weeks-old culture formed on 2% MEA plates at 25 °C were flooded with sterile distilled water and the surface was scraped. The suspension was filtered and adjusted to 10^5 conidia/mL with a haemocytometer. Ten healthy leaves of *Q. acutissima* were selected, half being inoculated with the suspension obtained from one strain and five with the other. Inoculated leaves were left in plastic bags to keep humidity levels high and left in an incubator at 25 °C under a 12-h photoperiod. Additionally, five healthy leaves were treated with sterile water and kept under the same conditions to serve as controls. Leaf spot symptoms, identical to those observed in the field, developed on the inoculated leaves 7 days after the inoculation, while no symptoms were observed on the control. Re-isolations of the fungus from the leaf spots developed on the inoculated leaves were successfully made, fulfilling Koch's postulates.

To our knowledge, this is the first report of *T. dryina* causing leaf spots on *Q. acutissima* in Korea. The symptoms developed by *T. dryina* attack are similar to those

Fig. 2 Phylogenetic relationship between *Tubakia dryina* isolates and some reference isolates retrieved from the NCBI database, inferred by the Maximum Likelihood method using the rDNA ITS regions. Bootstrap values ($\geq 50\%$) based on 1000 replications are indicated. The scale bar represents 0.01 nucleotide substitutions per site. The isolates used in this work are indicated in bold

caused by anthracnose and other causes and may have been underestimated (Kowalski 2006). Thus, more rigorous identification of the pathogen is needed to better

access the relevance of the disease and decide about the need for control measures.

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