

RESEARCH ARTICLE

Occurrence in Korea of Rust Disease on *Tilia mandshurica* Caused by *Pucciniastrum tiliae*

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ABSTRACT

Tilia mandshurica trees with rust symptoms have consistently been noticed during disease surveys in Korea since 2006. Based on the morphological examination and molecular sequence analysis of the internal transcribed spacer and large subunit of ribosomal DNA, *Pucciniastrum tiliae* was identified as the causal fungus of rust disease. This is the first report of *P. tiliae* infection on *T. mandshurica* in Korea.

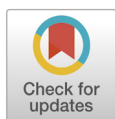
Keywords: Mandshurian linden, Teliospore, Urediniospore

INTRODUCTION

Tilia spp. (Malvaceae) are widely distributed throughout the temperate northern hemisphere including Asia, Europe, and eastern North America [1]. *T. mandshurica* Rupr. & Maxim., commonly named 'mandshurian linden', is a deciduous tree and native to northeastern Asia including China, Japan, Korea, and Russia (Siberia). In Korea, the tree is one of the most common trees in gardens and parks and a nectar source of small pollinators such as honeybees and flies [2].

On *T. mandshurica*, a rust fungus (*Pucciniastrum tiliae*) has been recorded from Japan and China, along with a dieback and canker-causing fungus (*Nectria dematiosa* from China) and two powdery mildew fungi (*Erysiphe clintonii* from USA and *E. oleosa* var. *zhengii* from Russia) [3]. *Pucciniastrum tiliae* is known to form spermatogonial and aecial stages on *Abies* spp. but uredinial and telial stages on *Tilia* spp. [4].

After a rust disease was observed on *T. mandshurica*, planted in an experimental forest of Korea University (37°30'20"N; 127°41'56"E), Yangpyeong, Korea in August 2006, it has been consistently observed in Goseong, Hoengseong, and Incheon. The rust disease on *T. mandshurica* caused premature defoliation (Fig. 1A). The affected leaves were discoloured and became yellow. Uredinia were formed on the lower leaf surfaces (Figs. 1B and C). The present study aimed to record this new rust disease on *T. mandshurica* in Korea and to identify the causal fungus based on morphological characteristics and molecular sequence data.



OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2023 March, 51(1): 1-6
<https://doi.org/10.4489/KJM.20230001>

Received: January 03, 2023

Revised: March 13, 2023

Accepted: March 21, 2023

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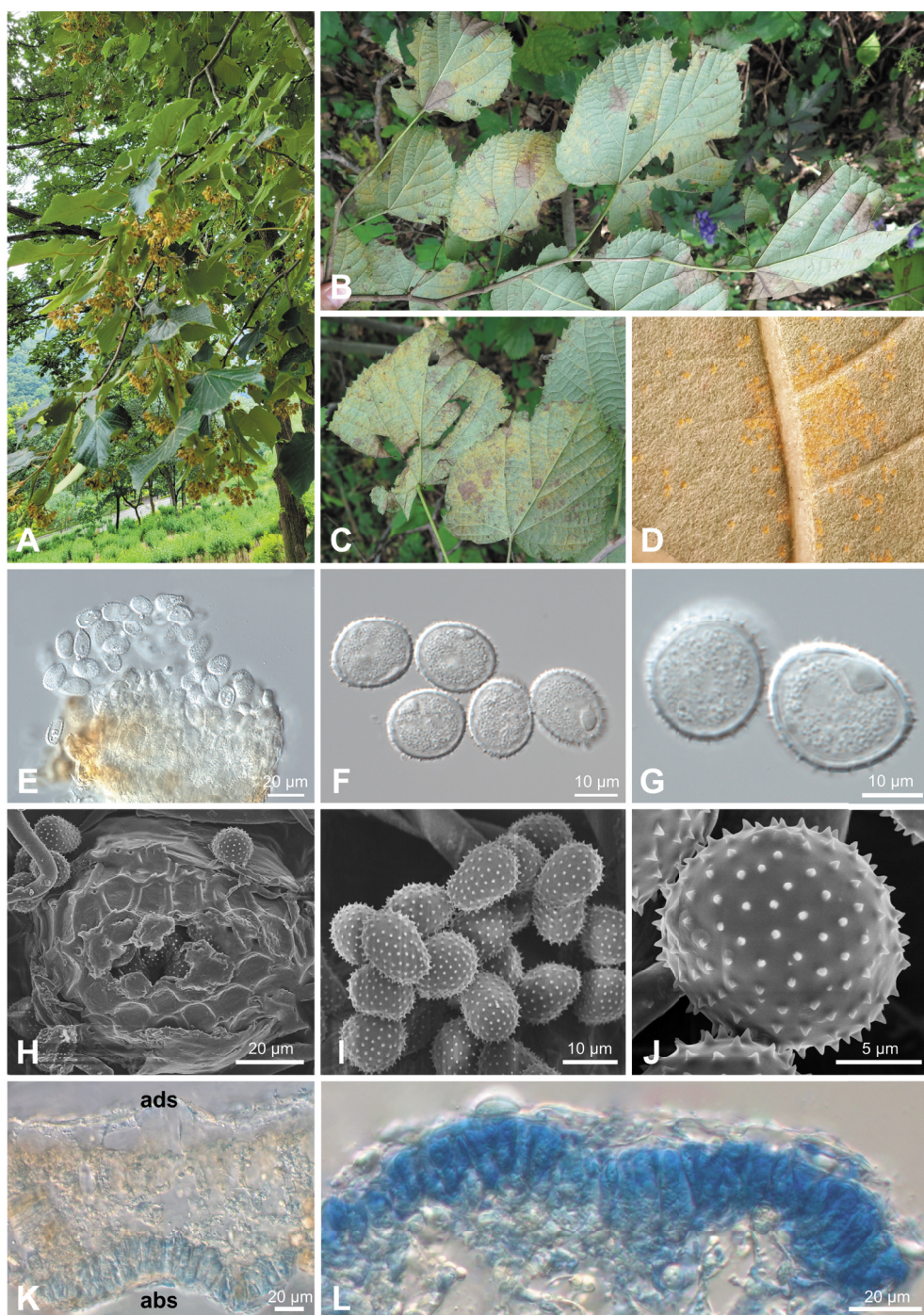


Fig. 1. Rust disease caused by *Pucciniastrum tiliae* on *Tilia mandshurica*. (A) Early defoliation of an affected tree. (B and C) Rust symptoms on the lower surface of leaves. (D) Uredinia formed on the lower surface of a leaf. (E) Uredinium under a differential interference contrast (DIC) microscope. (F and G) Urediniospores under a DIC microscope. (H) Uredinium under a scanning electron microscope (SEM). (I and J) Urediniospores under a SEM. (K) Vertical section of telium (abs, abaxial side; ads, adaxial side). (L) Teliospores in a leaf epidermis.

MATERIALS AND METHODS

For morphological investigation, rust-infected leaves were observed under a dissecting microscope (M205C; Leica, Wetzlar, Germany), a DIC light microscope (Axio Imager 2, Carl Zeiss, Oberkochen, Germany) and a scanning electron microscope (S-4800+EDS, Hitachi, Tokyo, Japan). All voucher specimens were deposited at the Korea University herbarium in Seoul, Korea. Information of all voucher specimens used for morphological and molecular phylogenetic analyses is provided in Table 1.

To confirm morphological identification, genomic DNA was extracted from urediniospores on infected leaves using MagListo 5M plant Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The internal transcribed spacer (ITS) and large subunit (LSU) rDNA regions were amplified using primer pairs ITS5u/ITS4rust [5,6] and LRust1R/LRust3 [5], respectively. The PCR products were purified using AccuPrep® PCR/Gel Purification Kit (Bioneer, Daejeon, Korea) and sequenced by a DNA sequencing service (Macrogen, Seoul, Korea) with the primers used for amplification. The resulting sequences were edited using the DNASTAR software package (Lasergen, Madison, WI, USA) and deposited in GenBank (OL519187–OL519191 for ITS and OL519193–OL519197 for LSU). Phylogenetic trees were constructed by the maximum-likelihood (ML) method using MEGA 7 [7], with the default settings of the program, with the Tamura-Nei model. The robustness of individual branches was estimated by bootstrapping 1,000 replicates.

Table 1. Rust specimens on *Tilia mandshurica* used for morphological and molecular analyses in this study.

Voucher no.	Locality in Korea	Collection date	GenBank accession no.	
			ITS	LSU
KUS-F21970	Experimental Forest of Korea University, Yangpyeong	11 Aug. 2006	OL519187	OL519193
KUS-F25288	Mt. Cheontaesan, Hoengseong	13 Sep. 2010	OL519188	OL519194
KUS-F25522	Incheon Arboretum, Incheon	20 Oct. 2010	OL519191	OL519196
KUS-F29484	Jinburyeong hill, Goseong	20 Sep. 2016	OL519190	OL519197
KUS-F31354	Incheon Arboretum, Incheon	22 Oct. 2019	OL519189	OL519195

ITS: internal transcribed spacer; LSU: large subunit.

RESULTS AND DISCUSSION

Uredinia were hypophyllous, scattered to grouped, bright yellow or orange, round, covered by the epidermis (Fig. 1D), and 50–100 µm in diameter (Figs. 1E and H). Urediniospores were subglobose to ellipsoidal, pale yellow, and measured (17.0–)18.3–20.7(–21.5) × (14.4–)15.0–16.2(–17.7) µm (av. 19.6 × 15.5 µm), with a wall of echinulate ornamentation and 0.7–1.6 µm thick (Figs. 1F, G, I, and J). Telia were hypophyllous, subepidermal, scattered to grouped, and brown. Teliospores were formed under the host plant epidermis, divided into 2–4 cells, ellipsoidal, angular or oblong, and measured (23.8–)27.3–35.4(–38.3) × (4.7–)6.4–9.7(–11.5) µm (av. 31.4 × 8.0 µm) with a thin and smooth wall of 0.5–1.0 µm thick (Figs. 1K and L). These morphological features matched those reported for *Pucciniastrum tiliae* [4,8].

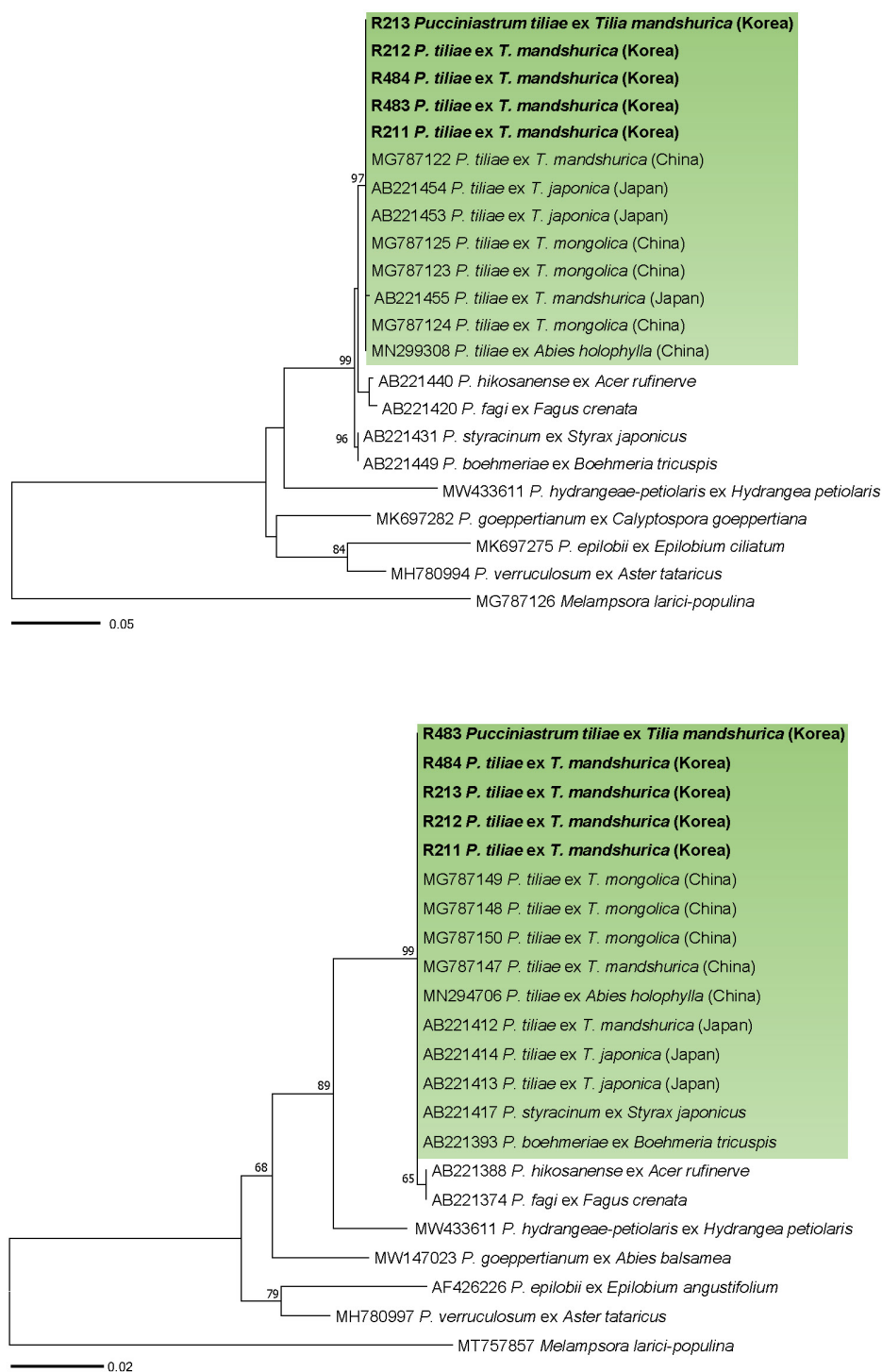


Fig. 2. Maximum likelihood tree of *Pucciniastrum* species inferred from the internal transcribed spacer (ITS) (A) and large subunit (LSU) (B) rDNA sequences. The numbers above the branches represent bootstrap values over 60%. The green boxes represent *Pucciniastrum tiliae*. The Korean specimens sequenced in the present study are shown in bold.

In BLASTn search, ITS rDNA sequences of the Korean specimens were identical with *P. tiliae* ex *T. mongolica* in China (MG787123, MG787124, MG787125), *T. japonica* in Japan (AB221453, AB221454), *T. mandshurica* in China (MG787122) and *Abies holophylla* in China (MN299308) but differed from *P. tiliae* ex *T. mandshurica* in Japan (AB221455) by only a nucleotide. LSU sequences revealed no nucleotide difference with all reference sequences of *P. tiliae*. In both maximum-likelihood phylogenetic trees of ITS (Fig. 2A) and LSU (Fig. 2B) sequences, the Korean specimens formed a well-supported clade with *P. tiliae* sequences ex *T. mongolica*, *T. japonica*, *T. mandshurica*, and *Abies holophylla* with high bootstrap values of 97% (ITS) and 99% (LSU).

Until now, *P. tiliae* had been recorded on eleven *Tilia* species, namely *T. americana* (Japan), *T. amurensis* (China, Japan, North Korea and Russia), *T. japonica* (Japan), *T. kiusiana* (Japan), *T. laetevirens* (China), *T. mandshurica* (China, Japan and Russia), *T. maximowicziana* (Japan), *T. miqueliana* (Japan), *T. mongolica* (China), *T. platyphyllos* (Japan) and *T. tuana* (China) [3,4,8]. Although *Tilia* spp. are widely distributed not only in Asia but also in Europe and Northeastern America, this rust fungus has yet been reported only in Northeast Asia. To our knowledge, this is the first report of *P. tiliae* on *T. mandshurica* in Korea. Since this pathogen has been previously recorded on *T. amurensis* in North Korea [8], and *T. mandshurica* in Northeast Asia (China, Japan, and Russia), the rust on *T. mandshurica* may have already existed in the Korean peninsula before the present study rather than newly emerging. The present study provides detailed information on morphological features and molecular data of this rust fungus on *T. mandshurica*. This finding will be useful for designing control measures against rust disease and conserving *T. mandshurica*.

CONFLICT OF INTERESTS

The authors declare no competing interest.

ACKNOWLEDGEMENTS

This work was supported by the National Academy of Agricultural Science grant (PJ014956) from the Rural Development Administration, Korea.

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