A new host genus and species (*Afzelia xylocarpa*) for *Phakopsora pachyrhizi* found in Thailand

Suneerat Seemadua · Tharntip Bhasabutra ·
Dean R. Beasley · Yu Pei Tan · Roger G. Shivas

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Abstract Leaves of *Afzelia xylocarpa* infected with rust were collected from the National Corn and Sorghum Research Centre, Rai Suwan, Nakhon Ratchasima, north-eastern Thailand in January 2011. The rust was identified as *Phakopsora pachyrhizi* based on morphological and DNA sequence data. *Afzelia xylocarpa* represents a new host genus and species for this rust fungus, which is commonly known as Asian soybean rust.

Keywords Soybean rust · Caesalpiniaceae · SSU

Afzelia xylocarpa is a leguminous tree (Caesalpiniaceae) native to the south-east Asian deciduous forests in Thailand, Vietnam, Cambodia, Laos and Myanmar. In Jan. 2011, leaves of *A. xylocarpa* infected with rust were found and collected from the National Corn and Sorghum Research Centre, Rai Suwan, Nakhon Ratchasima, Thailand.

Dry herbarium specimens of *A. xylocarpa* infected with rust were deposited at the Plant Pathology Mycological Herbarium, Department of Agriculture, Thailand (TTPH) as TTPH 002449. (Fig. 1a) The morphology of the rust was examined by cutting thin sections of uredinia and mounting in lactic acid on microscope slides that were gently heated to expel air bubbles. The sections were examined with an Olympus BX60 and images taken with a Nikon DS-Fi1 camera.

Examination of rust infected leaves showed that uredinia developed more frequently on the lower surface of the leaf

than on the upper surface. Uredinia were subepidermal, erumpent, dome shaped, ostiolate, pale brown, scattered on necrotic spots up to 2 mm diam. (Fig. 1b), enclosed by paraphyses that were cylindrical to clavate, 16–29 µm [av. = 22.7, SD = 4.7, $n=10$] × 6–9 µm [av. = 7.0, SD = 0.8, $n=10$], capitate apex, hyaline to subhyaline (Fig. 1d). Urediniospores were single-celled, subglobose, ellipsoidal, obovoid, subhyaline to pale brown, 22–39 [av. = 26.6, SD = 4.7, $n=20$] × 18–28 [av. = 21.9, SD = 2.3, $n=20$] µm, wall 1.5–3.0 [av. = 1.9, SD = 0.2, $n=20$] µm thick, echinulate, spines 0.5–0.1 µm high and 2–3 µm apart, germ pores inconspicuous (Fig. 1c).

Rust sori were excised from fresh host material, placed in 2 ml Bead Solution tubes of the UltraClean Microbial DNA Isolate Kit, and extracted according to the manufacturer's instructions (Mo Bio Laboratories). The small subunit (SSU) ribosomal RNA was amplified using the primers and PCR conditions recommended in Aime (2006), with the exception that Phusion High-Fidelity PCR Master Mix (Finnzymes) was used instead. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN), and the purified products were sequenced using the amplifying primers and additional primers recommended by Aime (2006) by Macrogen Incorporated (Korea) using the AB 3730xl DNA Analyser (Applied Biosystems). The sequence obtained was compared to the nucleotide sequence database (GenBank). The SSU sequence (GenBank accession JF990846) was a 99 % match to *Phakopsora pachyrhizi* BPI 871755 GenBank accession DQ354536.

On the basis of morphology and DNA sequence data the rust was identified as *Phakopsora pachyrhizi* Sydow and Sydow (1914), which causes Asian soybean rust. The Asian soybean rust pathogen was first identified in 1902 in Japan (Bromfield 1984). *P. pachyrhizi* is found throughout Asia and has spread to many soybean growing regions around the world, including North and South America (Ivancovich 2005; Schneider et al. 2005) and Africa (Levy 2005). Soybean rust is one of the most

S. Seemadua (✉) · T. Bhasabutra
Department of Agriculture, Plant Pathology Research Group,
Plant Protection Research and Development Office,
Chatuchak, Bangkok 10900, Thailand
e-mail: seemadua@yahoo.com

D. R. Beasley · Y. P. Tan · R. G. Shivas
Plant Pathology Herbarium, Agri-Science Queensland,
Dutton Park 4102, Australia

Fig. 1 *Phakopsora pachyrhizi*. (a) Symptoms on *Azelia xylocarpa*, (b) Uredinia on leaflets (bar=1 mm), (c) Urediniospores (bar=10 µm), (d) Paraphyses (bar=10 µm)

important diseases of soybean causing significant yield losses (Goellner et al. 2010).

Phakopsora pachyrhizi has a wide host range in the Fabaceae, with hosts in several genera including *Alysicarpus*, *Cajanus*, *Calopogonium*, *Canavalia*, *Centrosema*, *Clitoria*, *Coronilla*, *Crotalaria*, *Glycine*, *Lablab*, *Lespedeza*, *Lupinus*, *Lotus*, *Macroptilium*, *Melilotus*, *Pachyrhizus*, *Phaseolus*, *Pisum*, *Psophocarpus*, *Pueraria*, *Senna*, *Sesbania*, *Trifolium*, *Trigonella*, *Vicia* and *Vigna* (Ono et al. 1992). There is a report of an unidentified rust on *A. xylocarpa* from a seedling nursery in northern Thailand (Kawabe et al. 1998). Ours is the first confirmed record of *P. pachyrhizi* on *A. xylocarpa* in Thailand as well as the first report of *P. pachyrhizi* on a host in the genus *Azelia*.

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