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RESEARCH ARTICLE

Basil Tree, a New Host of Downy Mildew Pathogen *Peronospora belbahrii*

Hyun Ju Lee[1], Jae Sung Lee[2], Hyeon-Dong Shin[3], Young-Joon Choi[2]*

[1]Gyeonggido Agricultural Research and Extension Services, Hwaseong 18388, Korea

[2]Department of Biology, Kunsan National University, Gunsan 54150, Korea

[3]Division of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Korea

*Corresponding author: yjchoi@kunsan.ac.kr

ABSTRACT

Basil (*Ocimum* spp.) is a popular herb grown worldwide. During the past fifteen years, a downy mildew pathogen has caused considerable damage to basil cultivations. In August 2017, downy mildew disease symptoms were found on Basil Tree (or long foot Basil Tree), which was developed by the grafting of two basil varieties and is a continuous harvest plant with a woody trunk. The present study reports the occurrence of downy mildew disease in basil Tree and identifies the causal pathogen, as *Peronospora belbahrii*.

Keywords: Newly emerging disease, Oomycota, Phylogeny, Sweet basil

INTRODUCTION

Peronosporaceae, the largest obligate biotrophic family of the phylum Oomycota, cause downy mildew disease on a wide range of mono-and dicotyledonous plants [1]. Plants of the family Lamiaceae are the most common hosts of downy mildew [2]. These include economically relevant plants such as basil (*Ocimum basilicum*), coleus, and sage [3-5]. During the last 15 years, downy mildew has been the most prevalent disease in basil cultivations worldwide [6]. Thines et al. [4] have introduced a new species, *Peronospora belbahrii,* for the causal pathogen, while in Korea Choi et al. [7] have first reported the basil downy mildew and the causal pathogen.

Basil is one of the most popular and widely grown herbs worldwide. Normally, it is short-lived, lasting less than a year. Basil tree, also known as the long foot basil tree, which was developed by grafting two basil varieties, is a continuous harvest plant with a woody trunk comprising a ball-shaped head with aromatic leaves. Because the basil graft can be easily grown at home, there has been an increase in the production of basil trees in Korea. In August 2017, symptoms of downy mildew disease were found on basil tree plants in Korea. The present study aims to report the occurrence of downy mildew on basil trees and to identify the causal pathogen.

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MATERIALS AND METHODS

In August 2017, basil tree plants with typical symptoms of downy mildew disease were found in a plastic greenhouse in Yongin-si, Korea (37°05′56″N, 127°08′28″E). A representative sample was deposited in the Korea University Herbarium (accession no. KUS-F29876). In order to identify the causal pathogen, both morphological and molecular phylogenetic approaches were utilized. For microscopy, conidiophores and conidia were taken from underneath infected leaves, transferred to a drop of lactic acid (Sigma-Aldrich, St. Louis, MO, USA) on a microscope glass slide, covered with a glass cover slip, and briefly heated using an alcohol lamp. Morphological characteristics were examined under a model BX53F microscope (Olympus, Tokyo, Japan), and photographed with a DigiRetina 16M digital camera (Tucsen, Fuzhou, China).

For purposes of molecular phylogenetic analysis, both the internal transcribed spacer (ITS) rDNA and cytochrome oxidase II (*cox*2) mtDNA were amplified using procedures outlined by Choi et al. [8]. The PCR products were purified using an AccuPrep PCR Purification Kit (Bioneer, Daejeon, Korea) and sequenced via a DNA sequencing service (Macrogen, Seoul, Korea), with the same primers used for amplification. The ITS rDNA and the *cox*2 mtDNA sequences were edited using the DNASTAR software package version 5.05 (DNASTAR, Madison, WI, USA). Alignments of each locus were performed using MAFFT 7 [9] and the Q-INS-i algorithm [10], in addition to previously published reference sequences of *Peronospora* and an outgroup taxon, *Phytophthora megasperma*. To infer phylogenetic relationships, minimum evolution (ME) method was performed using MEGA 7.0 [11] with the default settings of the program, except for replacement with the Tamura-Nei model.

RESULTS AND DISCUSSION

Initial downy mildew symptoms on the basil tree appeared as yellowish to brownish spots on the upper surfaces of leaves, while greyish to brownish conidia occurred densely on the corresponding lower surfaces (Fig. 1A~1E). Disease incidence ranged from 50 to 70%. Conidiophores (n = 50) were hyaline, 200~400 μm in length, 4~6 μm in width (Fig. 1F), monopodially branched in 4~6 orders, while trunk was straight to slightly curved. Ultimate branchlets (n = 50) were mostly in pairs, slightly curved, 5~20 μm long and displayed subtruncate tips (Fig. 1G). Conidia (n = 100) were broadly ellipsoidal, greyish brown, and measured 23.0~28.5 × 19.0~22.5 μm (av. 25.3 × 20.6 μm) with a ratio of length to width of 1.15~1.30 (av. 1.23) (Fig. 1H, 1I). All morphological characteristics corresponded with those of *P. belbahrii* [4, 7], except for somewhat smaller conidia (25.3 × 20.6 μm vs. 31 × 24 μm). In downy mildew species, however, it is known that the host matrix may influence morphological characteristics including the size of conidia [12].

To confirm morphological identification, both ITS rDNA and *cox*2 mtDNA were sequenced,

and the resulting sequences were deposited in GenBank (accession nos. MG793199 for ITS, MG793198 for *cox*2). A BLASTn search revealed that, in regard to the ITS sequence, the Korean isolate differed only by a nucleotide from the *P. belbahrii* sequences from China (KP657570) and Korea (KX228833), and by two nucleotides from those of the Czech Republic (KJ960193), Cyprus (KF419290), Germany (FJ394335) and Switzerland (AY831719). The *cox*2 sequence revealed two nucleotide substitutions with those from Germany (FJ394344, KJ654229). In phylogenetic investigations for ITS rDNA (Fig. 2A) and *cox*2 mtDNA (Fig. 2B), the sequences of the downy mildew pathogen of the basil tree grouped strongly with the sequences of *P. belbahrii* of sweet basil, with high supporting values of 93% in ITS and 100%

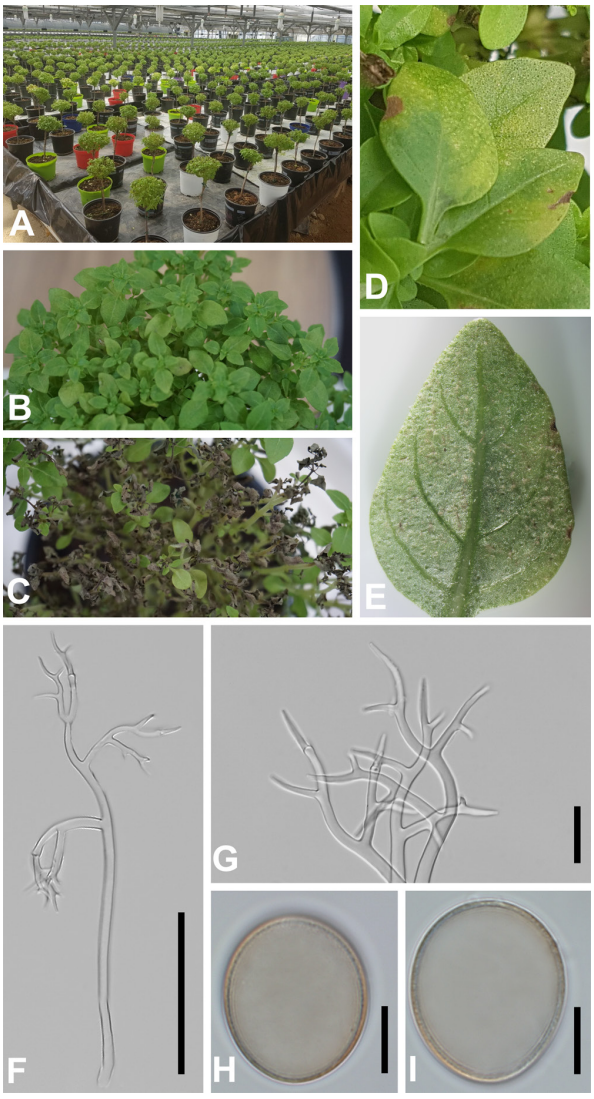

Fig. 1. Downy mildew disease caused by *Peronospora belbahrii* on basil tree. A, downy mildew occurred in a plastic house containing numerous basil tree pots; B, early symptom of yellowish, or yellow-green, leaves; C, late symptom of the leaves covered with greyish to brown conidia and often defoliation; D, yellowing and brownish spots on upper leaf surfaces; E, whitish conidiophores and greyish conidia on corresponding lower surfaces; F, conidiophore; G, ultimate branchlets; H, I, conidia (scale bars: F = 100 μm, G, H = 10 μm)

in *cox*2, proving its identification as *P. belbahrii*.

During the past fifteen years, *P. belbahrii* had been reported as a destructive downy mildew pathogen of sweet basil in countries on several continents [6, 13] including Korea [7]. In regard to the present morphological and molecular dataset, the pathogens found on sweet basil and basil trees in Korea were identical, confirming it as *P. belbahrii*, which suggests that it may have host-jumped between the two plants and may be in the process of spreading into different *Ocimum* plants. To our knowledge, this is the first record of a downy mildew affecting basil trees anywhere in the world. Considering the increasing demand for this crop, this disease may pose a serious risk to the management of basil tree cultivation.

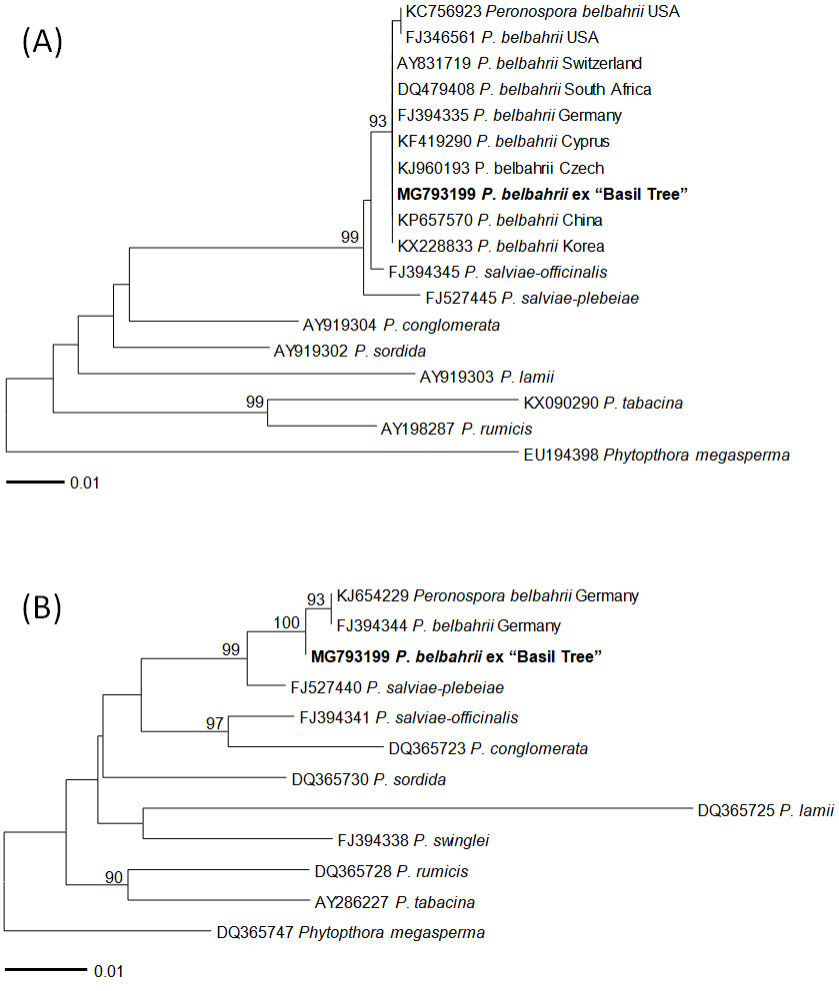

Fig. 2. Minimum evolution trees based on the internal transcribed spacer rDNA (A) and cytochrome oxidase II mtDNA (B) sequences. Boot strapping support values higher than 60% are given above the branches. Basil tree downy mildew specimens are shown in bold. The scale bar equals the number of nucleotide substitutions per site.

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