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Note

First Report of Powdery Mildew Caused by *Erysiphe cruciferarum* on *Arabidopsis thaliana* in Korea

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In November 2008, typical powdery mildew symptoms were observed on leaves of *Arabidopsis thaliana* ecotype Col-0 plants in a growth room under controlled laboratory conditions at Korea University, Seoul. The disease was characterized by the appearance of white powder-like fungal growth on the surface of infected leaves. As the disease progressed, infected leaves exhibited chlorotic or necrotic brown lesions, and leaf distortion and senescence. Conidiophores of the causal fungus were hyaline, unbranched, 3-4 celled, cylindrical, and 80-115×6-9 μm in size. Singly produced conidia (pseudoidium type) were hyaline, oblong to cylindrical or oval in shape, and 26-55×15-20 μm in size with a length/width ratio of average 3, angular/rectangular wrinkling of outer wall and no distinct fibrosin bodies. Appressoria on the hyphae were multi-lobed. These structures are typical of the powdery mildew *Oidium* subgenus *Pseudoidium*, anamorph of the genus *Erysiphe*. The measurements of the fungal structures coincided with those of *Erysiphe cruciferarum*. The phylogenetic analysis using ITS rDNA sequences revealed that the causal fungus *Erysiphe* sp. KUS-F23994 is identical to *E. cruciferarum*. The isolated fungus incited powdery mildew symptoms on the inoculated Arabidopsis leaves, which proved Koch's postulates. Taken all data together, we first report the occurrence of powdery mildew disease of *A. thaliana* caused by *Erysiphe cruciferarum* in Korea.

Keywords : *Arabidopsis thaliana*, *Erysiphe cruciferarum*, pathogenicity, powdery mildew

Arabidopsis thaliana has been used extensively as a genetic model host plant to unravel the molecular basis of plant-microbe interactions, due to its small genome size, its relatively short generation time, high fecundity and ease of mutagenesis (Micali et al., 2008). In particular, the publication of the entire *A. thaliana* genome sequence in 2000 have prompted the molecular genetic analysis of plant basal and race-specific disease resistance mechanisms (Glazebrook et al., 1997; Arabidopsis genome initiative, 2000). The recent identification of resistance or defense-related genes from crops and Arabidopsis further reinforces the use of Arabidopsis as a powerful tool for genetic dissection of plant defense response pathways.

Many fungal pathogens are known to infect *A. thaliana*, including members of Plasmodiophoromycetes, Oomycetes, Ascomycetes, and Basidiomycetes (Mauch-Mani and Slusarenko, 1993; Holub 2007). Among obligate biotrophic fungal pathogens, powdery mildews, such as *Erysiphe cruciferarum* Opiz ex Junell (Koch and Slusarenko, 1990) and *Golovinomyces* (syn. *Erysiphe*) *cichoracearum* V.P. Geljuta (Adam and Somerville, 1996) and *Golovinomyces* (syn. *Erysiphe*) *orontii* Castagne (Plotnikova et al., 1998) are able to infect *A. thaliana*. To date, however, there are no reports of occurrence of powdery mildew diseases on *Arabidopsis* plants in Korea. In November 2008, we first found typical powdery mildew symptoms on leaves of *A. thaliana* ecotype Col-0 plants which were placed in a growth room under controlled laboratory conditions at Korea University, Seoul. The *Arabidopsis* leaves infected by the powdery mildew fungus have been deposited as the herbarium KUS-F at the Mycological Herbarium of the Korea University, Seoul, Korea. To identify the causal agent of the powdery mildew disease, we performed morphological, cytological and molecular analyses, as well as the pathogenicity test.

Disease symptom and infection structures. Powdery mildew symptoms with white star-shaped colonies were observed on the rosette leaves of 5-6 week old Arabidopsis, mostly on upper surface of leaves, but rarely on the lower surface (Fig. 1A and B). The colonies increased in size and coalesced, which subsequently covered the entire leaf

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surface. As the disease progressed, infected leaves exhibited chlorotic or necrotic brown lesions, and leaf distortion and senescence (Fig. 1B). To visualize fungal development during the infection process, fungal structures on infected leaves were stained with trypan blue at various time points after inoculation (Fig. 1C to I). Within 12 h after inoculation, a conidium that has landed on a leaf germinated, producing a germ tube or an appressorial germ tube that differentiates a specialized infection structure, the appressorium (Fig. 1C and D). A mixture of enzymatic activities and pressure from the appressorium and penetration peg at the site, which the grass powdery mildew *Blumeria graminis* bleaches the underlying cell wall, are known to act on the epidermal cell to pave the way for plant cell invasion (Fig. 1E and F; Tucker and Talbot, 2001). Successful penetration of the powdery mildew fungus led to generation of the primary fungal feeding structure, so called the haustorium, and subsequent development of elongated secondary hyphae from the appressorium (Fig. 1G). Haustoria of biotrophic pathogens are thought to be responsible for the uptake of sugars and amino acids from the host plants to the fungal mycelium and the active delivery of effector proteins to suppress plant immune responses (Ellis et al., 2006). Formation of mature haustoria and elongated secondary hyphae supports a compatible interaction of *E.* sp. (KUS-F23994) with *A. thaliana* (Fig. 1G; Hwang and Heitefuss, 1982). Subsequent haustorial establishment and secondary hyphal proliferation occurred exclusively on the epidermal cells of the *A. thaliana* leaves (Fig. 1H). Along the hyphae, new appressoria formed and penetrated epiderma cells and then produced secondary haustoria. Completion of the asexual life cycle of *E.* sp. (KUS-F23994) was evidenced by the occurrence of sporulation (white powder) on inoculated rosette leaves of Arabidopsis (Figs. 1A and B). In general, powdery mildew fungi do not invade mesophyll cells, but rather limit their growth to the epidermal layer. The *E.* sp. (KUS-F23994) produced conidia singly on the conidiophores and sporulated little on *A. thaliana* leaves (Fig. 1A, B and I), which is consistent with the previous observation of a little sporulation of *E. cruciferarum* (Micali et al., 2008). In contrast to the *E. cruciferarum* sporulation, *G. cichoracearum* displays visibly abundant sporulation at 7 days after inoculation, and *G. orontii* requires 10 days for full sporulation (Micalli et al., 2008; Vogel and Somerville, 2002).

Morphological characteristics of the asexual state. To compare anatomical and developmental features of the putative powdery mildew fungus, microscopic examinations of the representative specimen (KUS-F23994) infected by a

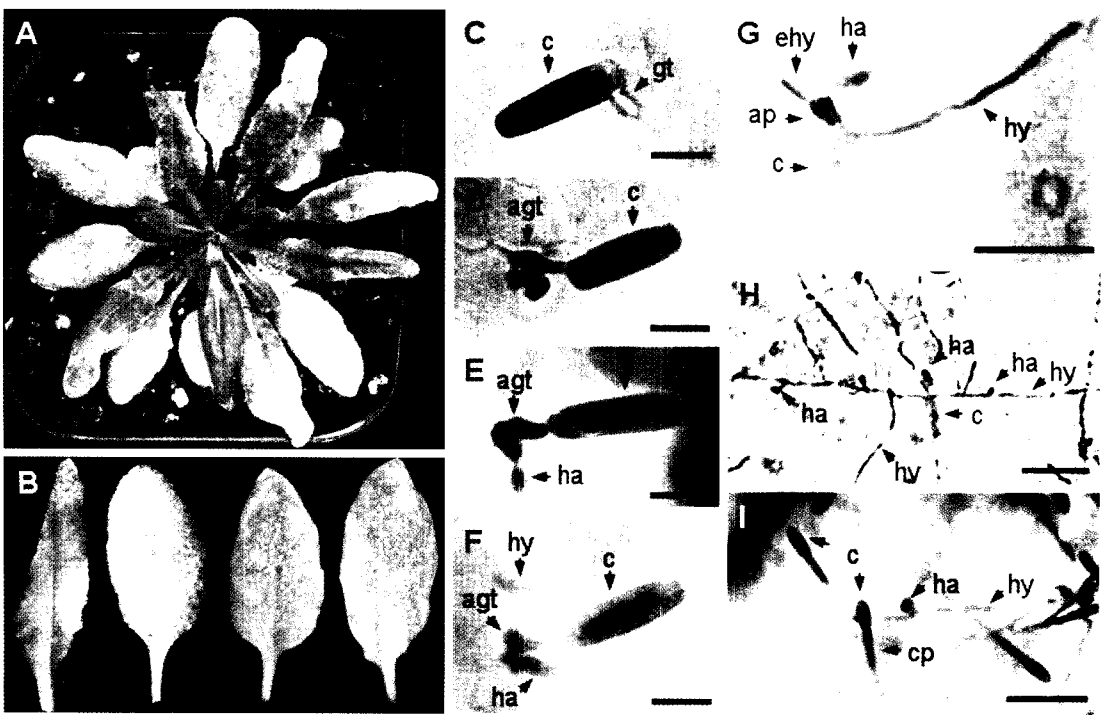

Fig. 1. Disease symptoms and fungal structures of *Erysiphe cruciferarum* KUS-F23994 on *Arabidopsis thaliana* ecotype Col-0. (A and B) Disease symptoms on leaves of *A. thaliana* plants infected with *E. cruciferarum*. (C-I) Trypan blue staining of fungal structures in *Arabidopsis* leaves (C and D) 12, (E and F) 24, (G) 72 and (H and I) 120 h after inoculation. Bars=20 μm (C-F) and 50 μm (G-I). ap: appressorium, agt: appressorial germ tube, c: conidium, cp: conidiophore, ha: haustorium, ehy: elongated secondary hyphae, gt: germ tube and hy: hyphae.

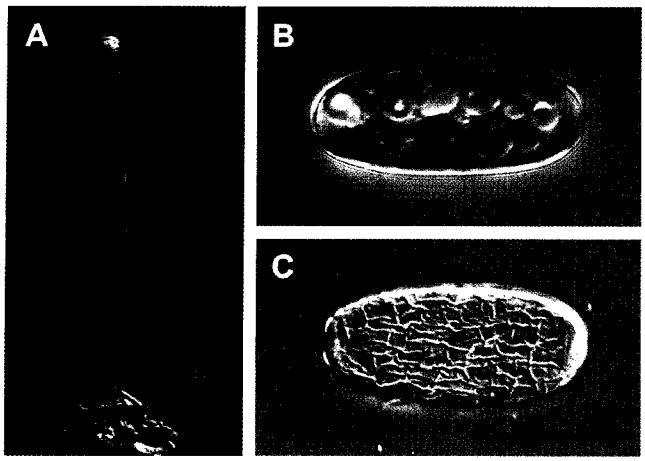

Fig. 2. Light microscope micrographs of conidiophore and conidium of *Erysiphe cruciferarum* KUS-F23994 from *Arabidopsis thaliana*. (A) conidiophore, (B) conidia and (C) surface feature of conidia of *E. cruciferarum* KUS-F23994. Bars=50 μm (A) and 20 μm (B and C).

powdery mildew fungus were done by bright field- and differential interference contrast (DIC)-light microscopy using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements and a Zeiss AX10 microscope (Carl Zeiss, Göttingen, Germany) mainly for photographs. Measurements were performed at 1000× for conidia and at 100-200× for other fungal structures. Conidiophores were hyaline, unbranched, 3-4 celled and cylindrical, and 80-115×6-9 μm in size (Fig. 2A). Singly produced conidia (pseudoidium type) were hyaline, oblong to cylindrical or oval in shape, and 26-55×15-20 μm in size with a length/width ratio of average 3, angular/rectangular wrinkling of outer wall and no distinct fibrosin bodies (Fig. 2B and C). Appressoria on the hyphae were lobed. No chasmothecia were found. These structures are typical of the powdery mildew *Oidium* subgenus *Pseudoidium*, anamorph of the genus *Erysiphe*. The measurements of the fungal structures coincided with those of *E. cruciferarum* previously described by Braun (1987).

Sequence analysis of the ITS rDNA. The rDNA gene sequences covering both highly conserved ribosomal subunits and variable internal transcribed spacer (ITS) regions are widely used to evaluate the phylogenetic relationships among related fungal species and races within species (Sanez and Taylor, 1999; Takamatsu and Kano, 2001). The PCR amplification and sequencing of the ITS rDNA was performed using the following primer set: ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (White et al., 1990). The resulting sequence of the complete ITS rDNA of *Erysiphe* sp. KUS-F23994 (accession no. FJ548627) has been deposited in the GenBank of National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). The nearly complete ITS rDNA of *Erysiphe* sp. KUS-F23994 was aligned with other *Erysiphe* spp. nucleotide sequences from

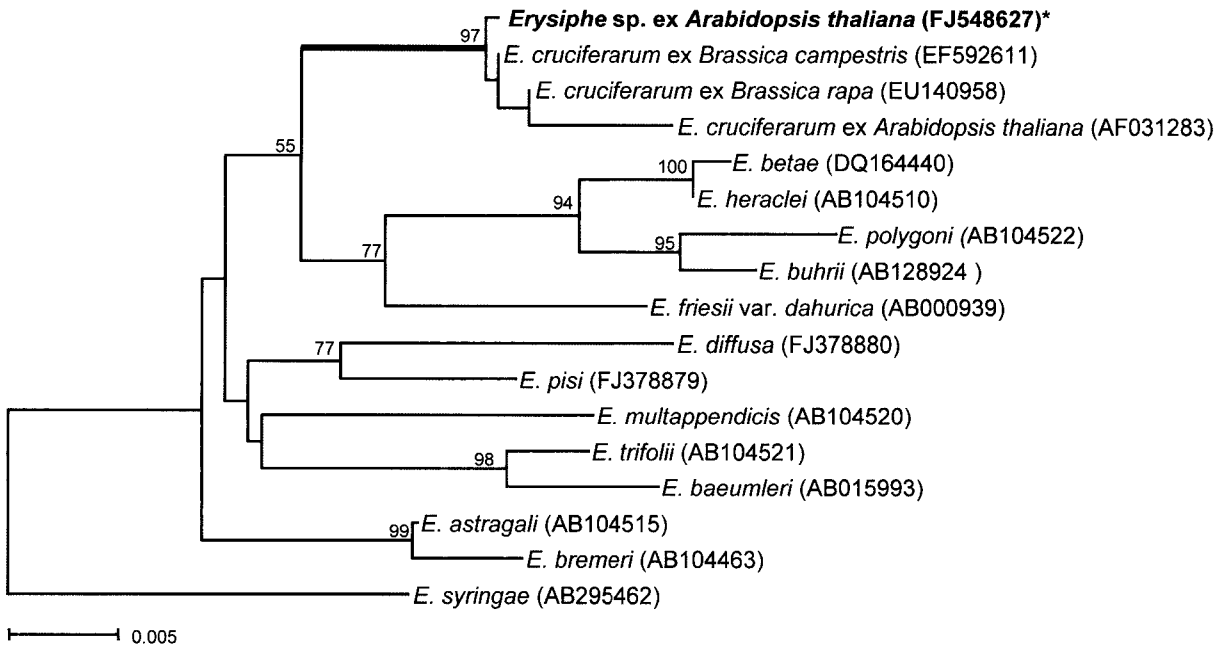

Fig. 3. Phylogenetic tree of *Erysiphe cruciferarum* KUS-F23994 (accession no. FJ548627) and other 16 *Erysiphe* spp., constructed by a neighbor-joining method based on the complete internal transcribed spacer (ITS) rDNA regions (ITS1, 5.8S rDNA and ITS2). Numbers above the branches represent the bootstrap values of over 50% obtained from 1,000 bootstrap replicates. Bar=Number of nucleotide substitutions per site. The GenBank accession numbers are represented in parentheses. Asterisk (*) denotes the isolate using in this study.

the NCBI GenBank database. Molecular phylogenetic reconstructions of these ITS rDNA sequences were done using MEGA4, version 4.0 (Tamura et al., 2007), for neighbor-joining analysis using Tajima-Nei distances. To test the reproducibility of results, 1000 bootstrap replications (Felsenstein, 1985) were performed by the parameters in default values. As shown in the ITS-based phylogenetic tree (Fig. 3), the *Erysiphe* sp. KUS-F23994 formed a well-supported group (a high bootstrap value of 97%) with three sequences of the powdery mildew fungus *E. cruciferarum* which is able to infect *Arabidopsis thaliana*, *Brassica campestris* and *B. rapa*. However, sequence divergences between the *Erysiphe* sp. KUS-F23994 and other *Erysiphe* species, except *E. cruciferarum*, was more than 3%. Taken together, the phylogenetic analysis revealed that the *Erysiphe* sp. KUS-F23994 is indeed identical to *E. cruciferarum*.

Pathogenicity test. To determine whether *Erysiphe* sp. KUS-F23994 infects Arabidopsis plants, we inoculated 4 to 5 week-old *A. thaliana* accession Col-0 plants with a conidial suspension of this powdery mildew fungus. Under controlled laboratory conditions, fungal infections were induced by spraying a conidial suspension or brushing infected leaf materials onto the *A. thaliana* leaves. For spray inoculation, a conidial suspension was adjusted to 10^5 conidia ml^{-1} in FC 43 (Fluorinert Electronic Liquid, Commercial Chemicals Division/3 M, St. Paul, MN) (Hwang and Heitefuss, 1982). The infected plants were placed in a growth room with 60% relative humidity at 25°C. The infected *A. thaliana* leaves showed typical powdery mildew symptoms, which first appeared as white-powdered colonies and subsequently coalesced with abundant growth on the entire leaf surface, as shown in Figure 1. Severe infections caused leaf distortion, withering and premature senescence.

Erysiphe cruciferarum on *A. thaliana* has previously been recorded from Europe and North America (Adam and Somerville, 1996; Allen et al., 2004; Xiao et al., 1997). In Korea, the fungus has been recorded to be parasitic to *Lepidium apetalum* (Shin and La, 1992) and Chinese cabbage (*Brassica campestris*) (Jee et al., 2008) of the family Brassicaceae, but not yet to *A. thaliana*. This is the first report of occurrence of powdery mildew on *A. thaliana* caused by *E. cruciferarum* in Korea. The causal agent *E. cruciferarum* of the powdery mildew disease on *A. thaliana* found in Korea will be useful as a valuable model organism for the study and understanding of biotrophic fungi-plant interactions in Korea.

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