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NOTE

Molecular Phylogenetic Status of Korean Strain of *Podosphaera xanthii*, a Causal Pathogen of Powdery Mildew on Japanese Thistle (*Cirsium japonicum*) in Korea

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Powdery mildew diseases are sensitive to climate change and spread can be favored by increased temperature and low moisture. During 2011 to 2012, a powdery mildew disease by a *Podosphaera* species was observed on the leaves of Japanese thistle (*Cirsium japonicum*) in Korea. The initial sign of this disease included scattered superficial white mycelia on leaves. As the disease progressed, abundant necrotic black spots exhibiting chasmothecia were formed on the leaves. rDNA ITS and 28S homologies of the fungus (EML-CSPW1) showed 100% identity values with those regions from many strains of *P. xanthii* (syn. *P. fusca*) via NCBI BLASTN search.

Keywords: Asteraceae, climate change disease, Japanese thistle, ITS and 28S rDNA sequences, powdery mildew

Powdery mildew is a climate change disease and can spread with the increase in temperature and low moisture (Osava, 2010; Siebold and Tiedemann, 2012). From August to October in both 2011 and 2012, a powdery mildew disease caused by a *Podosphaera* species was observed on the leaves of Japanese thistle (*Cirsium japonicum* Fisch. ex DC.) in most areas of Korea. Japanese thistle is a species of Asteraceae and is known commonly as a noxious weed throughout the world. Also, it has beneficial effects as an antioxidant, and has anticancer properties (Yu *et al*., 2008).

Powdery mildew diseases of various crops and other plants are caused by many different species of fungi grouped into main genera in Erysiphales which is an order including a single family, the Erysiphaceae. The Erysiphaceae is a group of plant pathogenic fungi that cause powdery mildew diseases on about 10,000 angiosperm species as obligate biotroph (Takamatsu *et al*., 2008; Ito and Takamatsu, 2010). The powdery mildew fungi are biotrophic parasites, with many species invading only epidermal cells by means of haustoria (Huckelhoven, 2005). *Podosphaera* is a genus of the powdery mildew fungi belonging to the tribe Cystotheceae of the Erysiphaceae (Braun and Takamatsu, 2000). *P.* (syn. *Sphaerotheca*) *xanthii* (or *fusca*) is also known to be a main causal agent of powdery mildew on various cucurbit plants and one of the most important limiting factors for cucurbit production worldwide (Shin *et al*., 2006; Perez-Garcia *et al*., 2009; Kousik *et al*., 2011; Park *et al*., 2011a, 2011b; Siddiqui *et al*., 2011). The species has been speculated to be a ubiquitous fungus with a broad host range. The taxonomy of the powdery mildew fungi recently underwent extensive revision based on rDNA sequence data (Mori *et al*., 2000; Heffer *et al*., 2006; Ito and Takamatsu, 2010). The nomenclature of these fungi has not yet been completely standardized in the literature. Furthermore, many basic aspects of the biology of *P. xanthii* remain unknown even though considerable efforts have been done to control the powdery mildew diseases it causes.

In Korea, various *Phodosphaera* species, including *P. tridactyla* on Japanese plum; *P. ferruginea* on goat's-beard; *P. phaseoli* on *Vigna* spp. including mung bean; *P. fusca* on *Lactuca sativa*, etc have been shown to cause powdery mildews (Shin, 2000). Only two powdery mildew pathogens, including *Erysiphe cichoracearum* (syn. *Golovinomyces cichoracearum*) in Japan and *Leveillula compositarum* in China, have been reported to occur on *Cirsium japonicum* (Farr and Rossman, 2012). The Japanese thistle plant is a species of the chrysanthemum family (Asteraceae) native to eastern Asia. The powdery mildew disease caused by *Podosphaera* species has been commonly observed on Japanese thistle leaves in most areas of Korea. However, there have been no detailed descriptions of the anamorph and teleomorph of Korean strains of *Podosphaera* species on Japanese thistle and detailed information on the molecular phylogenetic status of the fungal species is currently unavailable.

The objectives of this study were to investigate the outbreak of Japanese thistle leaf powdery mildew, to describe morphological characteristics (anamorph and teleomorph) of a Korean strain of *Podosphaera* species and to determine the molecular phylogenetic status of the causal pathogen based on ITS and 28S rDNA sequence analyses.

Occurrence of powdery mildew on Japanese thistle

From August to October in both 2011 and 2012, a powdery

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Table 1. ***Podosphaera*** **taxa used for molecular phylogenetic analysis in this study**

Taxon name	Collection no. (Isolate no.)	Source (host)	Origin	GenBank accession no.	
				ITS	28S
Podosphaera aphanis	R_Sco1b	*Rubus* subg. *idaeobatus* (raspberry)	UK	GU942462	
Podosphaera aphanis	S_Italy3	*Fragaria* x *ananassa* (strawberry)	Italy	GU942447	
Podosphaera aphanis	GOU1	NI**	NI	HQ918168	
Podosphaera balsaminae	MUMH2658	*Impatiens noli-tangere*	Japan	AB462805	AB462789
Podosphaera balsaminae	MUMH2841	*Impatiens noli-tangere*	Japan	AB462806	
Podosphaera balsaminae	MUMH245	*Impatiens textori*	Japan	AB040344	
Podosphaera balsaminae	MUMH1231	*Impatiens textori*	Japan		AB462787
Podosphaera cercidiphylli	MUMHS67	*Cercidiphyllum japonicum*	Japan	AB026140	
Podosphaera clandestina	MUMH327	*Spiraea japonica*	Japan	AB026150	
Podosphaera clandestina	VPRI 22119	*Collomia linearis*	USA	AF298546	
Podosphaera clandestina	MUMH1869	*Crataegus oxyacantha*	Argentina		AB525931
Podosphaera clandestina	NI	*Cydonia oblonga*	Iran		AB103367
Podosphaera clandestina	MUMH4968	*Amelanchier laevis*	Germany		AB525927
Podosphaera diclipterae	MUMH4056	*Peristrophe japonica*	Japan		AB462795
Podosphaera elsholtziae	MUMHS131	*Ajuga reptans*	Japan	AB026142	
Podosphaera epilobii	MUMH1873	*Epilobium ciliatum*	Argentina		AB525926
Podosphaera euphorbiae-hirtae	MUMH319	*Acalypha australis*	NI	AB040306	
Podosphaera euphorbiae-helioscopiae	CJLCC100	*Euphorbia pekinensis*	China		JF795491
Podosphaera ferruginea	NI	*Sanguisorba officinalis*	NI	AB027232	
Podosphaera fuliginea	PFZK2	*Cucumis melo*	China	JN627139	
Podosphaera fuliginea	Harbin1	*Cucumis melo*	China	EU294368	
Podosphaera fuliginea	HNZK4	*Cucurbita moschata*	China	JN860054	
Podosphaera fuliginea	MUMH303	*Veronicastrum japonicum*	Japan		AB462761
Podosphaera fugax	MUMH343	*Geranium thunbergii*	Japan	AB026134	AB525922
Podosphaera fusca	Sf202	NI	NI	EF137838	
Podosphaera fusca	MUMH108	*Syneilesis palmata*	Japan	AB040349	
Podosphaera fusca	MUMH1933	*Calendula officinalis*	Argentina	AB525914	
Podosphaera fusca	MUMH 2432	*Calendula officinalis*	Argentina	AB525915	
Podosphaera fusca	VPRI19332	*Abelmoschus ficulneus*	NI	AB040293	
Podosphaera fusca	NI	*Calendula officinalis*	Italy	EU140957	
Podosphaera fusca	NI	*Calendula officinalis*	Slovenia	EU159425	
Podosphaera fusca	81955	NI	NI	EF137860	
Podosphaera fusca	43116	NI	NI	EF088830	
Podosphaera fusca	MUMH304	*Calendula officinalis*	Japan		AB462762
Podosphaera fusca	MUMH311	*Arctium lappa*	Japan		AB462767
Podosphaera fusca	MUMH323	*Lactuca indica*	Japan		AB462773
Podosphaera fusca	MUMH328	*Lactuca raddeana* var. *elata*	Japan		AB462776
Podosphaera fusca	MUMHs142	*Rudbeckia* sp.	Japan		AB462798
Podosphaera intermedia	MUMH331	*Clerodendrum trichotomum*	Japan	AB026145	AB462777
Podosphaera leucotricha	N6-08	*Prunus persica* cv.*summerset*	Serbia	HM579841	
Podosphaera leucotricha	JH001	*Malus prunifolia*	Korea	HM242221	
Podosphaera leucotricha	BPI 879141	*Pyrus calleryana*	USA	GU122230	
Podosphaera leucotricha	VPRI 17729	*Malus domestica*	USA	AF073353	
Podosphaera lini	MUMH1392	*Linum usitatissimum*	Switzerland		AB525925
Podosphaera negeri	MUMH1479	*Escallonia rubra*	Argentina		AB525920
Podosphaera negeri	MUMH1478	*Escallonia rubra*	Argentina		AB525919
Podosphaera pannosa	P4-4	*Prunus persica*	France	HM579847	
Podosphaera pannosa	NI	Cherry tree	France	JN654341	
Podosphaera pannosa	NI	*Rosa* sp.	NI		AB022347
Podosphaera pannosa	MUMH 1476	*Rosa rubiginosa*	Argentina		AB525937
***Podosphaera* sp.**[a]	**EML-CSPW1**	***Cirsium japonicum***	**Korea**	**JX896686**	**JX896687**
Podosphaera spiraeae	MUMHS66	*Spiraea thunbergii*	Japan	AB026153	
Podosphaera spiraeae	NI	*Filipendula purpurea* var. *purpurea*	NI	AB022385	AB022384
Podosphaera tridactyla	VPRI 19006	*Prunus* sp.	USA	AF154321	
Podosphaera tridactyla	NI	*Prunus armeniaca* (apricot)	NI	AF011318	

Table 1. Continued

Taxon name	Collection no. (Isolate no.)	Source (host)	Origin	GenBank accession no.	
				ITS	28S
Podosphaera tridactyla	KUS-F26292	*Prunus salicina*	Korea	JQ517296	
Podosphaera tridactyla	NI	*Prunus japonica*	NI		AB022393
Podosphaera xanthii	97-24	*Cucumis sativus*	Japan	AB040330	
Podosphaera xanthii	MUMH68	*Melothria japonica*	Japan	D84387	
Podosphaera xanthii	MUMH808	*Verbenax hybrida*	USA	AB046985	
Podosphaera xanthii	97-12	*Cucumis sativus*	Japan	AB040324	
Podosphaera xanthii	MUMH65	*Cucumis sativus*	NI	AB026146	
Podosphaera xanthii	KUS-F25630	*Cucurbita moschata*	Korea	JQ409565	
Podosphaera xanthii	1C	*Senna occidentalis*	Mexico	JQ728480	
Podosphaera xanthii	MUMH309	*Cucurbita maxima*	Japan		AB462766
Podosphaera xanthii	MUMH326	*Dunbaria villosa*	Japan		AB462775
Podosphaera xanthii	MUMH434	*Trichosanthes kirilowii* var. *japonica*	Japan		AB462786
Podosphaera xanthii	MUMH339	*Glycine max* subsp. *max*	Japan		AB462783
Podosphaera xanthii	MUMH337	*Helianthus annuus*	Japan		AB462781
Podosphaera xanthii	MUMH338	*Zinnia elegans*	Japan		AB462782
Podosphaera xanthii	MUMH340	*Vigna unguiculata*	Japan		AB462784
Podosphaera xanthii	MUMH342	*Crotalaria juncea*	Japan		AB462785
Podosphaera xanthii	MUMH3864	*Zehneria japonica*	Japan		AB462792
Phyllactinia guttata	NI	*Corylus cornuta*	NI	AF011315	
Phyllactinia guttata	SMK17204	*Rhododendron schlippenbachii*	Korea		AB080435

[a] Korean *Phodosphaera* strain isolated from *Cirsium japonicum*. **NI: no information. BPI: U.S. National Fungus Collections (BPI), USDA-Agricultural Research Service. EML: Environment Microbiology Lab Herbarium, Chonnam National University, Korea. KUS: Herbarium of Korea University. MUMH: Mie University Mycological Herbarium, Japan. SMK: Mycological Herbarium, Division of Environmental Science & Ecological Engineering Korea University, Korea. VPRI: Plant Research Institute Herbarium, Australia.

mildew disease was observed on the leaves of Japanese thistle collected from several areas including Cheongyang, Gwangju, and Wando, South Korea (Fig. 1). Additional surveys showed that the disease was widespread in most areas of Korea, including Chungcheong and Jeonnam provinces, South Korea. The initial symptom of this disease was observed in late July to August, and included scattered superficial white mycelia, forming small circular colonies. As the disease progressed, the leaves turned blackish brown and had numerous necrotic black spots showing chasmothecia. When the disease developed, whole parts (leaf, stem, and petiole) of the plant were covered with fungal mats including white anamorphic mycelia and blackish teleomorphic structures. The ascocarps were abundantly formed on both surfaces of the leaves in late September to October, causing slight veinal necrosis and chlorosis. Three specimens examined in this

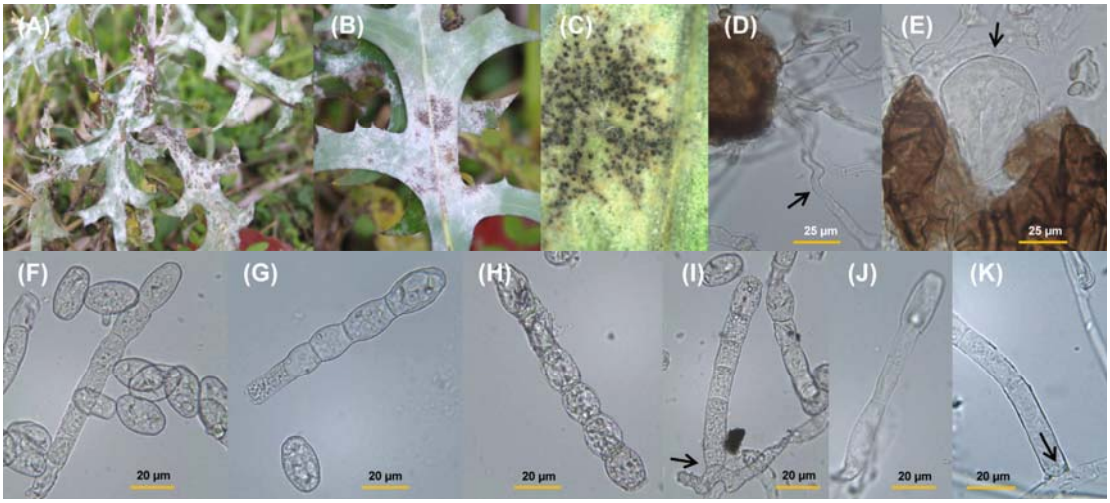

Fig. 1. Powdery mildew caused by *Podosphaera xanthii* on *C. japonicum* and morphology of the causative fungus, *Podosphera* sp. EML-CSPW1. (A) White superficial mycelia on leaves, stems and petioles, (B, C) Abundant necrotic black spots showing chasmothecia, (D) Chasmothecium with hyphoid appendages (400×), (E) Ruptured chasmothecium containing ascus (black arrow), but not bearing apparent ascospores, (F–K) Conidiophores and conidia; F–H, Euoidium types of conidiophores and ellipsoidal conidia; I–K, Conidiophores bent and cylindrical foot cells with a slight constriction at the base (black arrow I), and foot cell with basal septum adjacent to the mycelium (black arrow in K) (400×).

study were collected near Gwangju river in 2011. Fresh samples infected with the powdery mildew pathogen were examined using light microscopy. The dried specimens were deposited as EML-CSPW1, -CSPW2 and -CSPW3 at EMLH (Env. Microbiol. Lab Herbarium, Chonnam National University, Gwangju, Korea).

Morphological characteristics of powdery mildew fungus

As shown in Fig. 1, conidia of the powdery mildew pathogen were hyaline, ellipsoid and borne in long chains or formed singly on conidiophore, and measured 29.3–33.7 (av. 31.5) μm long × 14.3–17.1 (av. 15.8) μm wide. Fibrosin bodies were observed in the conidia. The fungus produced euoidium type (Figs. 1F–1H) and/or cylindrical (Figs. 1I–1K) conidiophores. Conidiophores were straight to slightly curved showing a slight constriction at the base (Fig. 1I). They had cylindrical foot cells with a basal septum adjacent to the subtending hypha (Fig. 1K). The size was 98.2–152.5 (av. 127.2) μm long. The cylindrical foot cells measured 15–30 (av. 23.2) μm long and were followed by 2–3 distal cells. Chasmothecia were present on both abaxial and adaxial surfaces of leaves. They were yellow to brown when young and turned black at maturity, and they were 70.8–94.4 (av. 85.4) μm long. Chasmothecia bore hyphoid appendages and contained asci, but not bearing apparent ascospores. Morphologically, the conidia and conidiophores of our strain fully agreed with previous records of *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (Braun and Takamatsu, 2000; Glawe, 2006; Liberato *et al*., 2006; Singh *et al*., 2009; Cosme *et al*., 2012) (Fig. 1).

Molecular phylogenetic analysis of powdery mildew fungus

To confirm the tentative identification based on the mor-

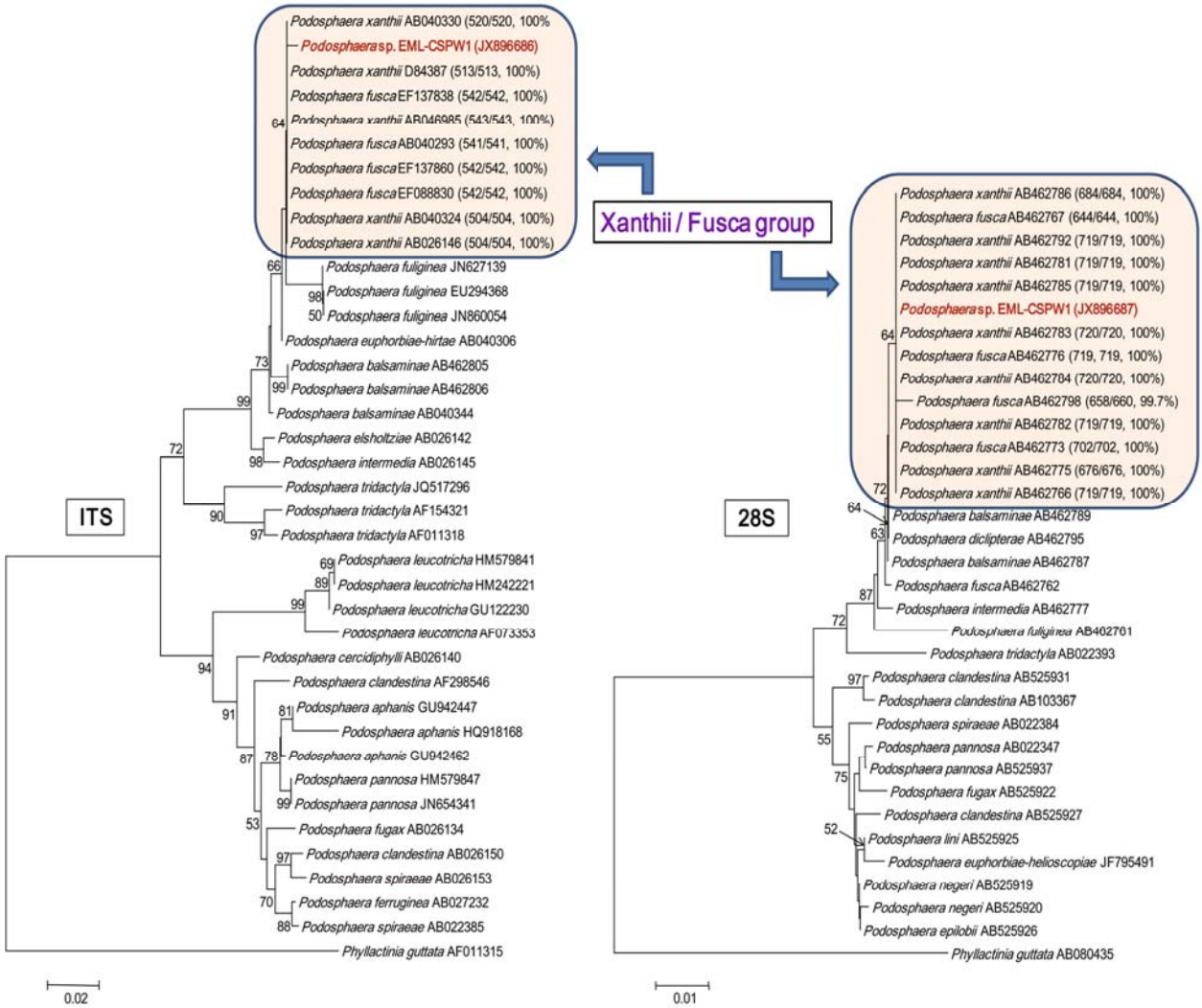

Fig. 2. Phylogenetic analysis of rDNA ITS (512 bp) sequences of 39 taxa and 28S (725 bp) sequences of 34 taxa, including EML-CSPW1 (GenBank accession nos. JX896686 and JX896687 for ITS and 28S, respectively). The percent sequence identity (the number of matches/the complete alignment length) values in the colored boxes were obtained via NCBI BLASTN search of each isolate. *Phyllactinia guttata* was used as an outgroup. Bootstrap values were shown above branches and were supported by more than 50% from 1,000 replications.

phological characteristics, rDNA sequence analysis of the three representative samples was performed. From extracted genomic DNA, the internal transcribed spacer (ITS) region including the 5.8S rDNA and 28S rDNA were amplified with the ITS1F (5′-CTTGGTCATTTAGAGGAAGT-3′) and LR5F (5′-GCTATCCTGAGGGAAAC-3′) primer set as described by Lee *et al.* (2011) and Lee (2012). The purified PCR products were sequenced using an ABI 3700 automated DNA sequencer. Out of three samples, the sequence information of EML-CSPW1 was successfully obtained. As shown in Table 1, phylogenetic analysis of rDNA ITS (512 bp) sequences of 39 taxa and 28S (725 bp) sequences of 34 taxa, including EML-CSPW1 (GenBank accession nos. JX896686 and JX896687 for ITS and 28S, respectively) were conducted, using BioEdit ver. 5.0.9.1, Clustal X ver. 1.83 (Thompson *et al.*, 1997). Their phylogenies were assessed by employing programs available in the MEGA 4 (Tamura *et al.*, 2007). The percent sequence identity (the number of matches/the complete alignment length) values in the colored boxes were obtained via NCBI BLASTN search of each isolate (Fig. 1).

When each of the rDNA ITS (GenBank accession no. JX 896686) and 28S (JX896687) sequences of the strain were compared with related species retrieved from NCBI GenBank, EML-CSPW1 was completely matched with *P. xanthii* (syn. *P. fusca*) which belongs to the *xanthii* group, showing 100% identity values with many related strains of *P. xanthii* or *P. fusca*, via NCBI BLASTN search (Fig. 2). Additional studies on the morphological and genetic variation within Korean *Podosphaera* species from different hosts are now under way.

This paper reports the occurrence of powdery mildew caused by *P. xanthii* on Japanese thistle in South Korea and shows its molecular phylogenetic status falling into a Xanthii/Fusca group and teleomorphic/anamorphic characteristics of Korean strain of *P. xanthii* on *C. japonicum* which is widely dispersed throughout Korea.

For phylogenetic analysis of the Erysiphaceae, many rDNA nucleotide sequence data have been commonly used. rDNA sequence data are especially useful for identifying powdery mildew fungi in the absence of teleomorphs (Okomoto *et al.*, 2002; Lee, 2012). The powdery mildew fungi were split into 5 major lineages including the *Pseudoidium* lineage consisting of 6 genera, i.e., *Erysiphe*, *Microsphaera*, *Uncinula*, *Uncinuliella*, *Brasiliomyces*, and *Typhulochaeta*; the *Eudoidium* lineage consisting of species formerly classified as *Erysiphe* species (such as *E. cichoracearum*, *E. orontii* and *E. galeopsidis*) and *Arthrocladiella mougeotii*; the endophytic lineage consisting of *Phyllactinia* species (such as *P. moricola* and *P. kakicola*), *Leveillula taurica* and *Pleochaeta shiraiana*; the fibrosin lineage consisting of genera *Sawadaea*, *Cystotheca*, *Podosphaera*, and *Sphaerotheca*; and the monocot lineage consisting of single species, *Blumeria graminis* based on nuclear ribosomal DNA sequences of (Mori *et al.*, 2000; Braun *et al.*, 2006). Recently, *Podosphaera* was divided into 2 clades: clade 1, consisting of the section *Podosphaera* on *Prunus* (*P. tridactyla* s.l.); and subsection Magnicellulatae; and clade 2, composed of the other member of section *Podosphaera* and subsection *Sphaerotheca* based on ITS and 28S rDNA sequence analyses (Takamatsu *et al.*, 2010). Although these data are sometimes insufficient to confirm identifications, some fragmentary molecular data including ITS rDNA sequence are becoming increasingly available and can be useful in complementing other data.

P. xanthii is commonly and widely distributed and has been designated variously as *Sphaerotheca fuliginae*, *Sphaerotheca fusca*, and *Podosphaera fusca*. Separation of *P. xanthii* from the *P. fusca* group was proposed based on morphological properties of teleomorph. It has been known that the natural variation of morphological characters within a species make it difficult to assess the phylogenetic status at the species level. Currently, many mycologists have considered *P. xanthii* (*fusca*) as a synonym of *P. fusca* (*xanthii*) although this separation has remained controversial. In the present study, the approach based on the molecular classification system was found to be useful for construction of the phylogenetic tree with regard to *Podosphera* species, showing that the two species belong to a distinct Xanthii/Fusca Group.

It is known that powdery mildew pathogens tend to thrive in conditions with less moisture and higher temperature. With respect to the climate change, we need to continuously monitor the occurrences of new plant diseases and epidemics of unrecorded or recorded plant diseases. The information gathered through our field surveys will be useful for better understanding of agricultural environment in relation to impacts of climate change and effectively responding to the matters.

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