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RESEARCH ARTICLE

Molecular Identification of *Podosphaera xanthii* and the Susceptibility of *Vigna* Species Genotypes to Natural Infection of Powdery Mildew

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Abstract Powdery mildew was observed on 20 genotypes of *Vigna unguiculata* subsp. *sesquipedalis* (yardlong beans) and 33 genotypes of *V. radiata* (mungbean) in Los Baños, Laguna, Philippines. Powdery mildew was collected and then subjected to molecular characterization to identify the species associated with the disease. Based on combined microscopic observations and molecular identification, the species causing powdery mildew to yardlong bean and mungbean was *Podosphaera xanthii* (Castagne) U. Braun & S. Takam. 2000 (Bas.: *Erysiphe xanthii* 1845). None of the yardlong beans and mungbean genotypes were resistant to powdery mildew, with disease incidence reaching up to 100% and with fungal colonies present on plant vines and both sides of leaves. This is the first report of powdery mildew disease caused by *P. xanthii* on yardlong bean and mungbean in the country. Both legume plant species are important food crops in the Philippines. The information from this study will be valuable in formulating other disease management approaches. The lack of resistant plants warrants further screening in legume germplasm collection to identify sources of resistance or tolerance.

Keywords: Yardlong bean, *Vigna unguiculata* subsp*. sesquipedalis,* mungbean, *Vigna radiata,* powdery mildew, *Podosphaera*.

Introduction

Yardlong Bean (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* (L.) Verdc.) and mungbean (*V. radiata (L.)* Wilczek) are two of the most important vegetable legumes in Asia. Production of yardlong beans in the Philippines is third globally, with 109,516 tons in 2020 valued at US$ 595.5 per ton [18, 50]. It is grown primarily for its long immature pods in home gardens, on dikes around paddy fields, and under partially shaded areas as a companion crop or commercial crop [17]. On the other hand, mungbean production in the Philippines posted at 35.5 thousand metric tons valued at US$ 40.9M [37, 50]. It is a raw material in processing products such as sprout production, starch, flour, and paste, used as fodder and cover crop, and grown as an intercrop, rotation, and relay crop [16].

These legume species are hosted and affected by various diseases, including powdery mildew. Powdery mildew is the most widespread leaf disease worldwide, caused by several fungal species in the family Erysiphaceae. Infection is evident by white mycelia and conidia on leaves, stems, fruits, and floral structures. It can spread well in high temperatures and low moisture [1, 36, 46]. Severely infected leaves may become chlorotic or necrotic and cause severe stunting and premature defoliation, thus, decreasing the photosynthetic rate and could crop quality and yield [49].

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In August 2021, powdery mildew was observed in twenty-yardlong bean and thirty-three mungbean genotypes grown at the Institute of Plant Breeding research station, University of the Philippines Los Baños, Philippines. Disease incidence was gathered, and leaf samples were collected from both plant species. This study aims to evaluate the response of the yardlong bean and mungbean genotypes to natural infection of powdery mildew. This study also aims to identify the pathogen associated with powdery mildew of yardlong bean and mungbean by microscopy and molecular technique, through multi-locus gene phylogeny.

Materials and Methods

Percent (%) Disease Incidence

The powdery mildew disease incidence of yardlong bean and mungbean was recorded by counting the number of powdery mildew-infected plants out of the total number of plants (in percentage) per genotype. A scale based on (%) disease incidence from [2] was employed: 0 (Immune): zero susceptibility, 1 (Highly resistant): <1-5%, 2 (Resistant): 6-10%, 3 (Moderately susceptible): 11-40%, 4(Susceptible): 41-70%, 5 (Highly susceptible): 71-100%.

Morphological Characterization

Powdery mildew leaf samples were collected on yardlong bean and mungbean plants for morphological examination. Morphological characters of the powdery mildew were viewed under a microscope (Olympus CX22, Japan), and conidiophores and conidial size were measured from randomly selected 15 conidiophores and 30 conidia. Images were analyzed using the ImageJ software (Version 1.51s, Wayne Rasband, National Institutes of Health).

PCR Assay

Fungal genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) extraction procedure [14, 20]. The extracted genomic DNA was used in subsequent polymerase chain reaction (PCR) assays to amplify fungal barcoding genes. The partial sequence of the internal transcribed spacer (ITS) and partial β-tubulin (*tub2*) gene regions were amplified using primers ITS5/ITS4 [58] and Bt2a/Bt2b [23], respectively. Amplification was performed using the PCR conditions described by [3] and [15]. The PCR products were resolved by gel electrophoresis [1.5% Agarose (Vivantis) 0.5X Tris-Acetate-EDTA buffer containing two µL GelRed solution (Biotium) (PowerPac™ and Sub-Cell GT, (Bio-Rad Laboratories)] and visualized using the GelDoc™ XR+ with Image Lab software (Bio-Rad Laboratories, USA). Apical Scientific Sdn performed DNA sequencing. Bhd., Malaysia.

Phylogenetic Analysis

A consensus DNA sequence was derived from the forward and reverse sequences using the sequence editing software Geneious. An initial analysis was done using the BLASTN program [62, 63] to determine the isolate's closest fungal genera based on the highest percent similarity e-value and highest query cover. Then, a phylogenetic analysis was performed using the Maximum likelihood (ML) method in MEGA-X software [30]. The concatenated sequence of the ITS and *tub2* was assembled and compared with the sequences of powdery mildew species (*Podosphaera, Golovinomyces, Erysiphe, Leveillula, Blumeria,* and *Oidium*) (Table 1) [24, 42, 21, 34, 60, 39, 40, 57, 26, 56]. *Oidium heveae* YN-201 [60], belonging to the similar family Erysiphaceae, was used as the outgroup. Sequences were aligned using CLUSTALW. The ML tree was generated using the Kimura-2 (K2) model [28] with gamma-distributed (G) rates among sites. Support values of the tree were evaluated with 1000 bootstrap replicates.

Table 1. Powdery mildew species used in phylogenetic analysis, their host, origin, and corresponding NCBI accession numbers.

Species	Isolate	Host	Locality	GenBank Accession No.		References
				ITS	β-tubulin	
Blumeria graminis f. sp. hordei	6	*Hordeum vulgare*	France	HM484333	HM538443	[57]
Blumeria graminis f. sp. secalis	3	*Secale cereale*	France	HM484331	HM538444	[57]
Blumeria graminis f. sp. tritici	14	*Triticum aestivum*	France	HM484334	HM538446	[57]
Erysiphe kenjiana	HMJAU-PM91841	*Ulmus pumila*	China	MK452611	MK452458	[42]
Erysiphe necator	BS-VS-G-1	*Vitis vinifera subsp. sylvestris*	Israel	MT920428	MT920478	[24]
Golovinomyces circumfusus	HAL 3300 F	*Eupatorium cannabinum*	Germany	MK452628	MK452459	[42]
Golovinomyces latisporus	HAL 3299 F	*Helianthus annuus*	Switzerland	MK452627	MK452497	[42]
Golovinomyces magnicellulatus	OH5	*Phlox paniculata*	USA	MN830824	MN822595	[21]
Golovinomyces orontii	IT_010	*Cucurbita pepo*	Italy	KR815766	KR815688	[40]
Leveillula contractirostris	EREM1313	*Alcea rugosa*	Israel	LC565506	LC565595	[56]
Leveillula chrozophorae	EREM8192	*Chrozophora tinctoria*	Armenia	LC565501	LC565569	[56]
Leveillula cylindrospora	EREM6625	*Salsola australis*	Armenia	LC565528	LC565557	[56]
Leveillula duriaei	EREM9974	*Nitraria schoberi*	Armenia	LC565505	LC565574	[56]
Leveillula golovinii	EREM 8367	*Nepeta sulfurea*	Armenia	LC565537	LC565551	[56]
Leveillula guilanensis	EREM6629	*Chondrilla juncea*	Armenia	LC565548	LC565555	[56]
Leveillula lanuginosa	HAI05141	*Pimpinella peregrina*	Israel	LC565535	LC565554	[56]
Leveillula lappae	HAI04213	*Cynara cardunculus*	Israel	LC565524	LC565589	[56]
Leveillula linariae	KW50683	*Linaria pontica*	Ukraine	LC565507	LC565587	[56]
Leveillula picridis	HAI05193	*Helianthus sp*	Israel	LC565540	LC565597	[56]
Leveillula taurica	EREM8849	*Scrophularia cineranescens*	Armenia	LC565495	LC565590	[56]
Leveillula saxaouli	KW2S	*Haloxylon sp.*	Uzbekistan	LC565531	LC565558	[56]
Leveillula verbasci	EREM8284	*Verbascum songaricum*	Armenia	LC565532	LC565549	[56]
Podosphaera cerasi	Quincy	*Prunus avium*	USA	MG183669	MK097249	[34]
Podosphaera fusca	Prosser IAREC Roza	*Taraxacum sp.*	USA	MG062864	MK097241	[39]
Podosphaera leucotricha	Puyallup REC GH	*Sorbus sp.*	USA	MF967413	MK097242	[34]
Podosphaera pannosa	Roza	*Prunus persica*	USA	KX842349	MK097253	[34]
Podosphaera prunicola	RR2	*Prunus virginiana*	USA	MF770746	MK097255	[34]
Podosphaera xanthii	BRIP:71599	*Vigna radiata*	Australia	MW293885	MW401671	[26]
Oidium heveae	YN-201	*Hevea brasiliensis*	China	KT935259	KU253575	[60]

Results and Discussion

Symptoms of Infection

In both yardlong bean and mungbean, symptoms consisted of extensive circular to irregular white, dusty colonies on upper and lower leaf surfaces. In addition, newly infected leaves had partial and light coverings of the colonies, which coated the entire leaf surface and vines as the disease progressed and caused premature defoliation. (Figure 1a).

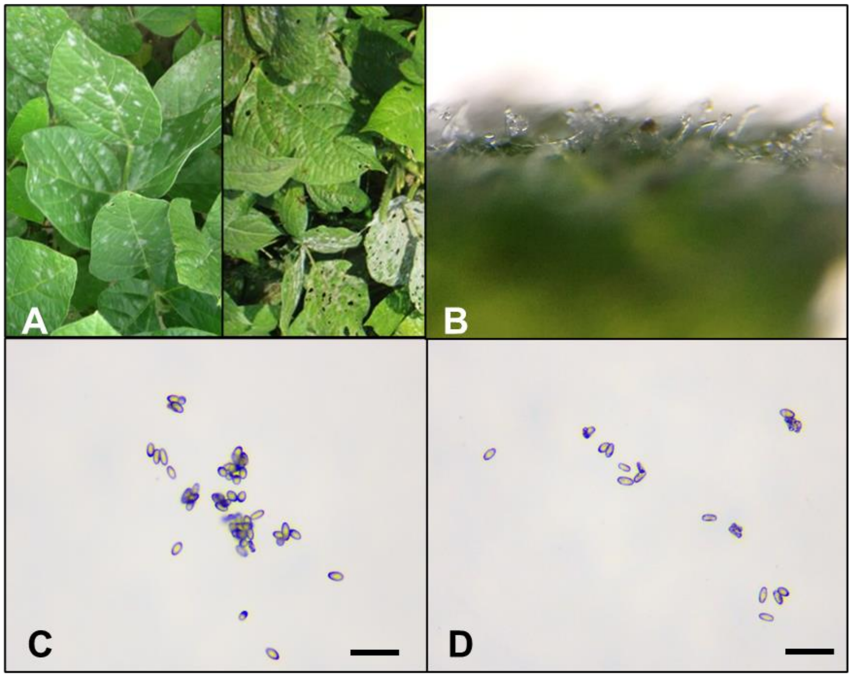

Figure 1. *Podosphaera xanhtii* : (a) symptoms on leaves on yardlong bean (left) and mungbean (right) leaves, (b) conidia and conidiophores (100X magnification), and conidia from (c) yardlong bean and (d) mungbean. Bar= 100 µm.

Powdery Mildew Incidence

Evaluation of the natural infection of powdery mildew on mungbean and yardlong bean observed in the research station of the Institute of Plant Breeding, University of the Philippine Los Baños, Laguna, Philippines, revealed that all of the 33 genotypes of mungbean and 20-yardlong bean genotypes were highly susceptible (5) and had a high incidence (88-100%) of powdery mildew disease (Figure 2).

In this study, all of the 20 genotypes of yardlong bean and 33 genotypes of mungbean were susceptible to powdery mildew, with some plants showing stunted growth due to lacking photosynthetic ability. Severe premature defoliation was also observed. Only a few yardlong beans and mungbean genotypes are reported to have a resistant response to powdery mildew [43, 12, 29, 11, 47, 59]. Thus, it is necessary to further evaluate the performance of other legume genotypes to powdery mildew from the germplasm collections.

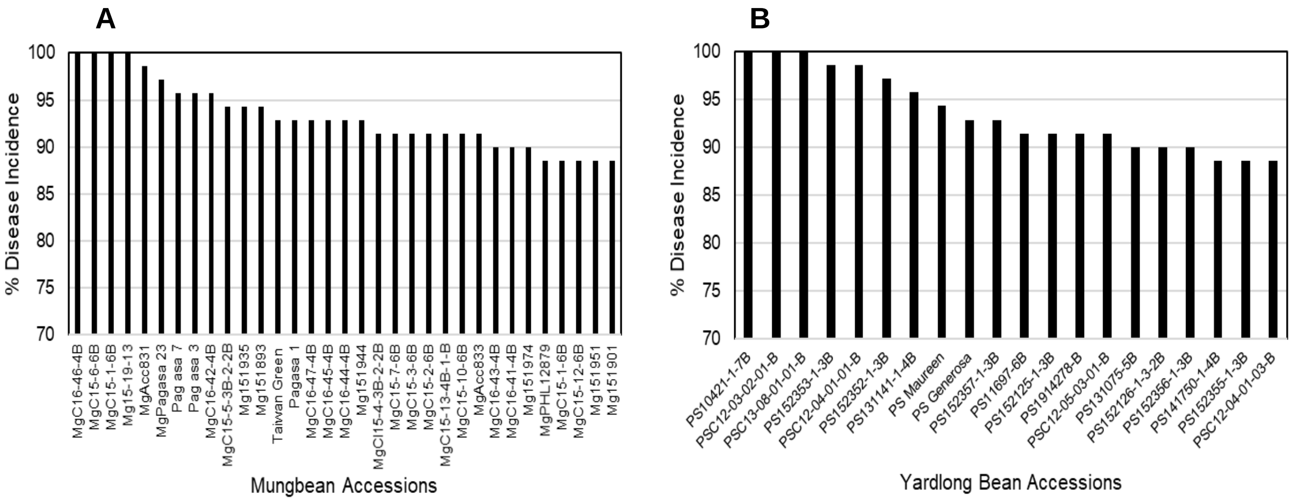

Figure 2. Percent (%) Incidence of the natural infection of powdery mildew at the 6-weeks-old (A) mungbean and (B) yardlong bean genotypes.

Morphological Characterization

Hyphae were branched, septate, flexuous to straight with indistinct to slightly nipple-shaped appressoria. Conidiophores (Figure 1b) were unbranched, and erect, measuring 85.15 to 195.17 µm × 10.31 to 13.70 µm ($\bar{x}$= 129.98 ×12.16 µm, *n=15*) in size in yardlong bean, while measuring 82.53 to 191.22 × 9.81 to 13.71 µm ($\bar{x}$= 137.39 ×12.26 µm, *n=15*) in mungbean. Conidia were hyaline, formed in chains of 2 to 6 cells with conspicuous fibrosin bodies visible in their cytoplasm, ellipsoid-ovoid to dolliform, measuring 31.88 × 17.79 µm (ranging from 28.2-36.73 µm × 15.47-20.97 µm, *n=30*), with a length to width ratio of mostly 1.75 to 2.0 in yardlong bean (Figure 1c) and measuring 32.99 × 17.52 µm (ranging from 28.57-36.7 µm × 15.77-20.06 µm, *n=30*), with a length to width ratio of mostly 1.8 to 2.0 in mungbean (Figure 1d). No sexual structures (chasmothecia) were observed on collected mungbean and yardlong bean leaf samples.

Molecular Identification

The nucleotide sequences of the ITS and partial β-tubulin gene regions were determined for the yardlong bean and mungbean specimens. Comparison based on the initial BLASTn analysis of the ITS gene region of 458bp amplicon of MBPM01a isolates and 459bp amplicon MBPM01c isolate revealed that 91.67% sequence identity with rDNA sequence of *P. xanthii (syn. P. fusca)* HUVU-08 (*Vigna unguiculata*, MH143485) and 89.35% sequence identity with rDNA sequence of *P. xanthii* FUCN (*Solanum melongena,* MG270574), respectively. BLASTn analysis of the β-tubulin gene region of 456bp amplicon (MBPM01a) and 459bp amplicon (MBPM01c) revealed a 99.74% and 99.94% sequence identity with rDNA sequence of *P. xanthii* BRIP:71599 (*Vigna radiata,* MW401671), respectively. The result of the phylogenetic analysis using the concatenated sequences of the ITS and β-tubulin gene regions of MBPM01a and MBPM01c revealed that both isolates from yardlong bean (MBPM01a) and mungbean (MBPM01a) grouped within the *Podosphaera xanthii* clade (Figure 3).

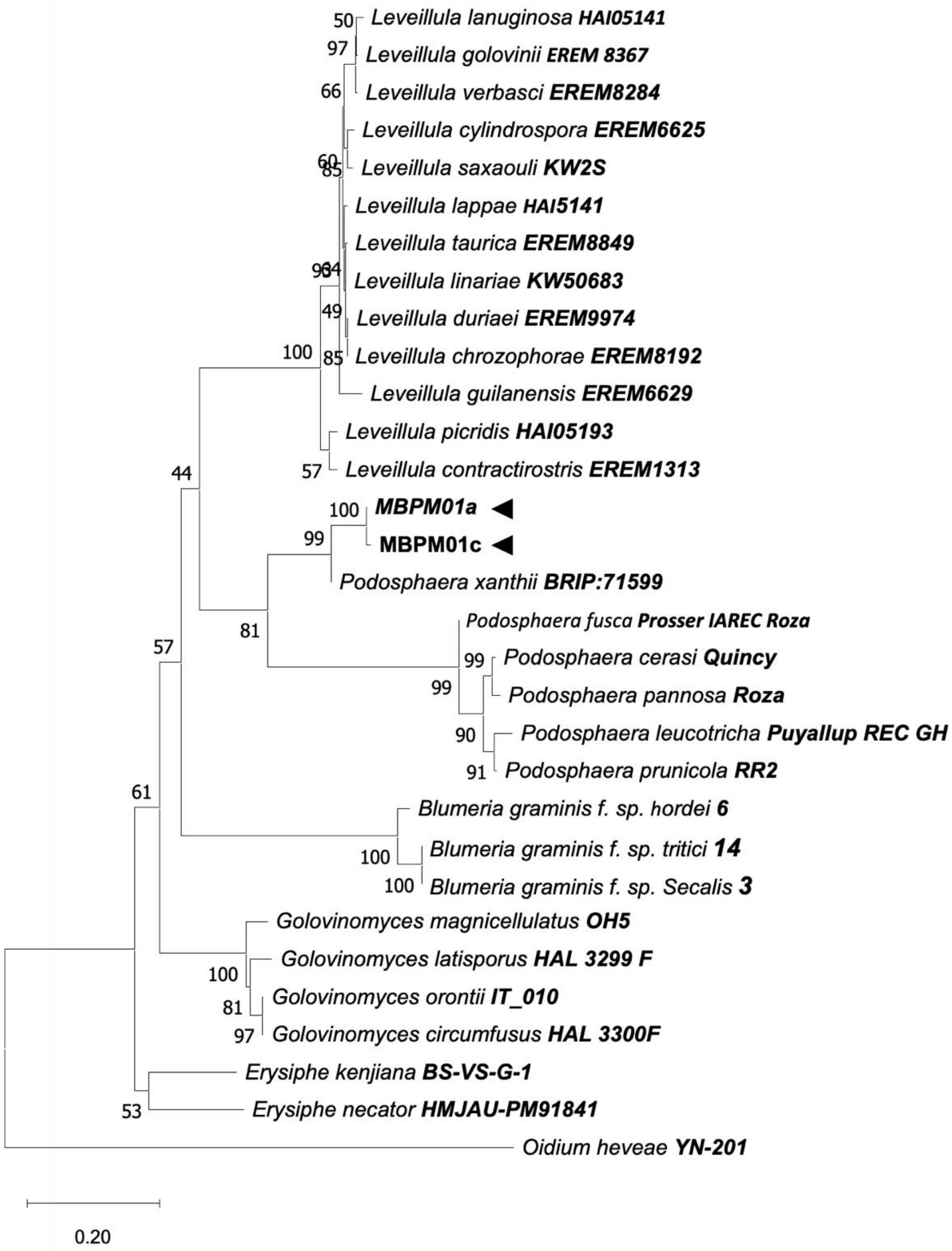

Figure 3. Maximum likelihood tree generated from the concatenated sequences of ITS and *tub2* genes of isolates MBPM01a and MBPM01c (arrowhead) and other powdery mildew species. *Oidium heveae* YN-201 was used as an outgroup.

The morphological characteristics of the *Podosphaera* identified on yardlong bean and Mungbean, in this study, were similar to the morphological descriptions for *Podosphaera* species [25], and of *Podosphaera xanthii* [12] and *P. fusca* [6, 45] except for the absence of sexual structures (chasmothecia) in the yardlong bean and mungbean leaf samples. The differentiation of *P. xanthii* from the *P. fusca* group was in accordance with the morphological species concept based on the sexual reproductive stage (teleomorph) by which the former has larger ascomata and oculi [15-25 (-30) µm diam., Ø 20 µm] that the latter [8-15 µm diam., Ø 10µm] [7, 9, 10]. The absence or lack of sexual structures is a common observation in the field [30] associated with the non-conducive environment for chasmothecia. [34]. Nonetheless, production of it can be induced in the laboratory [4]. Therefore, solely relying on morphological characters to define fungal species is ambiguous and confusing. In this study, the initial BLASTn analysis using phylogenetic analysis generated from the concatenated gene sequences of the two genes revealed that both powdery mildew specimens from yardlong bean and mungbean were grouped within the *Podosphaera xanthii* clade. This separated the *P. xanthii* from *P. fusca* without accounting for the size of chasmothecia. Furthermore, using ITS alone in identifying fungi to a species level is insufficient and arguable. Thus, we used two gene regions to verify and support the identification of the *P. xanthii* as the causal organism infecting yardlong bean and mungbean. This supports Taylor [53] in delineating fungal species using phylogenetic analysis, which should not be based only on a single gene phylogeny but on the consensus of multiple gene genealogies.

The species *Podosphaera xanthii* is previously referred to as *Sphaerotheca fuliginea* or *S. xanthii* and was reclassified and recognized in the genus *Podosphaera* [10]. This powdery mildew species is commonly found in Rosaceae [25] but was also reported on *Vigna* species in Myanmar [55], Australia [27], Taiwan [44], and Thailand [32], while other records on *E. polygoni* causing powdery mildew on *Vigna* radiata and *V. unguiculata* subsp. *sesquipedalis* are from the USA [22], Fiji [19], and Brazil [33].

Based on the combined morphological characteristics and molecular identification, the powdery mildew species infecting both yardlong bean and mungbean was *Podosphaera xanthii* (Castagne) U. Braun & S. Takam. 2000 (Bas.: *Erysiphe xanthii* 1845). In the Philippines, the powdery mildew on *Vigna unguiculata* subsp. *sesquipedalis* was reported to be caused by *Erysiphe poylgoni* [35, 52]. In *V. radiata,* powdery mildew is associated. with *Golovinomyces cichoracearum* (Bas.: *Erysiphe cichoracearum*) and *Erysiphe polygoni* [e.g., 41, 54, 13, 47, 48, 5]. Thus, this study is the first confirmed report of *Podosphaera xanthii* causing powdery mildew disease of yardlong bean and mungbean in the Philippines.

Conclusions

Through a combined morphological and molecular characterization, the occurrence and identity of *Podosphaera xanthii* causing powdery mildew in yardlong bean (*Vigna unguiculata subsp. sesquipedalis*) mungbean (*Vigna radiata*) were first confirmed and reported in the Philippines. A comprehensive survey and evaluation with accurate species identification on *P. xanthii* (syn. *P. fusca*) together with the previously reported *G. cichoracearum* and *E. polygoni* causing powdery mildew on yardlong bean and mungbean in the Philippines is warranted to determine which species is causing the majority of incidence and significant damage in legumes in the country, and ultimately used the information in the formulation of disease management approaches and breeding for resistance.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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