Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
9(1): 1–14 (2019) ISSN 2229-2225

www.creamjournal.org

Article
Doi 10.5943/cream/9/1/1

Diversity of sporulating rice endophytic fungi associated with Thai rice cultivars (*Oryza sativa* L.) cultivated in Suphanburi and Chainat Provinces, Thailand

Su-Han NH[1], Songkumarn P[1], Nuankaew S[2], Boonyuen N[2] and Piasai O[1]*

[1] Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, 50 Ngamwongwan Road, Chatuchak, Bangkok 10900, Thailand
[2] BIOTEC, National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Thanon Phahonyothin, Tambon Khlong Nueng, Amphoe Khlong Luang, Pathum Thani 12120, Thailand

Su-Han NH, Songkumarn P, Nuankaew S, Boonyuen N, Piasai O 2019 – Diversity of sporulating rice endophytic fungi associated with Thai rice cultivars (*Oryza sativa* L.) cultivated in Suphanburi and Chainat Provinces, Thailand. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 9(1), 1–14, Doi 10.5943/cream/9/1/1

Abstract

During the last 10 years, studies on endophytic fungal diversity in various plant host varieties have been investigated increasingly. However, very little empirical research focused on the diversity of sporulating rice endophytic fungi (SREF) associated with Thai rice cultivars (*Oryza sativa* L.) has been performed. The aim of the present study is to explore the SREF associated with Pathumthani 1 and Rice Department 47 rice cultivars using the culture-dependent method from selected sublocations in the Suphanburi and Chinat Provinces of Thailand. Two hundred forty-two SREFs were preliminarily identified based on their morphological traits, and the internal transcribed spacer (ITS) and large subunit (LSU) rRNA gene sequence similarity comparisons validated 21 species in 17 genera. The four most frequently isolated SREF species were *Nigrospora oryzae*, *Curvularia lunata*, *Daldinia eschscholtzii*, and *Exserohilum* sp.3. The results suggest that SREF abundance, richness, distribution, and communities are predominantly influenced by different tissue segment types, rice cultivars, collection areas, and collection times. Our results also provide an insight into SREF diversity and contribute to our basic knowledge of the relationship between fungal diversity and host/location origin.

Keywords – fungal endophytes – occurrence frequency – species diversity

Introduction

Rice (*Oryza sativa* L.) is grown in almost every part of Southeast Asia (Khush 2005, Lin et al. 2014). Production of rice on a commercial scale makes Thailand one of the major rice-cultivating countries in Southeast Asia. Thailand is the second largest country of rice growing area (8677627 ha) after Indonesia's rice growing area (14275211 ha) in Southeast Asia according to FAOSTAT data of 2016 (http://www.fao.org/faostat/en/#home). Intensively cultivated lands for commercial rice production are widely found in irrigated areas of the central region of Thailand. Non-photoperiod-sensitive cultivars, such as Pathumthani 1 (PT1), and Rice Department 47 (RD47), are commonly grown in the central part of Thailand (Kupkanchanakul 2000, Stuart et al. 2018). Pathumthani 1 is one of the aromatic rice varieties with a delicate flavor, high cooking

Submitted 6 August 2018, Accepted 29 November 2018, Published 28 January 2019
Corresponding Author: Onuma Piasai – e-mail – agromj@ku.ac.th

quality, long grains, high amylose content, and a soft texture (Ariyaphanphitak et al. 2005, Laohakunjit & Kerdchoechuen 2007). Rice Department 47 is a non-glutinous and photoperiod-insensitive cultivar that expresses resistance to brown plant hoppers and blast disease but is susceptible to bacterial blight disease (Theerakarunwong & Phothi 2016).

Endophytic fungi are microorganisms that live inside plant tissues without causing any deleterious symptoms (Jiang et al. 2013, Cao et al. 2015). They are distributed throughout various plant species and are associated with plant structures such as leaves, branches, stems, roots, and shoots (Stone & Bacon 2000, Porras-Alfaro & Bayman 2011). They spend the whole or parts of their lifecycle colonizing inter- and/or intracellular spaces of healthy plant tissues (Rodriguez et al. 2008). In addition, fungal endophytes play a major role in plant growth-promoting activities, such as nitrogen fixation and the production of plant growth regulators (Hamayun et al. 2009, Khan et al. 2012, Zheng et al. 2017). These fungi also produce antibiotic and antifungal substances that protect against insects, nematodes, and plant diseases (Zhang et al. 2014, Hartley et al. 2015, Amin 2016).

Endophytic fungal diversity has been widely studied in several plant hosts (You et al. 2012, Bonfim et al. 2016, Cosoveanu et al. 2016, Shi et al. 2016, Ibrahim et al. 2017, Correia et al. 2018). Studies investigating endophytic fungal diversity have been conducted in rice (Yuan et al. 2010, Laskar et al. 2012, Atugala & Deshappriya 2015, Potshangbam et al. 2017, Zhou et al. 2017). However, previous information about the diversity of sporulating rice endophytic fungi associated with Thai rice cultivars (*Oryza sativa* L.) cultivated in Thailand has been limited since 2004-2018. Thus, the aims of this study were as follows: (1) to study the diversity and distribution of sporulating endophytic populations associated with PT1 and RD47 rice cultivars from two provinces located in the central part of Thailand, based on morphological characteristics and ITS and LSU sequence data, (2) to assess the frequency occurrence of sporulating endophytic fungi in Thai rice cultivars and (3) to compare their common and rare species based on their fungal abundances and the richness in the differences of these two rice host varieties and two collecting sites of origin.

Materials & Methods

Study area, sample collection, isolation, and morphological identification

Eight selected rice field sites were surveyed on October 19, 2016 and January 28, 2017, including 4 sites in the Si Prachan and Sam Chuk districts: Suphanburi (SP) (site 1: 14˚36'10.07" N, 100˚10'32.54"E; site 2: 14˚36'10.83" N, 100˚10'50.99" E; site 3: 14˚45'31.63" N, 100˚05'50.42" E; and site 4: 14˚45'45.52" N and 100˚05'50.42" E) respectively; and another 4 sites in the Mueang and Han Kha districts: Chainat (CN) (site 5: 15˚09'36.89"N, 100˚11'59.73" E; site 6: 15˚10'42.98" N, 100˚06'43.36" E; site 7: 15˚04'24.62"N, 99˚58'05.22" E; and site 8: 15˚04'28.62" N, 99˚57'06.20" E) respectively. Samples of PT1 and RD47 rice cultivars were randomly collected and transported to the laboratory.

Three hundred twenty segments (1x1 cm^2) from healthy leaves and 320 segments from healthy roots of each rice cultivars collected from eight sites were used to isolate endophytic fungi. Each segment was washed with 70% ethanol (w/v) for 2 min, followed by sodium hypochlorite solution (2% chlorine) for 5 min, 70% ethanol for 30 s, and two final rinses in sterile distilled water. Sterilized leaf and root segments were placed in 9-cm petri dishes containing 2% Difco™ potato dextrose agar (PDA) medium supplemented with streptomycin (250 mg L^{-1}) to inhibit bacterial growth. The fungal isolates were incubated at 28ºC for 2-3 days (Naik et al. 2009). Hyphal tips were then selected and inoculated onto slant PDA as kept as pure culture for identification. The fungal isolates were examined and identified based on their morphological characteristics using a stereomicroscope (Olympus SZ-PT, Tokyo, Japan) and a compound microscope (Carl Zeiss: Scope.A1, Jena, Germany) according to standard mycological manuals, guide books, and papers (Ellis 1971, 1976, Barnett & Hunter 1987, Seifert et al. 2011). All cultures were deposited at Kasetsart University Culture Collection, the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, as KUFC-ERL/ERR.

DNA extraction, amplification, sequencing, and sequence identification

Total genomic DNA was extracted directly from mycelium using a CTAB method modified and previously described by Boonyuen et al. (2011). The genomic DNA was amplified using the polymerase chain reaction (PCR) in a 50-µL reaction mixture. Two regions of rDNA sequences including the internal transcribed spacer (ITS) and the large subunit (LSU) were amplified using primer pairs ITS5 and ITS4 (White et al. 1990) and LROR and LR7 (Vilgalys & Hester 1990), respectively. Each PCR contained 25 µL of DreamTaq DNA polymerase (1.25 U), 21 µL of ddH2O, 1 µL of each primer (0.2 µM), 1 µL of dNTPs (0.2 mM), and 1 µL genomic DNA extract. The amplifications were performed in a DNA Engine DYAD ALD 1244 Thermocycler (MJ Research, USA) with the following profile: an initial denaturation at 96 °C for 2 min (ITS) or 95 °C for 2 min (LSU), followed by 35 cycles of denaturation at 96 °C for 1 min, annealing at 53 °C for 1 min (ITS) or 53 °C for 1.30 min (LSU), and extension at 72 °C for 1.3 min (ITS) or 72 °C for 2.30 min (LSU), and a final extension at 72 °C for 10 min. The amplicons were visualized by loading 5 µL on 1% agarose electrophoresis gels. The PCR products were sequenced by Macrogen Inc. (South Korea). Consensus sequences for the ITS and LSU sequences were assembled in BioEdit Sequence Alignment Editor v.7.2.3 (Hall 2004). The consensus nucleotide sequences were used to perform individual nucleotide-nucleotide searches with the BLASTn algorithm at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/) to obtain the closest matched sequences. The highest BLAST hit sequences of the ITS and LSU sequence data were compared with morphological data identified to at least the genus or the species level and taxonomic position at the family level (Doilom et al. 2017, Jeewon et al. 2017). New sequences generated in this study are deposited in NCBI, and the accession numbers of the two rDNA sequences (ITS and LSU) are shown in Table 1.

Diversity and data analysis

Diversity of SREF, frequency of occurrence percent (FO%), and rate of fungal infection (IR%) were calculated according to Naik et al. (2009), Fang et al. (2013), Nalini et al. (2014).

$$FO\% = \frac{\text{Total number of segments yielding given fungus}}{\text{Total number of segments incubated}} \times 100$$

$$IR\% = \frac{\text{Total number of segments yielding} \geq 1 \text{ isolate in a plant/variety}}{\text{Total number of segments incubated in that plant/variety}} \times 100$$

Shannon-Weaver (H), Simpson (D), and Evenness (J) indices were calculated with the Species Diversity and Richness software package as described by Henderson & Seaby (1998).

Results

Fungal distribution and dominant and infrequent species of SREF

A total of 242 SERF isolates, including 21 species in 17 genera and 10 families, were identified using morphological characteristics and the BLAST search results of ITS and LSU sequence data with the most closely related representative isolates (Table 1). As shown in Table 2, comparison of the FO% among the two provinces and the two rice cultivars showed that the highest number of SREFs (74) was collected from the PT 1 cultivar in CN, while the lowest number (48) was collected from the PT1 cultivar in SP. Based on Table 2, H, D, and J were highest in the root segments of RD 47 in SP (H=1.90, D=8.57 and J=0.62) and lowest in the leaf segments of RD47 in SP (H=0.15, D=1.07 and J=0.05). The fungal species for which only one isolate was found were identified with an overall percentage of occurrence (OP^{FO}) of 0.08%: *Cladosporium tenuissimum*, *Humicola grisea*, *Pestalotiopsis* sp., *Acrophialophora levis*, *Trichoderma asperellum*, and *Ulocladium chartarum*. In contrast, the four most dominant species were *Nigrospora oryzae*,

Curvularia lunata, *Daldinia eschscholtzii*, and *Exserohilum* sp.3, with OP^{FO} values of 9.76%, 2.03%, 1.01%, and 0.85%, respectively (Fig. 1).

Table 1 Morphological data authenticated with sequence data of ITS and LSU results based on the closest match using BLAST search of the NCBI GenBank nucleotide database.

No.	Morphological identification and isolates number	Numbe of isolates[a]	Nearest BLAST match (Accession numbers of ITS sequence)	GenBank Accession number of ITS sequence[b]	Identity (%) of ITS	Nearest BLAST match taxonomic classification (Accession numbers of LSU sequence)	GenBank Accession number of LSU sequence[b]	Identity (%) of LSU	Proposed fungal ID in this study
1	*Acrophialophora* sp. ERR5-5	1	*Acrophialophora levis* (KM995890)	MH443356	99	Chaetomiaceae (FJ666356)	MH443377	99	*Acrophialophora levis*
2	*Chaetomium* sp. ERL9-9	2	*Chaetomium cupreum* (KF305757)	MH443340	99	Chaetomiaceae (FJ666356)	MH443364	99	*Chaetomium cupreum*
3	*Cladosporium* sp. ERL12-9	1	*Cladosporium tenuissimum* (MF473305)	MH443341	99	Cladosporiaceae (MH047202)	MH443363	100	*Cladosporium tenuissimum*
4	*Curvularia* sp. ERR14-6	26	*Curvularia lunata* (MF380920)	MH445914	100	Pleosporaceae (GU017540)	MH443366	99	*Curvularia lunata*
5	*Exserohilum* sp.1 ERR2-7	2	*Exserohilum rostratum* (LT837845)	MH443342	100	Pleosporaceae (AY016368)	MH443365	99	*Exserohilum* sp.1
6	*Exserohilum* sp.2 ERL12-5	2	*Exserohilum rostratum* (LT837845)	MH443346	100	Pleosporaceae (AY016368)	MH443367	99	*Exserohilum* sp.2
7	*Exserohilum* sp.3 ERR15-5	11	*Exserohilum rostratum* (LT837845)	MH443347	100	Pleosporaceae (AY016368)	MH445917	99	*Exserohilum* sp.3
8	*Fusarium* sp.1 ERR7-7	10	*Fusarium incarnatum* (MH290472)	MH443349	100	Nectriaceae (KU140624)	MH443369	100	*Fusarium incarnatum*
9	*Fusarium* sp.2 ERR14-9	6	*Fusarium* sp. (MH021985)	MH445916	100	Nectriaceae (KU140624)	MH443368	100	*Fusarium* sp.2
10	*Humicola grisea* ERR5-2	1	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	*Humicola grisea*
11	*Nigrospora oryzae* ERL2-10	125	*Nigrospora oryzae* (MF288748)	MH443350	100	Trichosphaeriaceae(EU852533)	MH443370	99	*Nigrospora oryzae*
12	*Nodulisporium*-like sp. ERL13-6	13	*Daldinia eschscholtzii* (KY792621)	MH443353	100	Hypoxylaceae (KY610440)	MH443372	99	*Daldinia eschscholtzii*
13	*Penicillium* sp. ERL13-5	6	*Penicillium oxalicum* (KY781806)	MH443352	100	Aspergillaceae (EF411064)	MH443374	99	*Penicillium oxalicum*
14	*Pestalotiopsis* sp. ERL3-3	1	*Pestalotiopsis* sp. (KF746141)	MH445915	100	Sporocadaceae (KY366173)	MH443375	99	*Pestalotiopsis* sp.
15	*Pyricularia oryzae* ERL15-9	3	*Pyricularia oryzae* (KT693184)	MH443357	100	Pyriculariaceae (KY173527)	MH478121	99	*Pyricularia oryzae*
16	*Talaromyces* sp.1 ERR11-5	8	*Talaromyces stipitatus* (MG461620)	MH443359	100	Aspergillaceae (AF510496)	MH443378	99	*Talaromyces stipitatus*
17	*Talaromyces* sp.2 ERR11-7	6	*Talaromyces* sp. (JX122729)	MH443358	99	Aspergillaceae (AF510496)	MH443380	99	*Talaromyces* sp.2
18	*Thielavia terricola* ERR7-5	6	*Thielavia terricola* (KY965442)	MH443360	99	Chaetomiaceae (FJ666356)	MH443379	99	*Thielavia terricola*
19	*Trichocladium* sp. ERR5-4	10	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	*Trichocladium* sp.
20	*Trichoderma* sp. ERR11-12	1	*Trichoderma asperellum* (MH356723)	MH443362	100	Hypocreaceae (HM466686)	MH478122	99	*Trichoderma asperellum*
21	*Ulocladium chartarum* ERR10-4	1	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	ND[c]	*Ulocladium chartarum*

[a]This number of isolates is based on a total of 242 SERF isolates; [b]New sequences generated in this study are deposited in NCBI; [c] ND=not determined (Unfortunately, *Humicola grisea* ERR5-2, *U. chartarum* ERR10-4 and *Trichocladium* sp. ERR5-4 did not germinate on synthetic media. Thus, this fungus was only identified using morphological data).

Comparison of SREF assemblages (species) between SP and CN provinces showed a difference in species composition. In SP, there were 15 species representing 12 genera, including *Curvularia lunata*, *Fusarium incarnatum*, *Fusarium* sp.2, *Nigrospora oryzae*, *Penicillium oxalicum*, *Talaromyces stipitatus*, and *Trichocladium* sp., occurring in both rice cultivars. Interestingly, *Nigrospora oryzae* was the most common species in PT1 (6.9%) and RD47 (13.1%) (Table 2). In CN, 16 species representing 14 genera, including *Chaetomium cupreum*, *Curvularia lunata*, *Exserohilum* sp.3, *Fusarium* sp.2, *Nigrospora oryzae*, *Daldinia eschscholtzii*, *Talaromyces* sp.2, and *Thielavia terricola*, occurred in both rice cultivars. Among overlapping taxa, the dominant species isolated from CN in PT1 was *Nigrospora oryzae* (11.9%), while *Nigrospora oryzae* (7.2%) and *Curvularia*

lunata (4.1%) were identified in RD47 (Table 2). In addition, *Curvularia lunata* was isolated from CN in different tissue types in RD47 at two collection times, with FO=4.1%.

Overlapping/exclusive SREFs found in two provinces in two tissue segments, two cultivars, and at two collection times

Five and six SREFs were found exclusively in SP and CN, respectively, while 10 species were common to both provinces (Fig. 2A). In SP, each rice cultivar found 11 endophytic fungi, while only seven species overlapped in both rice cultivars (Fig. 2B). In addition, nine and 11 SREFs were found in the leaf and root segments, respectively. However, four fungal isolates were identified specifically from leaf, and six species were identified from root (Fig. 2D). Moreover, during the first and second data collection times, only five taxa were abundant in both periods (Fig. 2F). When we compared the species overlapping from SP province based on tissue types and rice cultivars, three species (*C. lunata*, *N. oryzae,* and *T. stipitatus*) were overlapped between leaf and root in PT1, while three species (*C. lunata, F. incarnatum,* and *N. oryzae*) were overlapped in RD47 (Fig. 2H).

In CN, the fungal species isolated from PT1 (15) showed a high number more than RD47 (9), and eight species were recorded in both rice cultivars (Fig. 2C). Seven species were common taxa in all leaf and root segments (Fig. 2E), and seven species were found at both data collection time points (Fig. 2G). As shown in Fig. 2I, five species (*Exserohilum* sp.3, *F. incarnatum*, *P. oxalicum*, *P. oryzae*, and *T. terricola*) were overlapped between leaf and root in PT1, while two species (*C. lunata* and *N. oryzae*) were overlapped in RD47.

Discussion

SREF diversity of two Thai rice cultivars, two rice segments, and two selected provinces

The results from this study suggested that different fungal communities occur in different tissue types in two rice cultivars (PT 1 and RD 47), as shown in Table 2, Fig. 2. For example, the results showed that more diverse fungal species found in CN Province were detected in PT 1 than in RD 4, but no differences were observed between fungal communities isolated from PT1 and RD47 cultivated in SP Province. SREF community structures differed significantly among the two collection sites and two tissue segment types, suggesting that site characteristics affected the community composition of SREFs colonizing the roots and leaves of *Oryza sativa* The nutrient content, anatomy of each tissue type, and duration of aerial spore exposure may explain endophytic community differences between the two tissue types (Gong & Guo 2009, Lv et al. 2010, Sun et al. 2011).

Differences in rice cultivars may influence differences in the fungal communities among the cultivars, rice genotypes, and rice growth stages examined in this study (Collado et al. 2000, Li et al. 2007, De Errasti et al. 2010, Shi et al. 2016). Additionally, the two different collection time points in this study may account for differences in fungal communities, potentially due to differences in the climatic and environmental conditions of the rice fields, such as temperature, rainfall, and humidity that are required for SREF colonization (Osono 2008, Jumpponen & Jones 2010). For example, according to our comparison of different fungal isolates, more SREFs were isolated in SP at the second collection time point than at the first collection time point, which differs from the results of Suryanarayanan et al. (1998) and Chagas et al. (2017), who found lower fungal diversity during the dry season than during the rainy season.

In both provinces, the soil is loamy with good irrigation. Differences in the geographical conditions of the two provinces may exert dominant effects on SERF communities (Hoffman & Arnold 2008, Suryanarayanan et al. 2011, Christian et al. 2016, Del Olmo-Ruiz & Arnold 2016, Lee et al. 2017). However, a number of SREFs in this study did not differ (CN=16, SP=15) based on different geographical factors.

Several factors, such as host species or cultivar, soil type, plant physiological status, and tissue or organ of the host plant, may account for the observed differences in SERF diversity

(Arnold 2007, Novas et al. 2007, Hashizume et al. 2010). However, we did not measure some physical parameters, and they cannot be correlated with SERF diversity in this study.

Table 2 Fungal species distribution and FO% of rice endophytic fungi in SP and CN.

No.	Taxon	No. of SREFs and FO%																				
		SP Province										CN Province										Overall FO%
		PT 1				FO %	RD 47				FO %	PT 1				FO%	RD 47				FO %	
		leaf		root			leaf		root			leaf		root			leaf		root			
		1st	2nd	1st	2nd		1st	2nd	1st	2nd		1st	2nd	1st	2nd		1st	2nd	1st	2nd		
1	*Acrophialophora levis* *	-	-	-	-	-	-	-	-	1 (1.3)	1 (0.3)	-	-	-	-	-	-	-	-	-	-	1 (0.08)
2	*Chaetomium cupreum*	-	-	-	-	-	-	-	-	-	-	-	1 (1.3)	-	-	1 (0.3)	-	1 (1.3)	-	-	1 (0.3)	2 (0.15)
3	*Cladosporium tenuissimum**	-	-	-	-	-	-	-	-	-	-	-	1 (1.3)	-	-	1 (0.3)	-	-	-	-	-	1 (0.08)
4	*Curvularia lunata*[#]	2 (2.5)	1 (1.3)	-	3 (3.8)	6 (1.9)	2 (2.5)	-	1 (1.3)	2 (2.5)	5 (1.6)	-	-	2 (2.5)	-	2 (0.6)	6 (7.5)	2 (2.5)	3 (3.8)	2 (2.5)	13 (4.1)	26 (2.03)
5	*Daldinia eschscholtzii*[#]	5 (6.3)	-	-	-	5 (1.6)	-	-	-	-	-	3 (3.8)	-	-	-	3 (0.9)	5 (6.3)	-	-	-	5 (1.6)	13 (1.01)
6	*Exserohilum* sp.1	-	-	1 (1.3)	-	1 (0.3)	-	-	-	-	-	-	1 (1.3)	-	-	1 (0.3)	-	-	-	-	-	2 (0.15)
7	*Exserohilum* sp.2	-	2 (2.5)	-	-	2 (0.6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 (0.15)
8	*Exserohilum* sp.3[#]	-	2 (2.5)	-	-	2 (0.6)	-	-	-	-	-	-	3 (3.8)	2 (2.5)		5 (1.5)	-	-	3 (3.8)	1 (1.3)	4 (1.3)	11 (0.85)
9	*Fusarium incarnatum*	-	-	2 (2.5)	-	2 (0.6)	-	1 (1.3)	1 (1.3)	1 (1.3)	3 (0.9)	1 (1.3)	-	2 (2.5)	2 (2.5)	5 (1.5)	-	-	-	-	-	10 (0.78)
10	*Fusarium* sp.2	-	1 (1.3)	-	--	1 (0.3)	-	-	1 (1.3)	-	1 (0.3)	-	-	1 (1.3)	1 (1.3)	2 (0.6)	-	-	-	2 (2.5)	2 (0.6)	6 (0.47)
11	*Humicola grisea**	-	-	-	-	-	-	-	-	1 (1.3)	1 (0.3)	-	-	-	-	-	-	-	-	-	-	1 (0.08)
12	*Nigrospora oryzae*[#]	-	20 (25)	-	2 (2.5)	22 (6.9)	11 (13.8)	27 (33.8)	-	4 (5)	42 (13.1)	9 (11.3)	29 (36.3)	-	-	38 (11.9)	9 (11.3)	12 (15)	-	2 (2.5)	23 (7.2)	125 (9.76)
13	*Penicillium oxalicum*	-	-	-	2 (2.5)	2 (0.6)	-	-	-	2 (2.5)	2 (0.6)	-	1 (1.3)	-	1 (1.3)	2 (0.6)	-	-	-	-	-	6 (0.47)
14	*Pestalotiopsis* sp.*	-	-	-	-	-	1 (1.3)	-	-	-	1 (0.3)	-	-	-	-	-	-	-	-	-	-	1 (0.08)
15	*Pyricularia oryzae*	-	-	-	-	-	-	-	-	-	-	-	2 (2.5)	1 (1.3)	-	3 (0.9)	-	-	-	-	-	3 (0.23)
16	*Talaromyces stipitatus*	1 (1.3)	-	-	3 (3.8)	4 (1.3)	-	-	-	4 (5)	4 (1.2)	-	-	-	-	-	-	-	-	-	-	8 (0.62)
17	*Talaromyces* sp.2	-	-	-	-	-	-	-	-	-	-	-	-	-	3 (3.8)	3 (0.9)	-	-	-	3 (3.8)	3 (0.9)	6 (0.47)
18	*Thielavia terricola*	-	-	-	-	-	-	-	-	1 (1.3)	1 (0.3)	1 (1.3)	-	-	1 (1.3)	2 (0.6)	-	-	1 (1.3)	2 (2.5)	3 (0.9)	6 (0.47)
19	*Trichocladium* sp.	-	-	1 (1.3)	-	1 (0.3)	-	-	4 (5)	-	4 (1.2)	-	-	5 (6.3)	-	5 (1.5)	-	-	-	-	-	10 (0.78)

Table 2 Continued.

No.	Taxon	No. of SREFs and FO%																				
		SP Province										CN Province										Overall FO%
		PT 1				FO %	RD 47				FO %	PT 1				FO %	RD 47				FO %	
		leaf		root			leaf		root			leaf		root			leaf		root			
		1st	2nd	1st	1st		1st	2nd	1st	2nd		1st	2nd	1st	2nd		1st	2nd	1st	2nd		
20	*Trichoderma asperellum**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (1.3)	-	1 (0.3)	1 (0.08)
21	*Ulocladium chartarum**	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (1.3)	1 (0.3)	-	-	-	-	-	1 (0.08)
	Total no. of SREF isolates	8	26	4	10	48	14	28	7	16	65	14	38	13	9	74	20	15	8	12	55	-
	Total no. of segments incubated	80	80	80	80	-	80	80	80	80	-	80	80	80	80	-	80	80	80	80	-	-
	Overall no. of segments incubated	1280																				-
	Infection rate (IR%)	17.65										20.15										-
	Total no. of species at each collection	3	5	3	4	-	3	2	4	8	-	4	7	6	6	-	3	2	4	6	-	-
	Total no. of species in each rice cultivar	11					11					15					9					-
	Total no. of species in each collection province	15										16										
	Shannon-Weaver (H)	0.90	0.84	1.03	1.37	-	0.65	0.15	1.15	1.90	-	0.99	0.94	1.62	1.67	-	1.06	0.62	1.25	1.74	-	-
	Simpson (D)	2.54	1.69	6	5.62	-	1.62	1.07	3.5	8.57	-	2.33	1.71	6	9	-	3.11	1.56	4.67	9.43	-	-
	Evenness (J)	0.29	0.27	0.34	0.44	-	0.21	0.05	0.37	0.62	-	0.32	0.31	0.53	0.55	-	0.35	0.20	0.41	0.57	-	-
	Total genera in this study	17																				
	Total species in this study	21																				
	Total no. of fungal sporulating isolates	242																				

Number sign (#): dominant species; asterisk (*): rare species.

Most common Thai SREFs compared with SREFs found in different locations

Table 3 lists the most common SREFs collected from different countries with varying dominant endophytic fungal species. Four taxa—*Nigrospora oryzae*, C*urvularia lunata*, *Daldinia eschscholtzii*, and *Exserohilum* sp.3—were the most prevalent species in this study. For the most frequently observed species was *N. oryzae*, which is a plant pathogen and can cause potentially fatal diseases in humans (Al-Askar et al. 2014, Yew et al. 2014). It has also been isolated from several hosts worldwide (Varanda et al. 2016, Ferreira et al. 2017) and has been reported to produce antimicrobial activity and secondary metabolites (Gond et al. 2012, Thanabalasingam et al. 2015). Most of the endophytic fungal isolates in this study have been reported as plant-associated fungi, i.e., *Curvularia lunata* and *Pyricularia oryzae* (Khemmuk et al. 2016), suggesting that they may play an

ecological role in the plant. Of the five and six taxa found only in the SP and CN Provinces, respectively, most had low frequencies of occurrence (0.15-0.62%) or occurred only one time (0.08%). The Thai fungal endophytic community was not similar to that described by Naik et al. (2009), who investigated the endophytic fungi of rice in India, and differed from those found by Atugala & Deshappriya (2015), who studied SREFs in Sri Lanka. Additionally, as shown in other studies, some dominant endophytic fungal genera isolated from rice samples, specifically *Absidia*, *Cylindrocladium*, and *Paecilomyces*, reported by Atugala & Deshappriya (2015), were not observed in this study. Similarly, some SREFs, including the genera *Chaetomium*, *Cladosporium*, and *Penicillium*, discovered by Naik et al. (2009), were not previously found to be dominant endophytes, which contrasted with our findings.

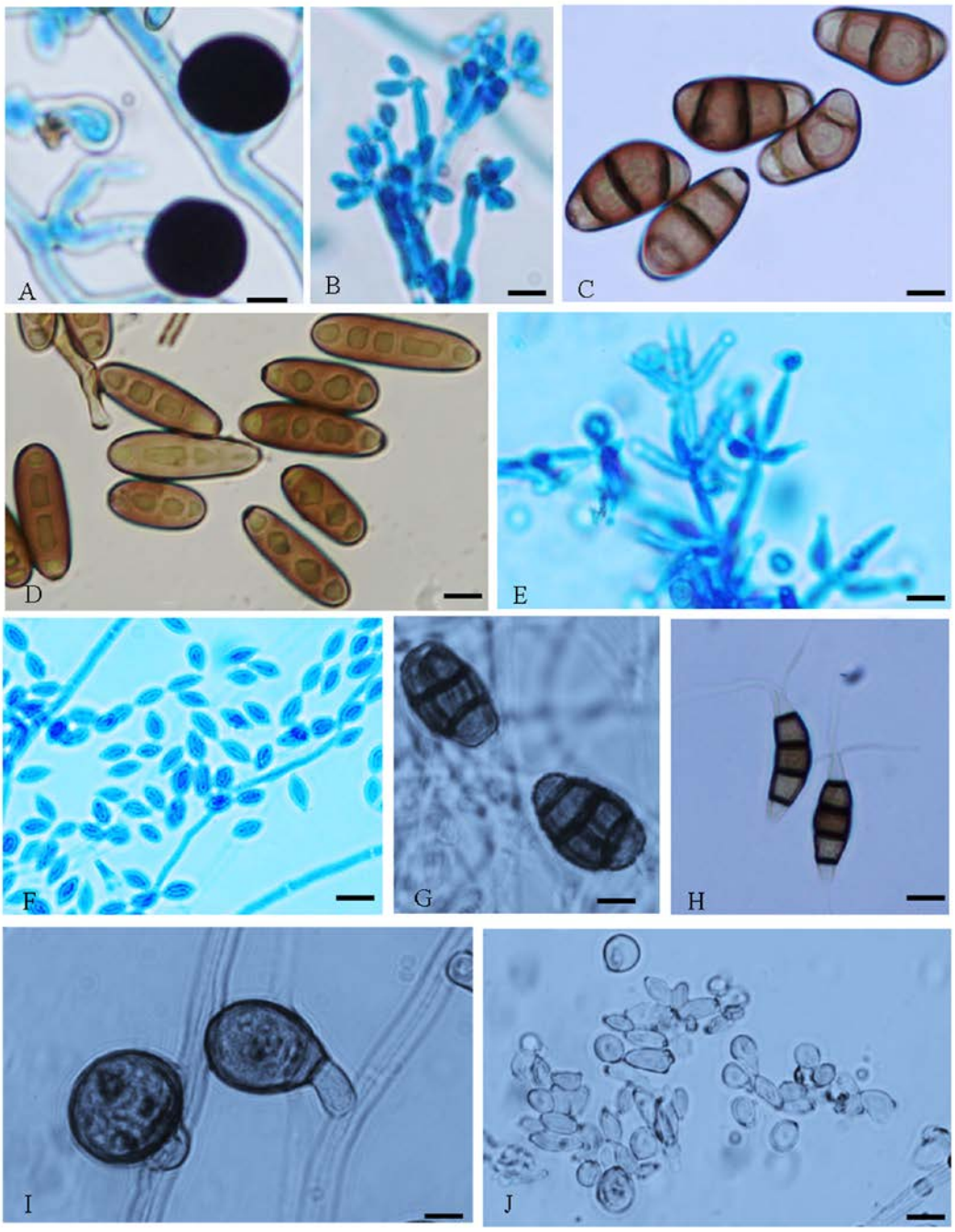

Fig. 1 – Four dominant fungi recorded in this study: A *Nigrospora oryzae*. B *Daldinia eschscholtzii*. C *Curvularia lunata*. D *Exserohilum* sp.3. E–J Rare species found in this study: E *Trichoderma asperellum*. F *Acrophialophora levis*. G *Ulocladium chartarum*. H *Pestalotiopsis* sp. I *Humicola grisea*. J *Cladosporium tenuissimum*. Scale Bars: A–J=10 µm.

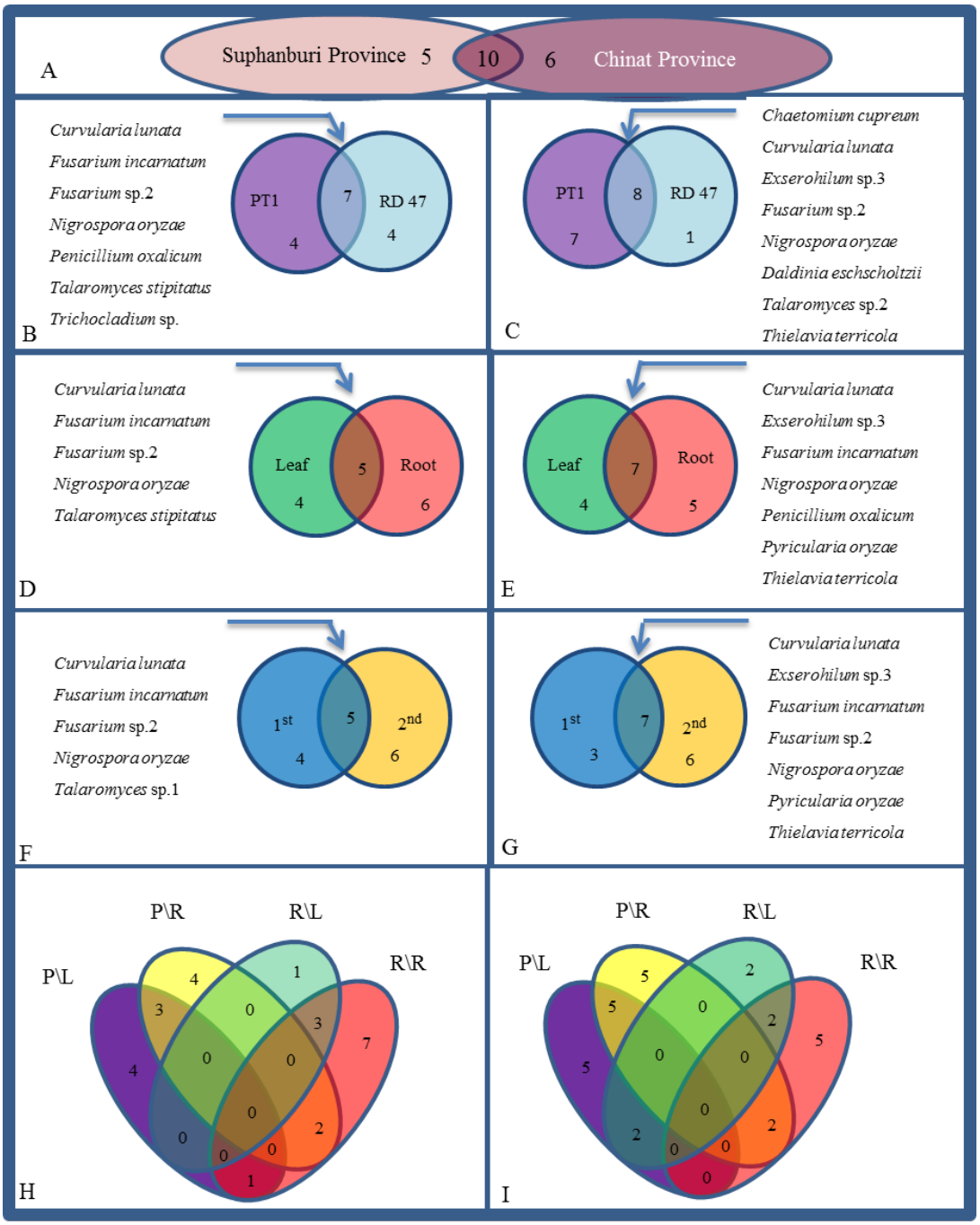

Fig. 2 – Distribution of rice endophytic fungi. A The two provinces. B, C Each rice cultivar. D, E Each tissue type. F, G Each collection time point. H, I Each tissue type in two rice cultivars in the SP and CN Provinces (P\L=leaf segments of PT 1, P\R=root segments of PT 1, R\L=leaf segments of RD 47 and R\R=root segments of RD 47).

Table 3 The most common SREFs from rice (*Oryza sativa* L.) recorded in published studies.

References	Collection sites and country	The most common species
This study	Central Thailand	*Nigrospora oryzae* *Curvularia lunata* *Daldinia eschscholtzii* *Exserohilum* sp.3
Potshangbam et al. (2017)	Northeast India	*Fusarium* sp. *Sarocladium* sp.

Table 3 Continued.

References	Collection sites and country	The most common species
		Aspergillus sp.
		Penicillium sp.
Atugala & Deshappriya (2015)	Sri Lanka	*Absidia* sp.
		Cylindrocladium sp.
Laskar et al. (2012)	Southern India	*Cladosporium* sp.
		Fusarium sp.
		Aspergillus sp.
		Penicillium sp.
		Helminthosporium sp.
Yuan et al. (2010)	China	*Exopkiala,*
		Cladopkialophora,
		Harpophora,
		Periconia macrospinosa,
		Ceratobasidium/Rhizoctonia
Naik et al. (2009)	Southern India	*Chaetomium globosum*
		Penicillium chrysogenum
		Fusarium oxysporum
		Cladosporium cladosporioides
Vallino et al. (2009)	Vercelli, North Italy	*Neotyphodium,*
		Stagonospora
		Penicillium
Tian et al. (2004)	Southern China	*Fusarium* sp.

Some of the endophytic genera that we identified were similar to those identified in several other studies (Tian et al. 2004, Naik et al. 2009, Laskar et al. 2012, Wang et al. 2016, Potshangbam et al. 2017). Those researchers reported *Fusarium* sp. as the most common taxon. Based on our data, *Fusarium* sp. occurred in both rice cultivars, although it was not isolated as the dominant species. Unfortunately, there are few reports describing SREFs from temperate zones to compare with our data (Vallino et al. 2009). Additionally, host difference of rice species resulted in different endophytic fungal genera and numbers of fungal isolates; for example, in Dongxiang wild rice (*Oryza rufipogon* Griff.) and wild rice (*Oryza granulata* Nees & Arn. ex Watt.) of China (Yuan et al. 2011, Wang et al. 2015). In conclusion, differences in geographical conditions, different cultivars, and different tissue types may exert major effects on the endophytic fungal community of rice. In this study, several species of *Curvularia*, *Exserohilum*, *Fusarium*, *Nigrospora,* and *Pyricularia* were identified as endophytic fungi, but they have been reported to cause major diseases in rice. Thus, further studies for the investigation and isolation of endophytic fungi in other rice varieties of Thailand will be used to monitor outbreaks of these latent pathogens. In addition, the study of the diversity of endophytic fungi will be used to identify and screen beneficial strains. These findings may be used as a source of potential biocontrol agents to test their antagonistic effects to rice pathogens in the future.

Acknowledgements

The research reported in this study was supported by the SEARCA (Southeast Asia Regional Centre for Graduate Study and Research in Agriculture) scholarship organization. This paper constitutes a partial fulfillment of the Graduate Program in the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University. We express our thanks to Dr. Satinee Suetrong (BIOTEC) for figure editing and are particularly grateful to Prof. E.B. Gareth Jones for his kind and invaluable suggestions on the paper. BIOTEC is thanked for providing facilities for DNA molecular work and research support. We would like to thank the editor and the two anonymous reviewers for their constructive comments, which helped us to improve the manuscript.

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