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Molecular Identification of Fungal Species Causing Brown Circular Leaf Spot Disease in Seedlings of Siamese Rosewood (*Dalbergia cochinchinensis* Pierre ex Laness)

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ABSTRACT

Morphological and pathological features of fungi isolated from leaves of *Dalbergia cochinchinensis* Pierre ex Laness seedlings (Siamese rosewood) with brown circular leaf spot disease have previously been reported. According to morphology, there are different fungi that cause this disease but information is only available on the fungi genera e.g. *Curvularia* sp., *Alternaria* sp., and *Colletotrichum* sp. including an unknown rust fungus. In order to identify the species of these fungi, molecular identification using information derived from the amplification of internal transcribed spacer sequences (ITS) via ITS1, ITS4 and ITS5 primers and phylogenetic analysis was performed. According to the results obtained from the analysis using Neighbor–joining method, the isolated fungal species from the diseased leaves were revealed as *Alternaria alternata*, *Colletotrichum siamense, Curvularia aeria* and *Maravalia pterocarpi,* with supportive bootstrap scores. This research reports the occurrence of the fungal species on this plant to provide additional data for controlling the causal agents of the disease in a more specific and targeted manner.

Keywords: Economic plant; Leave spot; Plant pathogenic fungi; Rosewood; Seedlings

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1. Introduction

The economic plant known as Siam rosewood (*D. cochinchinensis*) in Thailand is highly valuable, at approximately 250,000 Baht per cubic meter and is also included in the IUCN Red List of threatened species [1]. Seedlings of this plant found with severe brown circular leaf spot disease during the seedling stage were collected from two nurseries, Mahasarakham Forest Nursery Center and Khon Kaen Forest Nursery Station. It has been reported that this disease is caused by multiple fungi, morphologically identified as *Curvularia* sp., *Alternaria* sp., *Colletotrichum* sp., and an unknown rust fungus [2]. They are common plant pathogenic fungi able to cause diseases in many plants. *Curvularia* is a fungal genus consisting of more than 40 species, most being phytopathogenic, and are able to bring significant losses to agricultural operations [3]. Many of the fungi in this genus are reported as causes of foliar diseases; for example, in China, *C. microspore* is a new species causing leaf diseases of *Hippeastrum striatum* [4], *C. eragrostidis, C. geniculata*, *C. hawaiiensis*, *C. aeria* and *C. lunata* are causing rice leaf spots in Malaysia [5], *C. verruculosa* is causing leaf spots on *Cynodon* sp. in Hubei, China [6] and leaf spots on lettuce are being caused by *C. aeria* [7] as well as many *Curvularia* species causing leaf blight and spots in oil palm seedlings in Thailand [8]. Another common fungal leaf-disease relating agent is *Alternaria*, which can cause leaf spot and blight diseases in various plants e. g. *A, alternate*, *A. brassicicola* and *A. brassicae* [9-13]. *Colletotrichum* species are also significant pathogenic fungi leading to major damage in different plants e. g. citrus, tulip and rubber trees [14-16]. Apart from these common fungi, rusts such as basidiomycetes are reported as another prominent phytopathogen e.g. *Austropuccinia psidii* causing leaf spot rust on *Rhodamnia rubescens* and *Eugenia reinwardtiana, Thekopsora minima* on blueberry, *Puccinia pelargonii-zonalis* on geranium, and *P. arachidis* on *Arachis hypogaea* [17-19].

Seedlings are vulnerable to infection because of the environment for nursing the seedlings. They are usually maintained in moist conditions and are put close to the ground where there are easily exposed to pathogens [20-21]. In these two nurseries, the plant seedlings were found to be dramatically invaded by the fungi on their leaves, resulting in poor quality of the seedlings before being distributed to farmers. Although the pathological aspects have been studied, the species identification of these fungi was still left to question. According to the morphology of the fungi reported in a previous study [2], the species was unable to be identified. Thus, this gap in knowledge left unfilled by the previous study leads to the purpose of this study, aiming to decipher the species identity of the unidentified fungi, seeking better plant management strategies and control methods that are more precise and possibly species specific.

2. Materials and Methods

2. 1 Fungal isolation and genomic DNA extraction

The fungi were isolated and cultured in synthetic media according to previously used methods [2]. Spores of the rust fungus, taken directly from diseased leaves, were briefly washed in 95% ethanol and passed through sterile distilled water 3 times, and fresh fungal mycelia, approximately 1 g were also taken from the culture for genomic DNA extraction via a previously reported method [22]. Briefly, the fungal mycelia were cultured for 7-10 days on potato dextrose agar with cellophane paper and the mycelia on the surface of the paper were scrapped out before the addition of liquid nitrogen. The mycelia were finely ground with cetyltrimethyl ammonium bromide (CTAB) lysis buffer (600 µl), and then transferred into new Eppendorf tubes. The tubes were incubated at 60° C for 60 min and then 700 ul of chloroform: isoamyl (24:1) was added

before centrifugation at 12,000 rpm for 5 min at 4° C. In new tubes, only supernatants were collected and combined with isopropanol, 0.7 volume of the supernatant. The tubes were gently mixed then stored at -20° C for 20 min before 5-20 min of centrifugation at 12,000 rpm at 4° C in order to get DNA pellets. Ethanol (500 µl, 70%) was added to the tubes to clean the precipitated pellets again and left at room temperature until dry. Finally, TE buffer made up of Tris-HCl (10 mM) and EDTA (1 mM), 40 µl was added to dissolve the dried pellets and was then stored at -20° C for further use.

2.2 PCR amplification

To amplify the informative region for species identification, different primers were used, primers for general fungal identification ITS1-TCCGTAGGTGAACCTGCGG, ITS4-TCCTCCGCTTATTGATATGC and ITS5-GGAAGTAAAAGTCGTAACAAGG [23] for *Curvularia* sp. , *Alternaria* sp. , and *Colletotrichum* sp. isolates, and primers specific to rust fungi ITS1rustF10d-TGAACCTGCAGCAGGATCATTA, ITS1rustR3C-TGAGAGCCTAGAGATCCATTGTTA [24] for the unknown rust isolates because the ITS primers were unsuccessful. Before performing PCR, the extracted genomic DNA was diluted 10-fold with autoclaved dH_2O. The 25-µl PCR reaction was composed of 5 µM of each 20 mM primer solution, 2.5 ul of 20mM dNTP, 2.5 ul of 10x Immo buffer, 0.75 ul of 50 mM $MgCl_2$, 0.15 ul of *Taq* DNA polymerase, 2 ul of diluted genomic DNA and 14.6 ul of dH_2O. The PCR conditions were set as follows: 95°C for 7 min then 35 cycles of 95° C for 1 min, 55°C for 1.5 min then 72°C for 2 min and final extension at 72°C for 10 min. Electrophoresis with 1.2% agarose in TBE buffer (0.01 M EDTA, 0.9 M boric acid, 1 M Tris-base, pH 8.3) was done for 1h to investigate the successful PCR yields then stained with Redsafe™ Nucleic acid staining solution and visualized under UV light. Samples with products were in-gel purified and sequenced at 1st BASE, Apical Scientific Sdn Bhd, Selangor, Malaysia.

2.3 Phylogenetic analysis

Prior to phylogenetic analysis, DNA sequences were trimmed and evaluated from unclear chromatograms on Sequence Scanner Software v2.0. According to BlastN search, fungal DNA sequences of Internal transcribed Spacer (ITS) regions were selected from GenBank (www.ncbi.nlm.nih.gov) in order to construct the phylogenetic tree (Table 1). The sequence alignment of the sequences from the database with the ones from this study was performed using ClustalW and edited manually using MEGA 6.06 [25]. The analysis parameters of Neighbor- joining methods with 1,000 bootstrap replicates were as follows: Jukes-Cantor method was defaulted for processing the distances in the units of the number of base substitutions per site. To model the alteration rate of sites, gamma distribution was set for the calculation and the elimination of gaps was applied to all positions. The same software was also used to visualize the resulting trees. Bootstrap scores shown on tree nodes greater than 50 were considered as being supportive [26].

Table 1. Sequences of fungal species and their accession numbers from GenBank.

Fungal species	Accession no.	References
Alternaria alternata	KP124317	[27]
	KP124318	
	KP124322	
	KP124370	
Alternaria brassicae	KP115600	[27]
	KP115601	
	KP115602	
Alternaria brassicicola	JX499031	[28]
	JX648198	
	JX499030	
	JF417686	[29]
Alternaria cassiae	KJ718135	[30]
	KJ718136	
	KJ718137	
	KJ718138	

Fungal species	Accession no.	References
Alternaria passiflorae	KJ718209	[30]
Alternaria sp.	This study	*Alternaria* 001, 002, 003
Colletotrichum kahawae	KC735140 KC735141 KC735142 KC790932	[31]
Colletotrichum siamense	KP703383 KP703384 KP703386 KP703390	[32]
Colletotrichum gloeosporioides	JX010151 JX010152 JX010153	[33]
Colletotrichum gigasporum	KF687723 KF687724 KF687725 KF687726	[34]
Colletotrichum acutatum	JQ948395 JQ948396 JQ948397 JQ948398	[35]
Colletotrichum cereal	JX625161	[36]
Colletotrichum sp.	This study	Collet 1, 2, 3
Curvularia aeria	KF941270 KP340066 KP340067	[37] [38]
Curvularia affinis	JX966602 JX966610 KR012898	[39] [40]
Curvularia eragrostidis	KP340047	[38]
Curvularia geniculata	KP131873 KP131874 KP131875	[41]
Curvularia lunata	JX960584 JX960585 JX960594	[42]
Curvularia oryzae	KP400650 MH857148 MH859835	[43]
Curvularia sp.	This study	Cur 1, 2, 3
Puccinia coronata	GU598112 GU598111 GU598110 HM131335	[44] [45]
Puccinia monoica	AF182994 AF182996 AF182997 AF182993	[46]
Puccinia graminis	HQ012445 HQ012444 HQ012440 HQ012441	[47]
Melampsora larici-populina	DQ228110	[48]
Maravalia pterocarpi	KU301795	[49]
Rust	This study	Rust1, 2, 3

3. Results and Discussion

The Neighbor-Joining analysis revealed these 3 *Curvularia* isolates derived from the seedlings were *Curvularia aeria* (Fig. 1). The total branch length was 0.20514756. The supportive bootstrap scores above 50 are shown at the tree nodes. In the analysis, there were 19 nucleotide sequences with a total of 638 positions in the final dataset. Among *Curvularia* species, the consensus outcome from the analysis was highly supported by the bootstrap at 81. It can be concluded that the fungus is *Curvularia aeria*.

As illustrated in Fig. 2, the phylogenetic inference using the Neighbor-Joining method with the total branch length of 0.09244556 revealed the species of *Alternaria* isolates 001, 002, and 003 as *Alternaria alternata* supported by the high bootstrap score of 100. This analysis involved 20 nucleotide sequences. There were 481 informative positions total in the final dataset included in the analysis.

The identification of *Colletotrichum* isolates was suggested by Neighbor-Joining analysis and yielded the phylogenetic tree with the total branch length of 0.17737448. It suggested the *Colletotrichum* isolates Collet 1, 2, and 3 were clustered inside *Colletotrichum siamense* branch with a supportive bootstrap score of 84. For the final dataset put in the analysis, there were 23 nucleotide sequences with a total of 622 positions (Fig. 3).

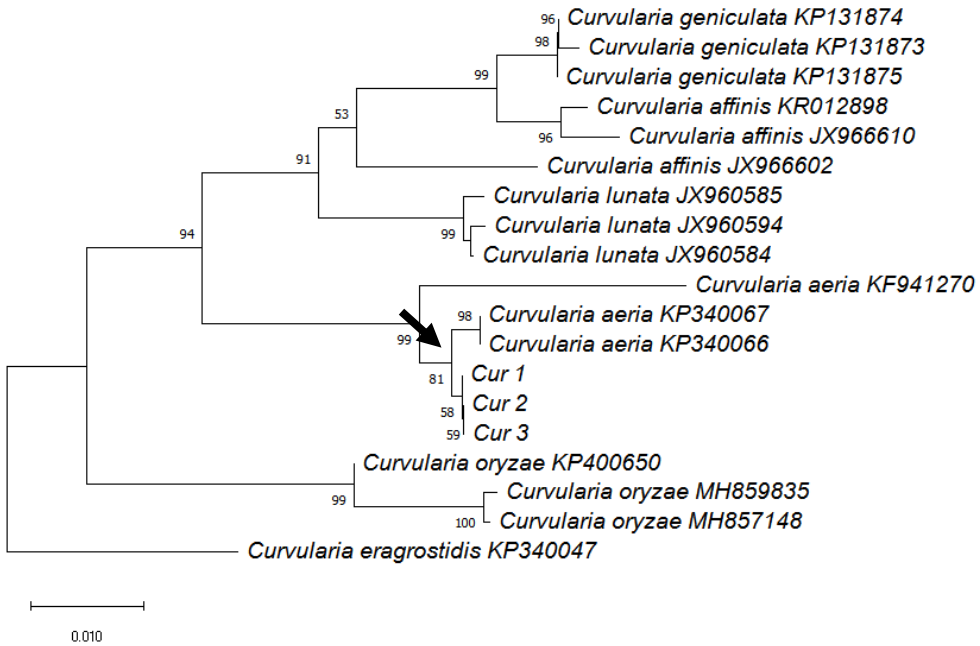

Fig. 1. The phylogenetic tree for *Curvularia* isolates using the Neighbor-Joining method with bootstrap test of 1000 replicates suggested that the fungal isolates were *Curvularia aeria*. The inference was supported by the bootstrap score of 81 (arrowhead).

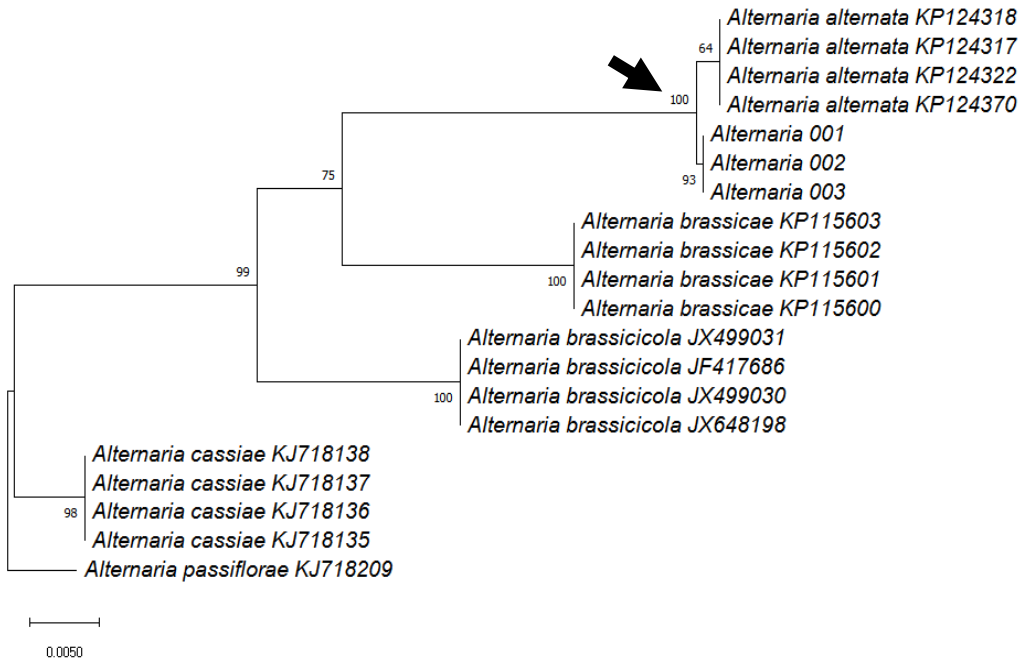

Fig. 2. The phylogenetic tree for *Alternaria* isolates using the Neighbor-Joining method with bootstrap test of 1000 replicates suggested that the fungal isolates were *Alternaria alternata*. The inference was supported by the bootstrap score of 100 (arrow head).

The unknown rust fungus was identified by the Neighbor-Joining inference with a highly supportive bootstrap score of 100 concluding that the rust fungus found on the plant leaves was *Maravalia pterocarpi*. The total length of the tree was 4.09256894. The total positions of this dataset were 216 from selected 16 nucleotide sequences (Fig. 4). *Melampsora larici-populina* was used as a root of the tree of rust fungi because it is a close relative to other members in Pucciniales including *Puccinia* and *Maravalia* [50].

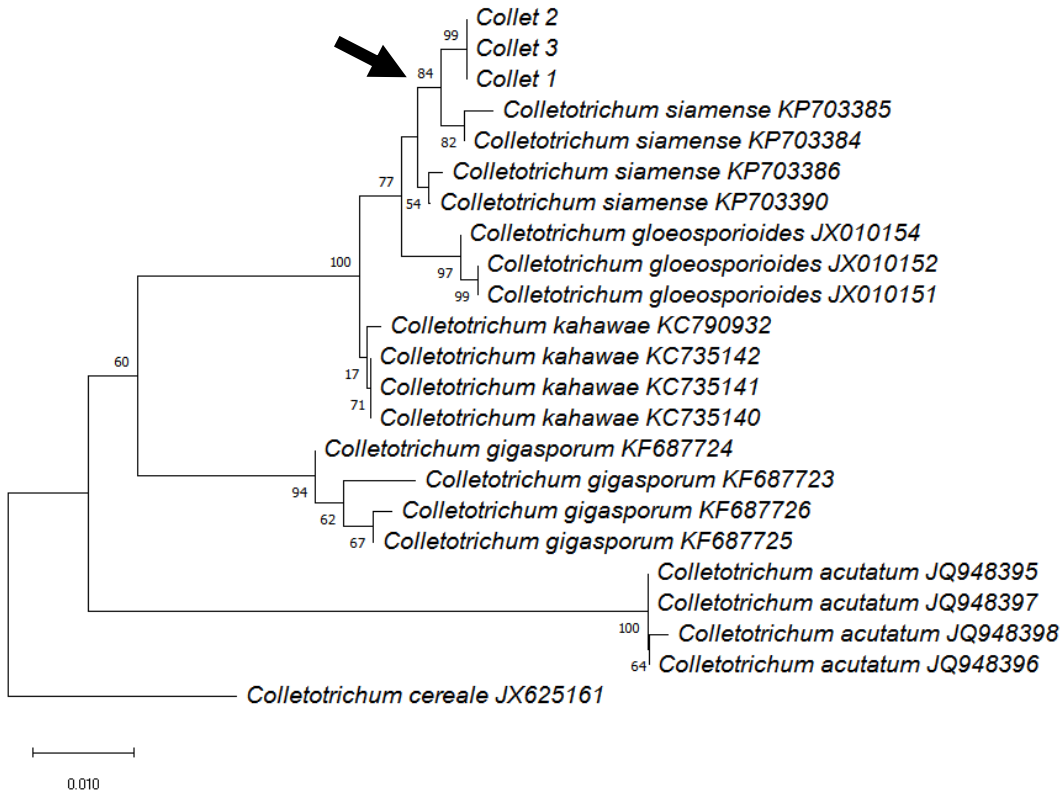

Fig. 3. The phylogenetic tree for *Colletotrichum* isolates using the Neighbor-Joining method with bootstrap test of 1000 replicates suggested that the fungal isolates were *Colletotrichum siamense*. The inference was supported by the bootstrap score of 84 (arrowhead).

There are a large number of *Alternaria*, *Curvularia,* and *Collectotrichum* species reported as plant pathogenic fungi causing leaf spot disease in various host plants as previously mentioned [3-13]. Exclusively in the *Dalbergia* genus, *A. alternata* and *C. gloeosporioides* were found to be the causal agents of leaf blight and leaf anthracnose on *D. sissoo* Roxb in India and Bangladesh [51-52]. Other *Alternaria* species, *A. porri* and *C. lunata,* have also been recorded from different parts of *D. sissoo* in Pakistan [53]. Similarly, in this study, it was molecularly proven that *A. alternata*, *C. siamense,* and *C. aeria* are the fungi significantly causing the serious leaf spot disease on leaves of the plant. Additionally, the rust fungus identified as *M. pterocarpi* is the first report using molecular methodology for identification. This fungus is also a significant risk to seedlings as a plant pathogenic agent e.g. leave spot

diseases of another *Dalbergia* species (*D. tonkinensis*) in China [54]. It can be said that these are common fungal pathogenic agents leading to the damage of this valuable plant, both in terms of plant survival rates and wood quality.

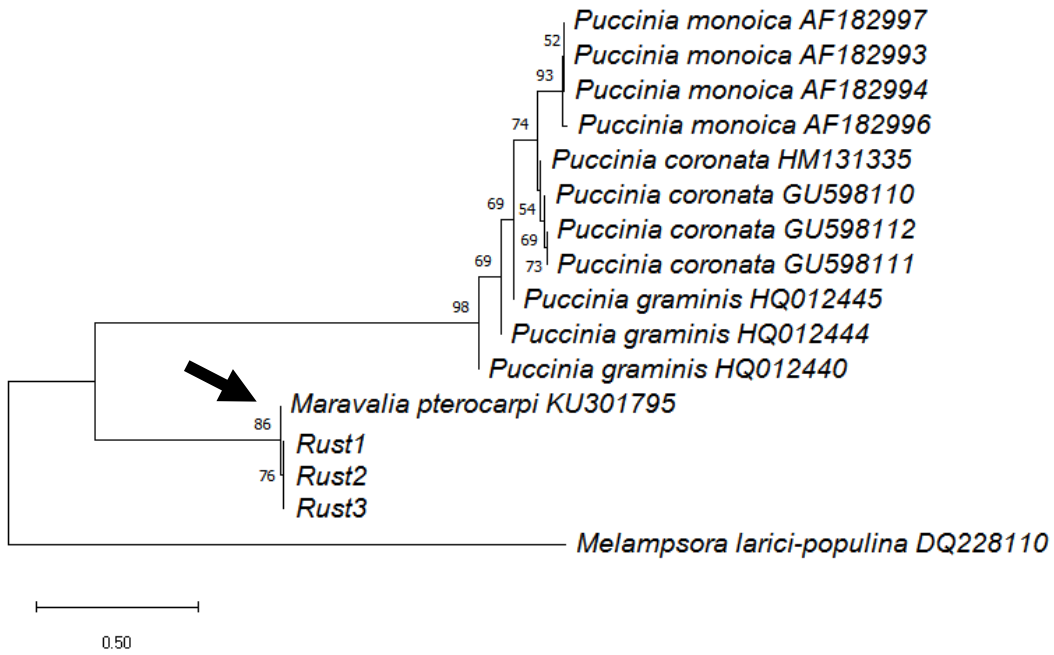

Fig. 4. The phylogenetic tree for the rust isolates using the Neighbor-Joining method with bootstrap test of 1000 replicates suggested that the fungal isolates were *Maravalia pterocarpi*. The inference was supported by the bootstrap score at 86 (arrowhead).

4. Conclusion

The brown circular leaf spot disease in Siamese rosewood seedlings is caused by a combination of fungi in one spot and the rust fungus, *M. pterocarpi*. It is very severe in seedlings and leads to low plant quality which could further bring about a reduction in the survival rate when planted in the field. Thus, in further studies, specific management of the disease caused by the fungi should be considered to overcome the disease.

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References

[1] Rungrattanagul W, Jantragul S. Siamese Rosewood Situation in Thailand [Internet]. [Cited 2020 August 5]. Available from: http://www.dnp.go.th/DNPResearch1/Files/Publication/25570508/%E0%B8%88%E0%B8%B8%E0%B8%A5%E0%B8%AA%E0%B8%B2%E0%B8%A3%E0%B8%9B%E0%B8%B5%E0%B8%97%E0%B8%B5%E0%B9%886%E0%B8%89%E0%B8%9A%E0%B8%B1%E0%B8%9A%E0%B8%97%E0%B8%B5%E0%B9%882.pdf

[2] Chairop W, Sanoamuang N. Diseases in seedling of Siamese rosewood (*Dalbergia*

cochinchinensis Pierre ex Laness) and fungi associated with brown circular leaf spot. Khon Kaen Agr J 2016;44:977-82.

[3] Santos PRR, Leão EU, Aguiar RWS, Melo MP, Santos GR. Morphological and molecular characterization of *Curvularia lunata* pathogenic to andropogon grass. Bragantia 2018;77:326-32.

[4] Liang Y, Ran SF, Bhat J, Hyde KD, Wang Y, Zhao DG. *Curvularia microspora* sp. nov. associated with leaf diseases of *Hippeastrum striatum* in China. MycoKeys 2018;29:49-61.

[5] Kusai NA, Mior Zakuan Azmi M, Zulkifly S, Yusof MT, Zainudin, NAI. Morphological and molecular characterization of *Curvularia* and related species associated with leaf spot disease of rice in Peninsular Malaysia. Rend Fis Acc Lincei 2016;27:205-14.

[6] Huang J, Zheng L, Hsiang T. First report of leaf spot caused by *Curvularia verruculosa* on *Cynodon* sp. in Hubei, China. Plant Pathol 2005;54:253.

[7] Pornsuriya C, Ito S, Sunpapao A. First report of leaf spot on lettuce caused by *Curvularia aeria*. J Gen Plant Pathol 2018;84:296-9.

[8] Kittimorakul J, Pornsuriya C, Sunpapao A, Petcharat V, Survey and incidence of leaf blight and leaf spot diseases of oil palm seedlings in southern Thailand. Plant Pathol J 2013;12:149-53.

[9] Sharma G, Dinesh Kumar V, Haque , Bhat SR, Prakash S, Chopra VL. *Brassica coenospecies*: a rich reservoir for genetic resistance to leaf spot caused by *Alternaria brassicae*. Euphytica 2002; 125:411-7.

[10] Mirkova E, Konstantinova P. First Report of *Alternaria* Leaf Spot on Gerbera (*Gerbera jamesonii* H. Bolux ex J. D. Hook) in Bulgaria. J Phytopathol 2003; 151:323-8.

[11] Al-Lami HF, You MP, Barbetti MJ. (2019), Incidence, pathogenicity and diversity of *Alternaria* spp. associated with alternaria leaf spot of canola (*Brassica napus*) in Australia. Plant Pathol 2019;68:492-503.

[12] Luo H, Jia GG, Ma DF, Sun ZX, Deng JX. First report of leaf spot disease caused by *Alternaria jacinthicola* on *Tagetes erecta* in China. Plant Dis 2018; 102:2375.

[13] Mangwende E, Kritzinger Q, Aveling TAS. Control of *Alternaria* leaf spot of coriander in organic farming. Eur J Plant Pathol 2019;154:575-84.

[14] Liu X, Li B, Cai J, Zheng X, Feng Y, Huang G. *Colletotrichum* species causing anthracnose of rubber trees in China. Sci rep 2018;8:10435.

[15] Choi O, Choi O, Kwak YS, Kim J, Kwon JH. Spot anthracnose disease caused by *Colletotrichum gloeosporioides* on Tulip tree in Korea. Mycobiology 2012;40:82-4.

[16] Guarnaccia V, Groenewald JZ, Polizzi G, Crous PW. High species diversity in *Colletotrichum* associated with citrus diseases in Europe. Persoonia 2017;39:32-50.

[17] Shivas RG, Beasley DR, McTaggart AR. Online identification guides for Australian smut fungi (Ustilaginomycotina) and rust fungi (Pucciniales). IMA Fungus 2015;5:195-202.

[18] Göre ME. Geranium rust disease caused by *Puccinia pelargonii- zonalis*: first report in Turkey. Plant Pathol 2008;57: 786.

[19] Fávero AP, Moraes SA, Garcia AAF, Valls JFM, Vello NA. Characterization of rust, early and late leaf spot resistance in wild and cultivated peanut germplasm. Sci Agr 2009;66:110-7.

[20] Narisawa K, Shimura M, Usuki F, Fukuhara S, Hashiba T. Effects of pathogendensity, soil moisture, and soil

pH on biological control of clubroot in Chinese cabbage by *Heteroconium chaetospira*. Plant Dis 2005;89:285-90.

[21] Larkin RP, Fravel DR. Effect of varying environmental conditions on biologi- cal control of Fusarium wilt of tomato by non-pathogenic *Fusarium* spp. Phytopathol 2002;92:1160-6.

[22] Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1987;19:11-5.

[23] White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: a guide to methods and applications. San Diego: Academic Press; 1990:315-22.

[24] Barnes CW, Szabo LJ. Detection and identification of four common rust pathogens of cereals and grasses using real- time polymerase chain reaction. Phytopathol 2007; 97:717-27.

[25] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Phylogenet Evol 2013;30: 2725-9.

[26] Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evol 1985;39:783-91.

[27] Woudenberg JHC, Seidl MF, Groenewald JZ, de Vries M, Stielow B, Thomma BPHJ, Crous PW. Alternaria section Alternaria: Species, formae speciales or pathotypes? Stud Mycol 2015;82:1-21.

[28] Naumann TA, Wicklow DT. Chitinase modifying proteins from phylogenetically distinct lineages of *Brassica* pathogens. Physiol Mol Plant Pathol 2013;82:1-9.

[29] Deng JX, Paul NC, Park MS, Yu SH. Molecular characterization, morphology and pathogenicity of *Alternaria panax* from araliaceous plants in Korea. Mycol Prog 2013;12:383-96.

[30] Woudenberg JH, Truter M, Groenewald JZ, Crous PW. Large-spored *Alternaria* pathogens in section Porri disentangled. Stud Mycol 2014;79:1-47.

[31] Doyle VP, Oudemans PV, Rehner SA, Litt A. Habitat and host indicate lineage identity in *Colletotrichum gloeosporioides* s.l. from wild and agricultural landscapes in North America. PLoS ONE 2013;8:E62394.

[32] Liu F, Wang M, Damm U, Crous PW, Cai L. Species boundaries in plant pathogenic fungi: a *Colletotrichum* case study. BMC Evol Biol 2016;16:81.

[33] Weir BS, Johnston PR, Damm U. The *Colletotrichum gloeosporioides* species complex. Stud Mycol 2012;73:115-80.

[34] Liu F, Cai L, Crous PW, Damm U. The *Colletotrichum gigasporum* species complex. Persoonia 2014;33:83-97.

[35] Damm U, Cannon PF, Woudenberg JH, Crous PW. The *Colletotrichum acutatum* species complex. Stud Mycol 202;73: 37-113.

[36] Tao G, Liu Z, Liu F, Gao YH, Cai L. Endophytic *Colletotrichum* species from *Bletilla ochracea* (Orchidaceae), with descriptions of seven new species. Fungal Divers 2013;61:139-64.

[37] Rivera- Vargas LI, Negron- Ortiz V. Root and soil-borne Oomycetes (Heterokontophyta) and fungi associated with the endangered conifer, *Torreya taxifolia* Arn. (Taxaceae) in Georgia and Florida, USA. Life Excit Biol 2013;1:202-24.

[38] Nor Azizah K, Madihan NZA, Shahrizim Z, Mohd TY, Nur Ain Izzati MZ. Morphological and molecular characterization of *Curvularia* and related species associated with leaf spot disease of

rice in Peninsular Malaysia Rend. Lincei Sci Fis Nat 2016;27:205-14.

[39] Yew SM, Chan CL, Lee KW, Na SL, Tan R, Hoh CC, Yee WY, Ngeow YF, Ng KP. A five-year survey of dematiaceous fungi in a tropical hospital reveals potential opportunistic species. PLoS ONE 2014;9:E104352.

[40] Parsa S, Garcia-Lemos AM, Castillo K, Ortiz V, Lopez-Lavalle LA, Braun J, Vega, FE. Fungal endophytes in germinated seeds of the common bean, *Phaseolus vulgaris*. Fungal Biol 2016;120:783-90.

[41] Irinyi L, Serena C, Garcia-Hermoso D, Arabatzis M, Desnos-Ollivier M, Vu D, Cardinali G, Arthur I, Normand AC, Giraldo A, da Cunha KC, Sandoval-Denis M, Hendrickx M, Nishikaku AS, de Azevedo Melo AS, Merseguel KB, Khan A, Parente Rocha JA, Sampaio P, da Silva Briones MR, Ferreira RC, de Medeiros Muniz M, Castanon-Olivares LR, Estrada-Barcenas D, Cassagne C, Mary C, Duan SY, Kong F, Sun AY, Zeng X, Zhao Z, Gantois N, Botterel F, Robbertse B, Schoch C, Gams W, Ellis D, Halliday C, Chen S, Sorrell TC, Piarroux R, Colombo AL, Pais C, de Hoog S, Zancope-Oliveira RM, Taylor ML, Toriello C, de Almeida Soares CM, Delhaes L, Stubbe D, Dromer F, Ranque S, Guarro J, Cano-Lira JF, Robert V, Velegraki A, Meyer W. International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database- the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol 2015;53:313-7.

[42] Funnell-Harris DL, Prom LK, Pedersen JF. Isolation and characterization of the grain mold fungi *Cochliobolus* and *Alternaria* spp. from sorghum using semiselective media and DNA sequence analyses. Can J Microbiol 2013;59:87-96.

[43] Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 2019;92:135-54.

[44] Beirn LA, Moy M, Meyer WA, Clarke BB, Crouch JA. Molecular analysis of turfgrass rusts reveals the widespread distribution of *Puccinia coronata* as a pathogen of Kentucky bluegrass in the United States. Plant Dis 2011;95:1547-57.

[45] Liu M, Hambleton,S. Laying the foundation for a taxonomic review of *Puccinia coronate* s.l. in a phylogenetic context. Mycol Prog 2013;12:63-89.

[46] Roy BA. Patterns of association between crucifers and their flower-mimic pathogens: host jumps are more common than coevolution or cospeciation. Evolution 2001;55:41-53.

[47] Liu M, Hambleton S. *Puccinia chunjii*, a close relative of the cereal stem rusts revealed by molecular phylogeny and morphological study. Mycologia 2012;104:1056-67.

[48] Yu Z, Cao Z, Liu X. ISSR marker and ITS sequence study of *Melampsora larici-populina* in China. Agr Sci China 2005;5:847-54.

[49] Wang J, Zhou G, Su S, He Y, Dong W, Zhang Q, Liu J. Identification of pathogens of the *Dalbergia tonkinensis* rust disease. Scientia Silvae Sinicae 2016;52:165-9.

[50] Duplessis S, Cuomo CA, Lin YC, Aerts A, Tisserant E, Veneault-Fourrey C, Joly DL, Hacquard S, Amselem J, Cantarel BL, Chiu R, Coutinho PM, Feau N, Field M, Frey P, Gelhaye E, Goldberg J, Grabherr MG, Kodira CD, Kohler A, Kües U, Lindquist EA, Lucas SM, Mago R, Mauceli E, Morin E, Murat C, Pangilinan JL, Park R, Pearson M, Quesneville H, Rouhier N, Sakthikumar S, Salamov AA,

Schmutz J, Selles B, Shapiro H, Tanguay P, Tuskan GA, Henrissat B, Van de Peer Y, Rouzé P, Ellis JG, Dodds PN, Schein JE, Zhong S, Hamelin RC, Grigoriev IV, Szabo LJ, Martin F. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proc Natl Acad Sci USA 2011;108:9166-71.

[51] Singh KP, Kumari P, Bhadauria S, Management of *Alternaria* leaf blight of *Dalbergia sissoo*. Indian J For 2009; 32:429-30

[52] Shamsi S, Sultana R, Azad R. Occurrence of leaf and pod diseases of sissoo (*Dalbergia sissoo Roxb)* in Bangladesh. Bangladesh J. Plant Pathol 2012;28:45-52.

[53] Rajput NA, Pathan MA, Rajput AQ, Jiskani MM, Lodhi AM, Rajput SA, Khaskhali MI. Isolation of fungi associated with shisham trees and their effect on seed germination and seedling mortality. Pak J Bot 2010;42:369-74.

[54] Wag J, Zhou G, Su S, He Y, Dong W, Zhang Q Liu J. Identification of pathogens of the *Dalbergia tonkinensis* rust disease. Sci Silvae Sin 2016;52:165-9.