Morphological Studies of Endophytic and Plant Pathogenic *Phomopsis liquidambaris* and *Diaporthe phaseolorum* (*P. phaseoli* anamorph) from Healthy Plants and Diseased Fruits

A. Danggomen, N. Visarathanonth*, L. Manoch and O. Piasai

Department of Plant Pathology, Faculty of Agriculture, Kasetsart University Bangkok 10900, Thailand

**Corresponding author, Email: agrnpv@ku.ac.th*

Abstract

The purpose of this study was to isolate and identify *Phomopsis* spp. from healthy and diseased plants from various sources. Substrates included 1) healthy leaves of jackfruit (*Artocarpus heterophyllus*), *Colubrina asiatica*, and *Antirhea lucida* and 2) rotted fruits of tamarind (*Tamarindus indica*), guava (*Psidium guajava*) and longkong (*Lansium domesticum*). The surface sterilization method with 70 % ethanol was used to isolate a *Phomopsis* endophyte from the healthy leaves. Direct isolation and the tissue transplanting methods were used to isolate *Phomopsis* from the rotted fruits. Morphological characteristics of the fungal species were examined as colony growth pattern and color, and the fruiting bodies, conidiophores, and conidia were examined under stereo and light microscopes. Camera lucida drawings were made. After identifying samples based on morphology, molecular analysis using 28sRNA sequence was used to confirm the results. One isolate of *Phomopsis liquidambaris* and two isolates of *Diaporthe phaseolorum* (*Phomopsis phaseoli* anamorph) were found as endophytes from healthy plant tissues, whereas two isolates of *P. liquidambaris* and one isolate of *D. phaseolorum* were found from the diseased fruits. *P. liquidambaris* is a new record for Thailand as an endophyte and a parasitic fungus.

Keywords: fruit rot, coastal forest tree, endophytic fungi, *Phomopsis liquidambri*s, *Diaporthe phaseolorum*

Introduction

The genus *Phomopsis* (Sacc.) Bubak (*Diaporthe* Nitschke teleomorph) contains more than 900 species names from a wide range of hosts (Uecker 1988; Rehner and Uecker 1994; Rossman et al., 2007; Rossman and Palm-Hernández 2008). Species of *Phomopsis* have been reported as plant pathogens, endophytes, and saprophytes and can even cause health problems in humans and other mammals (VanWarmelo and Marasas, 1972; Uecker 1988; Rehner and Uecker 1994; Sutton et al., 1998; Garcia-Reyne et al., 2011). Several species were reported as pathogens on various crops, as endophytes from healthy plant tissues of the same or different hosts, and as saprophytes on dead plant materials (Udayanga et al., 2011).

In identification of *Phomopsis* spp., classic mycology uses morphological and cultural characteristics, and biometrical values of anamorph and teleomorph structures. Delimitation of the species is uncertain because some *Phomopsis* spp. have pycnidia with two types of conidia (alpha and beta), whereas other species produce only one type of conidia. Moreover, the teleomorph stage of *Diaporthe* Nitschke was not determined in all described *Phomopsis* species. Significantly, one species can infect several botanically different hosts, and several *Phomopsis* spp. can be isolated from one plant species (Van Niekerk et al., 2005).

Phomopsis liquidambaris was first reported as an endophyte from healthy living branches of *Liquidambar formosana* Hance (Hamamelidaceae) in Fujian Province, China (Chang-Qing et al., 2005). *Phomopsis* spp. are common endophytic fungi that exist in a wide variety of plants (Murali et al., 2006; Li et al., 2010).

In Thailand, Sontirat et al. (1994) recorded 8 species of *Phomopsis* from various diseased plants. Visarathanonth (1999a; 1999b) reported *Phomopsis* spp. from fruit rot of tropical and temperate fruits. Kokaew et al. (2011) recorded *Phomopsis arnoldiae* as an endophyte isolated from leaves of the forest tree *Ascistrocladus tectorius* in Khao Yai National Park, Nakhon Ratchasima province and found 72 isolates of endophytic *Phomopsis* spp. from healthy wild plants and forest trees from various habitats.

The purposes of this study were to 1) isolate *Phomopsis* species from leaves of healthy plant, diseased fruits and wild plants and, 2) identify *Phomopsis* spp. using morphological characteristics.

Materials and Methods

Isolation for Endophytic Fungi

The healthy leaves of jackfruit (*Artocarpus heterophyllus*) from Kasetsart University, Bangkok as well as *Colubrina asiatica* and *Antirhea lucida* from Mu Koh Surin National Park, Phang Nga Province were collected (Table 1). The surface sterilization method was used to isolate endophytic fungi (Li et al., 2005). From each plant an asymptomatic leaf was chosen randomly. Leaf portions were thoroughly washed in running tap water, then surface sterilized by submerging in 70% ethanol for 2 min. After drying, each leaf was divided into four segments and placed on water agar (WA) supplemented with 50 mg L^{-1} streptomycin to suppress bacterial growth. All plates were incubated at 28°C for 3-4 weeks. Emerging fungi were transferred to fresh potato dextrose agar (PDA) plates, incubated for 1 week, and periodically checked for purity.

Isolation for Plant Pathogenic Fungi

Fruits of tamarind (*Tamarindus indica*), guava (*Psidium guajava*), and longkong (*Lansium domestricum*) showing symptoms of fruit rot were collected from the local market near Kasetsart University, Bankok, Thailand (Table 1). Direct isolation and the tissue transplanting method were used to isolate *Phomopsis* spp. pathogens (Agrios, 2005). For the tissue transplanting method, small pieces of specimen tissue (3-5 mm) were excised at the margin of the infected leaves, surface disinfected with 1% Sodium hypochlorite for 1 min., and placed onto potato dextrose agar (PDA). Samples were incubated at 28°C for 2-3 days and a fine needle was used to transfer the young mycelium to slant PDA. Direct isolation was also employed from diseased fruits incubated in a moist chamber in a Petri-dish or plastic box. A fine needle was used to transfer the growing mycelium from diseased fruits to slant PDA.

Table 1 Collection data of healthy and diseased plant samples for isolation of *Phomopsis* sp. and *Diaporthe* sp.

Plant part	Plant type	Family	Scienctific name	Common name	Location	Date
Leaves	Healthy plant	Moraceae	*Artocarpus heterophyllus*	Jackfruit	KU, Bangkok	13/6/ 2011
Fruits	Diseased plant	Myrtaceae	*Psidium guajava*	Guava	KU, Bangkok	27/10/2011
		Leguminosae	*Tamarindus indica*	Tamarind	KU, Bangkok	22/1/ 2012
		Meliaceae	*Lansium domesticum*	Longkong	KU, Bangkok	15/1/2012
Leaves	Healthy plant	Rhamnaceae	*Colubrina asiatica*	-	Mu Ko Surin National Park, Phang Nga	9/4/2012
		Rubiaceae	*Antirhea lucida*	-	Mu Ko Surin National Park, Phang Nga	9/4/2012

Identification of the Fungal Strains

Morphological Studies: Macroscopic features were studied including colony growth pattern, color, and texture, and fungal growth rate was measured on PDA. Colony characteristics and pigment production were observed after 7 days of growth at 28°C. Sporulation was found after 30 days incubation on PDA at 28ºC. Microscopic characters were observed on a slide preparation using sterile distilled water and lactophenol as mounting media and examined under stereo and light microscopes (Olympus BH-2 with Normaski Interference Contrast). Camera lucida drawings were made.

To induce sporulation, small pieces of grape branch were autoclaved, placed on PDA with the fungal inoculum, and incubated for 14 days at 28ºC.

Molecular analyses of *Phomopsis* strains were conducted by Dr. Takashi Mikawa, Mitsubishi, Japan using -28sRNA sequence to confirm their identity.

Results and Discussion

One isolate of *Phomopsis liquidambaris* C.Q. Chang, Z.D. Jiang & P.K. Chi and two isolates of *Diaporthe phaseolorum* (Cooke & Eills) Sacc. (*Phomopsis phaseoli* anamorph) were found from healthy plant tissues (jackfruit, *Antirhea lucida* and *Colubrina asiatica* respectively), whereas two isolates of *P. liquidambaris* and one isolate of *D. phaseolorum* were found from diseased fruits (tamarind, guava and longkong respectively) (Table 2).

Phomopsis liquidambaris C.Q. Chang, Z.D. Jiang & P.K. Chi (Figure 1-3)

Reference: Chang-Qing et al. (2005)

Specimens examined: leaf of jackfruit (KUFC 7070); fruit rots of tamarind (KUFC7066) and fruit rots of guava (KUFC7073). Kasetsart University, Bangkok.

Colonies of *P. liquidambaris* KUFC 7070 on PDA reaching 9 cm diam. in7 days at 28°C. Mycelium gray, immersed, branched, septate, hyaline. Conidiomata were eustromatic, immersed, brown to dark brown. Conidiophores were hyaline, simple branched and septate at the base. Conidia were extruded from ostioles in white mass. Conidia: α-conidia hyaline, unicellular, ellipsoid to fusiform 5.5-8.3×1.2-2.0 µm and β-conidia hyaline, unicellular, filiform and curved at one end 16.8-24.8×0.6-0.9 µm.

Macroscopic and microscopic features on PDA of endophytic *Phomopsis liquidambaris* (KUFC 7070) from jackfruit leaf were very similar to *P. liquidambaris* (KUFC 7066) and (KUFC7073) causing fruit rots of tamarind and guava fruits, but the sizes of α-conidia and β-conidia were slightly different (Table 2). However the size ranges of α and β-conidia of the three isolates (α-conidia 5.5-8.3×1.2-2.0 µm and β-conidia 16.8-24.8×0.6-0.9 µm) were similar to the sizes recorded for *P. liquidambaris* from China (α-conidia 6.5-8.1×1.7-2.2 and β-conidia 10.5-24.5×0.6-1.0 µm). In addition, the colony of *P. liquidambaris* on guava showed zonation. Chang-Qing et al. (2005) were first to report *Phomopsis liquidambaris* as an endophytic fungus from branches of the woody plants *Liquidambaris formosanae* Hance

Table 2 A comparision of α- and β-conidia sizes of *Phomopsis liquidambaris* and *Diaporthe phaseolorum* (*Phomopsis phaseoli* anamorph) isolated from healthy plants and diseased fruits cultivated on PDA at 28 °C for 30 days.

Plant type	KUFC[1/]	Fungal species	Host	Conidial size (µm)	
				α-conidia	β-conidia
Healthy plant (leaves)	7070	*P. liquidambaris*	Jackfruit	5.5-8.3×1.2-2.0	16.8-24.8×0.6-0.9
	7518	*D. phaseolorum*	*Antirhea lucida*	6.0-10.0×1.8-2.0	12.0-19.0×0.9-1.0
	7519	*D. phaseolorum*	*Colubrina asiatica*	7.0-10.0×1.5-2.0	14.0-28.0×0.8-1.0
Disease plant(fruits)	7066	*P. liquidambaris*	Tamarind	6.0-7.7×1.4-1.8	14.4-22.0×1.0-1.3
	7073	*P. liquidambaris*	Guava	5.9-7.1×1.6-2	16.7-22.7×1.0-1.4
	7069	*D. phaseolorum*	Long kong	4.9-6.8×0.9-1.6	14.8-23.9×0.7-0.9

[1/] KUFC = Kasetsart University Fungal Collection

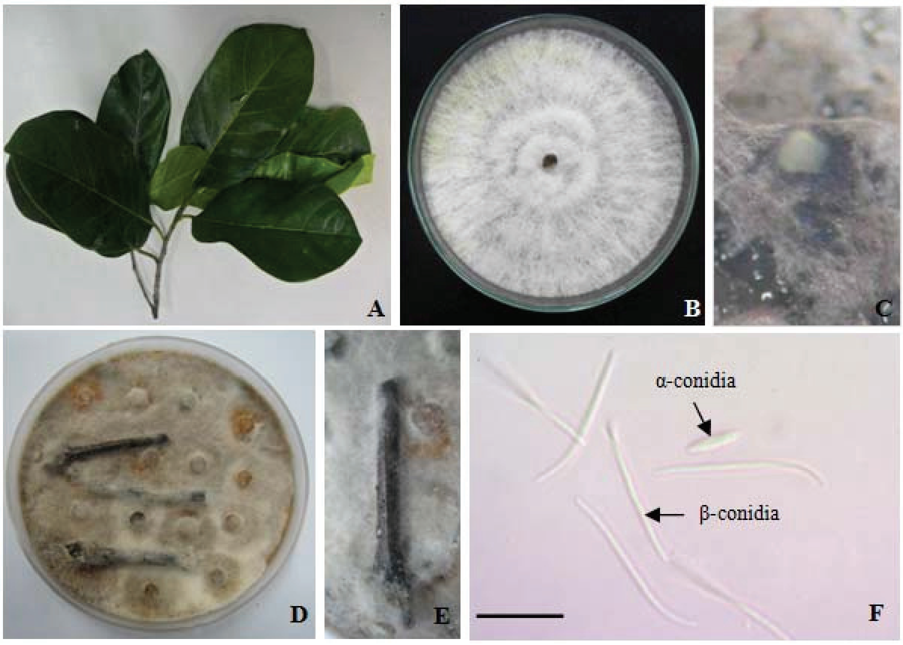

Figure 1 Morphology of *Phomopsis liquidambaris* (KUFC 7070): A) Healthy leaf of jackfruit (*Artocarpus heterophllus*); B) Colony on PDA 7 days at 28°C; C) Pycnidia and exudates on PDA; D, E) Pycnidia on branches of grape and F) α-conidia and β-conidia (Bar = 10 µm).

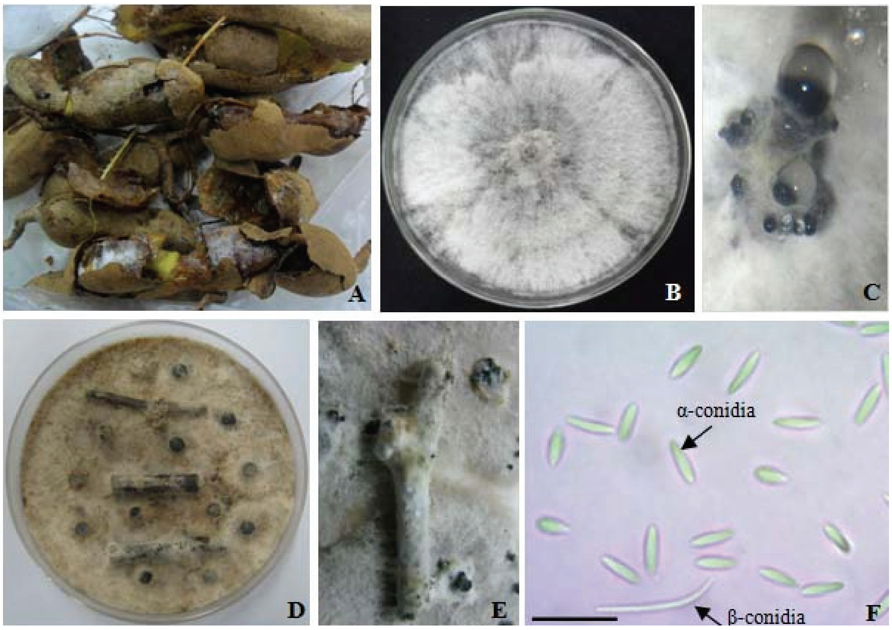

Figure 2 Morphology of *Phomopsis liquidambaris* (KUFC 7066): A) Tamarind fruit rot; B) Colony on PDA 7 days at 28°C; C) Pycnidia and exudates on PDA; D, E) Pycnidia on branches of grape and F) α-conidia and β-conidia (Bar = 10 µm).

(Hamamelidaceae) and the type specimens were deposited in the Mycological Herbarium of South China Agricultural University, Guangzhou, China. In the present study, only one isolate of *P. liquidambaris* (KUFC7070) was found as an endophytic fungus from a healthy leaf of jackfruit, whereas two isolates of *P. liquidambaris* were isolated from fruit rots of tamarind (KUFC 7066) and guava (KUFC 7073).

As a pioneer decomposer, *Phomopsis* spp. have been shown to accelerate leaf litter decomposition and soil nitrogen transformation (Dai et al., 2010;

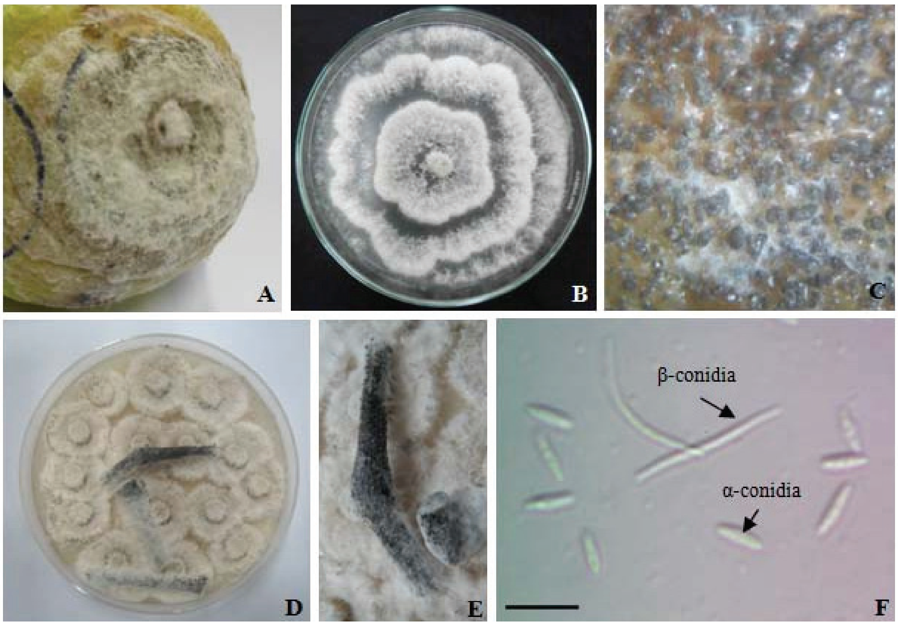

Figure 3 Morphology of *Phomopsis liquidambaris* (KUFC 7073): A) Guava fruit rot; B) Colony on PDA 7 days at 28°C; C) Pycnidia and exudates on PDA; D, E) Pycnidia on branches of grape and F) α-conidia and β-conidia (Bar =10 μm).

Chen et al., 2013). The endophyte *Phomopsis liquidambaris* (strain B3) is capable of growing with the phenolic compound 4-HBA (4-hydroxybenzoic acid) as the sole carbon and energy source *in vitro* (Chen et al., 2011).

In Thailand, Sontirat et al. (1994) recorded 8 species of *Phomopsis* including *P. ambiqua*, *P. asparagi, P. citri*, *P. heveae*, *P. hibisci*, *P. oryzae-sativae*, *P. sojae*, *P. vexans* from various diseased plants such as pear, asparagus, lime, para rubber and rice. Visarathanonth (1999a, 1999b) reported *Phomopsis* spp. causing fruit rot of tropical fruits, such as rambutan, durian, guava, tamarind, mango and mangosteen as well as temperate fruits, such as peach, persimmon, strawberry, pear and apple. Kokaew et al. (2011) reported *Phomopsis arnoldiae* as an endophyte isolated from leaves of *Ancistrocladus tectorius* (Ancistrocladaceae) at Khao Yai National Park, Nakhon Ratchasima province and also recorded 72 isolates of *Phomopsis* spp. from healthy wild plants in various habitats. Therefore, as an endophyte and fungal pathogen on tamarind and guava, *P. liquidambaris* is a new record for Thailand.

Diaporthe phaseolorum (Cooke & Eills) Sacc. (Figures 4-6)

Reference: Saccado (1882)

Anamorph: *Phomopsis phaseoli* Petch 1922

Specimens examined: Leaves of *Antirhea lucida* (KUFC 7518), and *Colubrina asiatica*, (KUFC 7519); Mu Koh Surin National Park, Phang Nga. and longkong fruit rot (KUFC 7069);

Kasetsart University, Bangkok.

Colonies of *D. phaseolorum* KUFC 7069 on PDA reaching 9 cm diam. on 7 days at 28°C. Mycelium was white, immersed, branched, septate, hyaline. Conidiomata were immersed, brown to dark brown. Conidiophores were hyaline, simple branched and septate at the base. Conidia were extruded from ostioles in white mass. Conidia: α-conidia hyaline, unicellular, ellipsoid to fusiform 4.9-6.8×0.9-1.6 μm and β-conidia hyaline, unicellular, filiform and curved at one 14.8-23.9×0.7-0.9 μm.

Endophytic *Diaporthe phaseolorum* isolates KUFC 7518 and KUFC 7519 were very similar in macroscopic and microscopic characteristics to *D. phaseolorum* isolate KUFC 7069, but α-conidia of *D. phaseolorum* (KUFC 7069) from diseased fruits was slightly smaller than the endophytic isolates (Table 2). Cheng et al. (2008) showed the first reported the endophyte *Diaporthe phaseolorum* var. *sojae* from *Kandelia candel* an estuarine mangrove on the South China Sea Coast. Pioli et al. (2003) studied morphologic characters of twenty-three

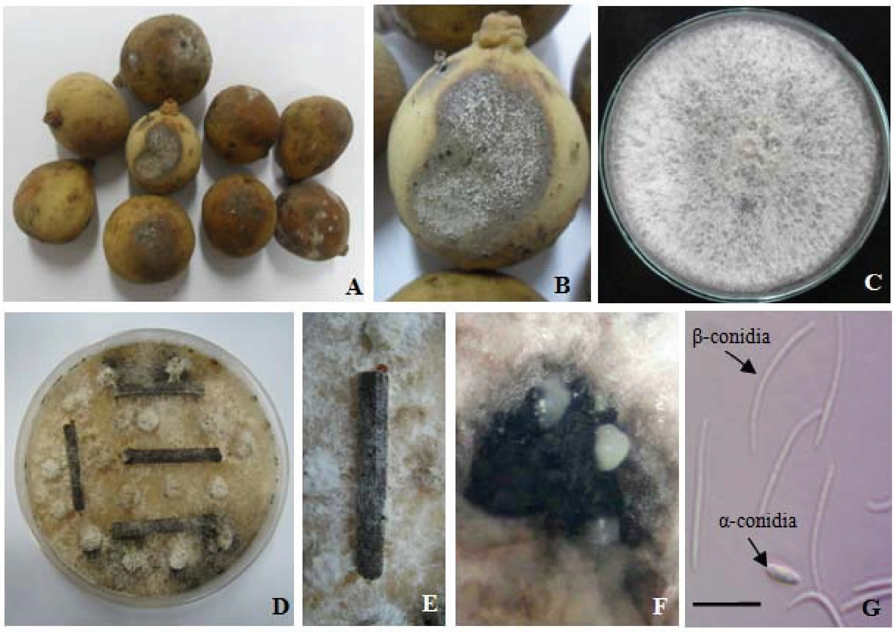

Figure 4 Morphology of *Diaporthe phaseolorum* (*P. phaseoli* anamorph)(KUFC 7069): A, B) Longkong fruit rot (*Lansium domesticum*); C) Colony on PDA 7 days at 28 °C; D, E) Pycnidia on branches of grape; F) Pycnidia and exudates on PDA and G) α-conidia and β-conidia (Bar = 10 µm).

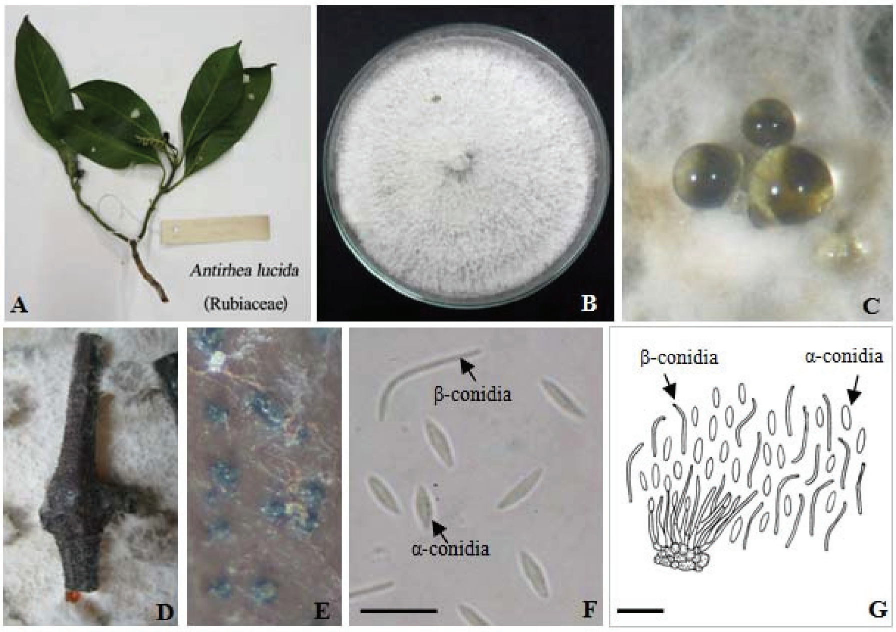

Figure 5 Morphology of *Diaporthe phaseolorum* (*P. phaseoli* anamorph)(KUFC 7518): A) *Antirhea lucida*; B) Colony on PDA incubated for 7 days; C) Pycnidia and exudates on PDA; D, E) Pycnidia on branches of grape; F) α-conidia and β-conidia G) Camera lucida drawings of conidiophores and conidia (Bars: F,G = 10 µm)

isolates of *D. phaseolorum* related to type of colonies, stroma, pycnidia and conidia, presence of perithecia and asci length by principal component analysis in the core soybean-producing area of Argentina. Santos et al. (2011) described the morphological characteristics of *Diaporthe* species including, *D. caulivora*, *D. novem*, *D. phaseolorum* and *D. longicolla* isolated from stems and seeds of soybean as well as from stems of sunflower (*Helianthus annuus*), *Arctium lappa*, *Asclepias syriaca* and *Dipsacus lacinatus* in Croatia. Iriart et al. (2011) recorded that *D. phaseolorum* (syn. *P.*

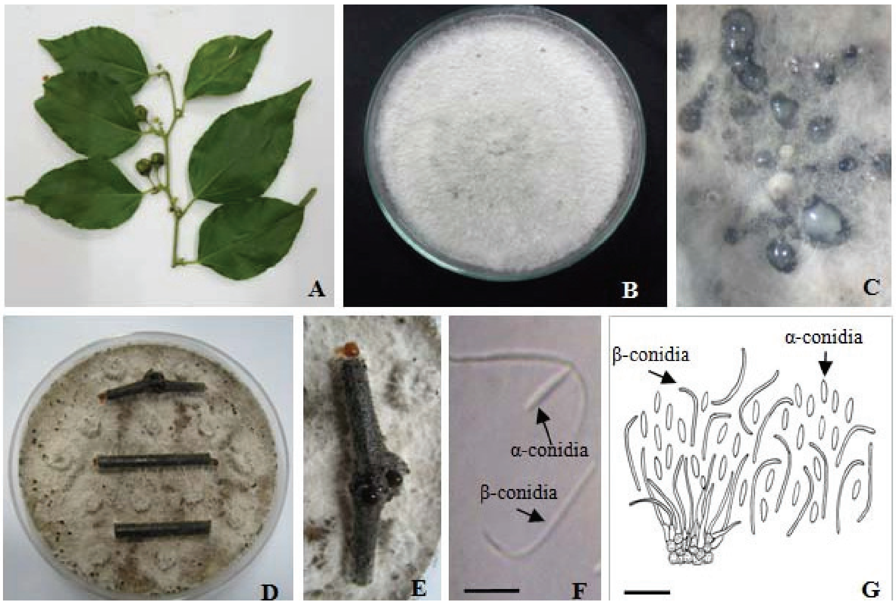

Figure 6 Morphology of *Diaporthe phaseolorum* (*P. phaseoli* ; anamorph) (KUFC 7519): A) *Colubrina asiatica*; B) Colonies on PDA 7 days at 28 °C; C) Pycnidia and exudates on PDA; D, E) Pycnidia on branches of grape; F) α-conidia and β-conidia and G) Camera lucida drawings of conidiophores and conidia (Bars: F, G = 10 μm)

phaseoli) is a frequent fungal parasite of plants, present on all continents around the world. Costamilan et al. (2008) reported *D. phaseolorum* var. *caulivora* causes stem canker of soybean in Brazil. Moreover, the *Phomopsis* anamorph has been associated with post-harvest fruit rot of kiwifruit in Italy (Luongo et al., 2011).

In Thailand, Sontirat et al. (1994) reported that *Diaporthe phaseolorum* was found in rotten pepper fruit. In this study, *D. phaseolorum* was found as an endophyte in the leaves of *Antirhea lucida* and *Colubrina asiatica* and as a plant pathogen from longkong fruit.

Conclusions

The endophytic fungus *Phomopsis liquidambaris* was isolated from jackfruit leaves from the Kasetsart University campus, whereas *Diaporthe phaseolorum* were found from healthy leaves of coastal forest tree, *Antirhea lucida* and *Colubrina asiatica* at Mu Koh Surin National Park, Phang Nga, Thailand.

Two isolates of *Phomopsis liquidambaris* were found from tamarind and guava fruit rots, whereas *Diaporthe phaseolorum* were found from diseased longkong fruit from a market near Kasetsart University, Bangkok, Thailand.

Phomopsis liquidambaris is a new record as an endophytic on jackfruit and plant parasitic fungus on tamarind and guava for Thailand whereas *Diaporthe phaseolorum* is a new record as an endophyte in *Antirhea lucida* and *Colubrina asiatica* and fungal pathogen in *Lansium domestricum* (Meliaceae) in Thailand.

Acknowledgments

We wish to acknowledge the Graduate Scholarship as of Fiscal Year 2010 for the financial support of this research for Miss Apiraporn Danggomen from the Graduate School, Kasetsart University. Grateful thanks is extended to the Plant Genetic Conservation Project under the Royal Initiative of HRH Princess Maha Chakri Sirindhornand and the Naval Special Warfare Command, the Royal Thai Fleet, the Royal Thai Navy for their assistance in collecting the plant samples at Mu Ko Surin National Park, Phang Nga province. We thank Dr. Takashi Mikawa, for molecular analysis of *Phomopsis* strains.

References

Agrios, G.N. 2005. Plant Pathology. 5th ed. Elsevier Academic Press, Burlington, Mass.

Chang-Qing, C., C. Ying-Hui, X. Mei-Mei, J. Zi-De and C. Pei-Kun. 2005. New species of *Phomopsis* on wood plants in Fujian province. Mycosystema 24: 6-11.

Cheng, Z., W. Tang, S. Xu, S. Sun, B.Y. Huang, X. Yan, Q. Chen and Y. Lin. 2008. First report of an endophyte (*Diaporthe phaseolorum* var. *sojae*) from *Kandelia candel*. Journal of Forestry Research 19: 277-282.

Chen, Y., Y. Peng, C.C. Dai and Q. Ju. 2011. Biodegradation of 4-hydroxybenzoic acid by *Phomopsis liquidambaris*. Applied Soil Ecology 51: 102-110.

Chen, Y., X.G. Xie, C.G. Ren and C.C. Dai. 2013. Degradation of N-heterocyclic indole by a novel endophytic fungus *Phomopsis liquidambaris*. Bioresource Technology 129:568-574.

Costamilan, L.M., J.T. Yorinori, A.M.R. Almeida, C.D.S. Seixas, E. Binneck, M.R. Araujo and J.A. Carbonari. 2008. First report of *Diaporthe phaseolorum* var. *caulivora* infecting soybean plants in Brazil. Tropical Plant Pathology 33: 381-385.

Dai, C.C., Y. Chen, L.S. Tian and Y. Shi. 2010. Correlation between invasion by endophytic fungus *Phomopsis* sp. and enzyme production. African Journal of Agricultural Research 5: 1324-1330.

Garcia-Reyne, A., F. López-Medrano, J.M. Morales, C. García Esteban, I. Martín, I. Eraña, Y. Meije, A. Lalueza, A. Alastruey-Izquierdo, J.L. Rodríguez-Tudela and J.M. Aguado. 2011. Cutaneous infection by *Phomopsis longicolla* in a renal transplant recipient from Guinea: first report of human infection by this fungus. Transplant Infectious Disease 13: 204-207.

Iriart, X., R. Binois, A. Fior, D. Blanchet, A. Berry, S. Cassaing, E. Amazan, E. Papot, B. Carme, C. Aznar and P. Couppie. 2011. Eumycetoma caused by *Diaporthe phaseolorum* (*Phomopsis phaseoli*): a case report and a mini-review of *Diaporthe/Phomopsis* spp. invasive infections in humans. Clinical Microbiology and Infection 17: 1492-1494.

Kokaew, J., L. Manoch, J. Worapong, C. Chamswarng, N. Singburaudom, N. Visarathanonth, O. Piasai and G. Strobel. 2011. *Coniochaeta ligniaria* an endophytic fungus from *Baeckea frutescens* and its antagonistic effects against plant pathogenic fungi. Thai Journal of Agricultural Science 44: 123-131.

Li, H., C. Qing, Y. Zhang and Z. Zhao. 2005. Screening for endophytic with antitumour and antifungal activities from Chinese medicinal plants. World Journal of Microbiology and Biotechnology 21: 1515-1519.

Li, S., G.L. Hartman and D.L. Boykin. 2010. Aggressiveness of *Phomopsis longicolla* and other *Phomopsis* spp. on soybean. Plant Disease 94: 1035-1040.

Luongo, L., A. Santori, L. Riccioni and A. Belisario. 2011. *Phomopsis* sp. Associated with post-harvest fruit rots of kiwifruit in Italy. Journal of Plant Pathology 93: 205-209.

Murali, T.S., T.S. Suryanarayanan and R. Geeda. 2006. Endophytic *Phomopsis* species: host range and implications for diversity estimate. Canadian Journal of Microbiology 52: 673-680.

Pioli, R.N., E.N. Morandi, M.C. Martinez, F. Lucca, A. Tozzini, V. Bisaro and E. Hopp. 2003. Morphologic, molecular and pathogenic characterization of *Diaporthe phaseolorum* variability in the core soybean-producing area of Argentina. Phytopathology 93: 136-146.

Rehner, S.A. and F.A. Uecker. 1994. Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Canadian Journal of Botany 72: 1666-1674.

Rossman, A.Y., D.F. Farr and L.A. Castlebury. 2007. A review of the phylogeny and biology of the *Diaporthales*. Mycoscience 48: 135-144.

Rossman, A.Y. and M.E. Palm-Hernández. 2008. Systematics of plant pathogenic fungi: why it matters. Plant Disease 92: 1376-1386.

Santos, J.M., K. Vrandecic, J. Cosic, T. Duvnjak and A.J.L. Phillips. 2011. Resolving the *Diaporthe* species occurring on soybean. Persoonia 27: 9-19.

Sontirat, P., P. Pitakpai, T. Khamhangridthirong, W. Choobamroong and U. Kueprakone. 1994. Host Index of Plant Diseases in Thailand. Mycology Section, Plant Pathology & Microbiology Division, Department of Agriculture, Bankkok, Thailand.

Sutton D.A, A.W Fothergill, M.G. Rinaldi. 1998. Guide to Clinically Significant Fungi. Commonwealth Mycological Institute, Kew, Surrey, England.

Udayanga, D., X.Z. Liu, L. Cai and K.D. Hyde, 2011. The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Diversity 50: 189-225.

Uecker, F.A. 1988. A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. Mycologia Memoir 13: 1-231.

Van Warmelo, K.T. and W.F.O. Marasas. 1972. *Phomopsis leptostromiformis*: the causal fungus of lupinosis, a mycotoxicosis, in sheep. Mycologia 64: 316-324.

Van Niekerk, J.M., J.Z. Groenewald, D.F. Farr, P.H. Fourie, F. Hallen and P.W. Crous. 2005. Reassessment of *Phomopsis* species on grapevines. Australian Plant Pathology 34: 1-13.

Visarathanonth, N. 1999a. Diseases of Tropical Fruits and Control. J. Film Process Co. Ltd., Bangkok, Thailand.

Visarathanonth, N. 1999b. Diseases of Temperate Fruits. J. Film Process Co. Ltd., Bangkok, Thailand.

Manuscript received 15 October 2013, accepted 18 December 2013