Identification of Morphology and Pathogenicity of *Pyricularia* sp. Causing Blast Disease in Grass

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Abstract Morphological of 27 *Pyricularia* sp. isolates obtained from grass expressing blast disease symptoms was characterized using RFA (Rice flour agar) media and 8 colony types were grouped. The colony color was greyish green. The conidia shapes were pyriform or pear shape with rounded base and narrow toward the tip which is pointed or blunt. Study of pathogenicity on 40 Thai elite rice varieties showed that only 3 out of 27 isolates could infect different Thai elite rice varieties. Spot lesion to eye shape symptoms were founded on rice leaves. The isolate LPG61102 could infect the most numbers of Thai elite rice varieties with the virulence index of 0.15. While 24 isolates were unable to infect any of 40 tested rice varieties. This first report in Thailand on *Pyricularia* sp. causing blast disease in grass provided preliminary information between the two host species.

Keywords: Blast disease, Blast fungus, Pathogenicity test

Introduction

Fungal species from the *Pyricularia* genus are associated with blast disease in plants from the Poaceae family, causing losses in economically important crops such as rice, oat, rye, barley, wheat and triticale. *Pyricularia grisea* (Cooke) Sacc. and *P. oryzae* Cav. attacks a wide variety of grasses (Poaceae) family. The species of *Pyricularia* share some morphological traits, while *P. oryzae* and *P. grisea* can be differentiated from other species by morphological traits (Klaubauf *et al.,* 2014). However, no detectable morphological characteristics that are distinct between *P. grisea* and *P. oryzae* have been reported until now. The name *P. grisea* has been widely used since one isolate from crabgrass was first identified in 1880 (Saccardo, 1880), another name, *P. oryzae* has been used since an isolate from rice broad, however, individual strains infect a limited number of grass species (Ou, 1985; Borromeo *et al*., 1993; Urashima *et al*., 1993).

The pathogenicity of *P. grisea* have been reported to caused responses in goose grass, weeping love grass, and rice that varied from no visible symptoms, through isolated discrete lesions with or without brown margins, to coalescing lesions that completely killed the inoculated areas of the leaf. The fungus sporulated under conducive conditions, but the degree of sporulation varied considerably, and produced visible symptoms (Heath *et al*., 1990). It has been reported the pathogenicity of *P. grisea* and *P. oryzae* on green bristlegrass and rice that, pathogenicity of *P. oryzae* isolates from rice and three of *P. oryzae* isolates from green bristlegrass showed cross-infectivity on green bristlegrass and rice, respectively. The results demonstrated that isolates from green bristlegrass and crabgrass consist of both *P. oryzae* and *P. grisea*, and that *P. oryzae* isolates showed cross-infectivity between rice and green bristlegrass, suggesting host shifts might occur in *P. oryzae* and *P. grisea* (Qi *et al*., 2019).

In Thailand, the data of grass infected *Pyricularia* sp. was limited. The objective of this study was aimed at identification of the blast isolates collected from area where rice blast disease epidemic was occurred. Their morphology was observed, and pathogenicity was assessed using Thai elite rice varieties. Preliminary results obtained in this study will be used for further analysis on the relationships between the 2 hosts of grass infected blast fungi.

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Materials and methods

Collection and Isolation of blast pathogen

Samples with typical blast symptoms on grass leaves were collected from different rice growing regions of Thailand during growing season in year 2018. For isolation of single spore, the infected leaves of diseased plants were cut into small pieces and placed on moist filter paper in Petri dishes, then incubated under light for 24 h at 25 °C. Single spore was picked with a fine glass needle under a binocular microscope. Each single spore was transferred onto rice flour agar (RFA) medium. In order to maintain blast isolates for further use, each isolate was grown on RFA where surface was covered with filter paper for 7-14 days. The filter paper was dried and stored at -20 °C.

Examination of morphological characteristics

Each isolate was cultured on RFA media at 25 °C for 5 days and mycelial disk was transferred to the center of a new RFA medium. The colony morphology and colony color of each isolate on RFA medium were examined daily for 14 days. Sporulation was induced by scraping mycelium in each plate and the plate was let for 48 hr. The spores of each isolate were harvested and mounted in lactophenol cotton blue. The spore morphology was observed, and spore diameter was measured from 100 spores for each isolate using image analyzer under microscope.

Plant Material

A total of 40 Thai elite rice varieties were used in pathogenicity test. The standard control varieties, JHN, IR64 and KDML105 were included (Table 1). Germinated seeds were planted in a seedling tray (33 x 11 x 11 cm) and fertilizer was supplied at 7 days after planting by adding ammonium sulphate at 1 g/tray. Seedling were in a greenhouse until use.

Rice Blast Inoculation

Each blast isolates to be used in pathogenicity test was grown on RFA medium and incubated at 25°C. After 8-10 days, sporulation was induced by scraping mycelium in each plate. Counting of spores was performed by using a hemocytometer and concentration of spore suspension was adjusted to 1×10^5 spores/ml in 0.5% gelatin for spray inoculation. Inoculation was done by spraying on 14-day-old rice seedlings. The inoculated plants were incubated for 24 h in a humid chamber and then transferred to a glasshouse. The experiment was repeated twice with 4 plants each. Blast symptoms were evaluated 7 days after inoculation using a 0 – 6 scale based on the type and size of lesions (Roumen *et al*., 1997). Rice lines that only showed scores from 0 to 2 (no sporulating lesion) were considered as resistant; rice lines were considered susceptible if the majority of the lesions developed were type 3 – 6. The virulence index (VI) of blast fungus was calculated using formula:

$$VI = \frac{\text{No. of susceptible variety}}{\text{No. of tested variety}}$$

Table 1. Forty Thai elite rice varieties used in pathogenicity test.

No.	Varieties	Region*	Blast** Resistance	No.	Varieties	Region*	Blast** Resistance
1	RD27	C	R	21	Chai Nat 1	All	R
2	RD41	N	R	22	Pathum Thani 1	C	R
3	RD6	N and NE	R	23	Leb Nok Pattani	S	S
4	RD43	All	R	24	Plai Ngahm Prachin Buri	C	R
5	RD59	N and C	R	25	Prachin Buri 2	C	R
6	RD7	All	S	26	Sang Yod Phattalung	S	S
7	RD61	N and C	R	27	Hahng Yi 71	NE	R
8	Jow Khao Chiangmai	N	R	28	Red Hawn Rice	NE	R
9	Phitsanulok 1	N and C	R	29	Yipun DOA1	N and NE	S
10	Khlong Luang 1	C	R	30	RD8	N	-
11	RD31	C	S	31	Homcholasit	C	-
12	Phitsanulok 60_1	All	S	32	RDP3	C	R
13	Suphan Buri 1	NE	R	33	Yipun DOA2	N	S
14	Niaw Ubon 1	All	S	34	RD29	N and C	R
15	Suphan Buri 2	C	R	35	Sunpatong 1	N	R
16	Suphan Buri 3	All	R	36	Phrae 1	N	R
17	Phitsanulok 2	C	R	37	RD10	N	S
18	Suphan Buri 60	C	R	38	RD14	N	R
19	Suphan Buri 90	C	R	39	KDML105	NE	S
20	Sakon Nakhon	NE	S	40	Jao Hom Nin	NE	R

*C (Central), N (North), NE (Northeast) and S (South)
**R (Resistances), S (Susceptible) and – (No data)

Results

Collection and Isolation of blast pathogen

Blast samples were collected from grass leaves grown around the edge of rice paddy field in 12 provinces of Thailand including Lampang (North), Khon Kaen (North East), Lopburi, Phichit, Phitsanulok, Phetchabun, Sing Buri, Uthai Thani (Central), Ratchaburi, Tak (West), Nakhon Si Thammarat and Phatthalung (South). Using single spore isolation technique, total number of 27 isolates were obtained. (Table 2)

Morphological characteristics of grass infected Pyricularia sp.

The 27 isolates of grass infected *Pyricularia* sp. were assigned to 8 morphological groups based on the differences in morphological characteristics (colony color and colony morphology). Various isolates produced circular colonies with rough and smooth margins on RFA media which the color was varied from light yellow, white, to grayish colors. The colony type of Group 1 was greenish gray to white gray in the middle and the edge of colony was circular smooth, Group 2 was black grayish to greenish gray color in the middle and the edge of colony was circular smooth, Group 3 was white colony color and white gray circular smooth at the edge, Group 4 was grayish to white gray color at the middle and the edge of colony was white, Group 5 was white gray to white color at the middle and the edge of colony was white circular smooth, Group 6 was greenish gray at the middle and the edge of colony was circular smooth, Group 7 was grayish white at the middle and the edge of colony was grayish yellow with circular smooth, Group 8 was white and the edge of colony was circular smooth. The conidia shape was pyriform (pear shaped) with rounded base and narrowed towards the tip which is pointed or blunt (Figure 1). The colony diameters of all groups were ranged from 6 to 9 cm (data not shown).

Table 2. Location, host, given isolate name, pathogenicity and morphology classification of 27 grass infected *Pyricularia* sp. isolates.

Province	Host	Isolate number	Pathogenicity group	Morphology group
Khon Kaen	grass	KKN61102	1	6
	grass	KKN61103	1	5
Lampang	grass	LPG61102	2	3
	grass	LPG61103	1	1
Lopburi	grass	LRI61104	1	5
Nakhon Si Thammarat	grass	NST61101	1	1
Phichit	grass	PCT61104	1	7
Phatthalung	grass	PL61121	1	6
	grass	PL61122	1	6
Phitsanulok	grass	PLK61106	2	5
	grass	PLK61107	1	1
	grass	PLK61110	1	7
Phetchabun	grass	PNB61105	1	8
	grass	PNB61106	1	6
	grass	PNB61107	1	2
	grass	PNB61110	1	2
Ratchaburi	grass	RBR61109	1	4
	grass	RBR61110	1	5
	grass	RBR61111	1	2
Sing Buri	grass	SBR61101	1	1
	grass	SBR61104	1	1
Tak	grass	TAK61104	1	8
	grass	TAK61105	2	6
Uthai Thani	grass	UTI61102	1	3
	grass	UTI61104	1	6
	grass	UTI61106	1	5
	grass	UTI61107	1	3

Pathogenicity test

Study of disease reaction on 40 Thai elite rice varieties showed that the pathogenicity of 27 isolates differed in pathogenicity. Blast symptoms were first appeared as small spot and later enlarge up to roundish, slightly elongated to narrow or slightly elliptical lesions more than 3 mm long with a brown. Based on the blast lesions and affected area of leaves, symptoms were scored, and the 27 isolates were designated into 2 groups. The members of the first group were avirulent to tested rice varieties consisting of 24 isolates including KKN61102, KKN61103, LPG61103, LRI61104, NST61101, PCT61104, PL61121, PL61122, PLK61107, PLK61110, PNB61105, PNB61106, PNB61107, PNB61110, RBR61109, RBR61110, RBR61111, SBR61101, SBR61104, TAK61104, UTI61102, UTI61104, UTI61106 and UTI61107. Members of the second group were virulent, consisting of 3 isolates including LPG61102, PLK61106 and TAK61105 (Table 1, Figure 2)

The results showed that the most virulence was the isolate LPG61102. The isolate could infect 6 varieties of Thai elite rice including RD59, Suphan Buri 60, Chai Nat 1, RDP3, RD29 and RD15 with the virulence index scored 0.15. The isolate TAK61105 could infect 5 varieties including RD43, Suphan Buri 1, Niaw Ubon 1, Phitsanulok 2 and Suphan Buri 60 with the virulence index scored 0.13. The isolate PLK61106 could infect 2 varieties of elite Thai rice varieties including Suphan Buri 3 and Sunpatong 1 with the virulence index scored 0.05. While 24 isolates were unable to infect any of 40 Thai elite rice varieties being tested (Table 3).

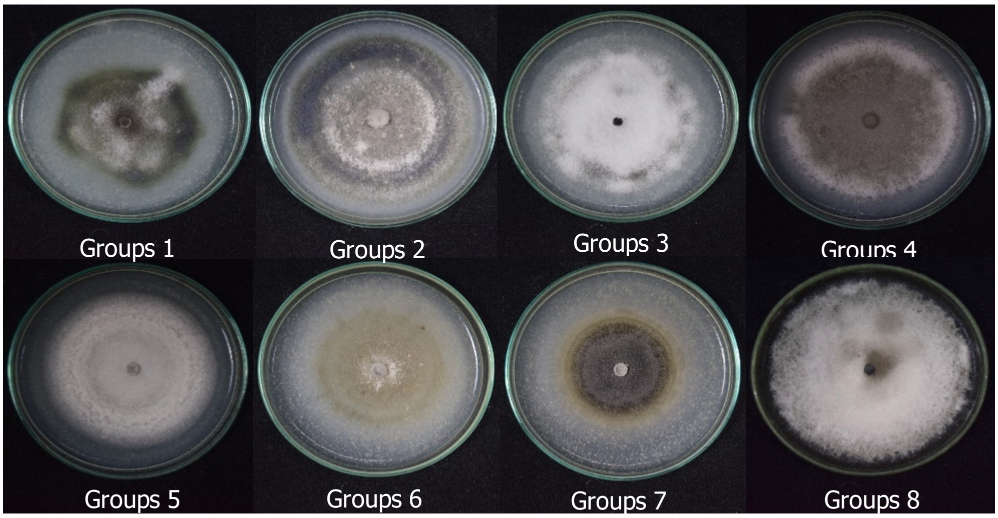

Figure 1. Morphological characteristics of 27 *Pyricularia* sp. isolated from grasses.

Table 3. The virulence index of blast fungus tested on Thai elite rice varieties.

Isolate	Virulance Index	Isolate	Virulance Index	Isolate	Virulane Index
KKN61102	0	PLK61106	0.05	RBR61111	0
KKN61103	0	PLK61107	0	SBR61101	0
LPG61102	0.15	PLK61110	0	SBR61104	0
LPG61103	0	PNB61105	0	TAK61104	0
LRI61104	0	PNB61106	0	TAK61105	0.13
NST61101	0	PNB61107	0	UTI61102	0
PCT61104	0	PNB61110	0	UTI61104	0
PL61121	0	RBR61109	0	UTI61106	0
PL61122	0	RBR61110	0	UTI61107	0

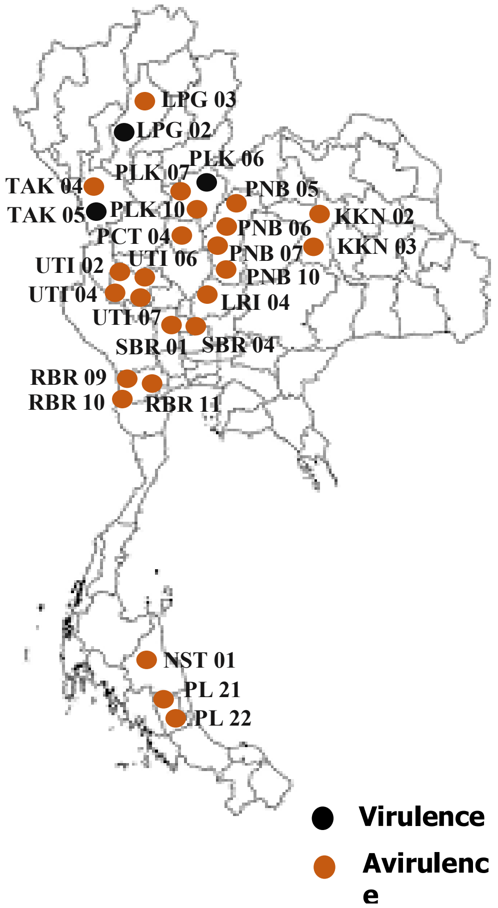

Figure 2. Distribution of 27 blast isolates collected from 12 provinces of Thailand, virulence isolates were indicated in black and avirulence isolates were in brown.

Discussion

The result show that 27 isolates of *Pyricularia* sp. collected from grasses were assigned to 8 morphological groups based on the differences in morphological characteristics (colony color, colony and morphology). Blast fungus circular colonies with rough or smooth margin on RFA media. The isolate showed light yellow color, white to grayish color. The colony diameter was ranged from 6 – 9 cm. The conidia shape was pyriform with rounded base and narrowed towards the tip, which is pointed or blunt. The colony morphology of grass infected *Pyricularia* sp. found in this study was similar to the observation reported in morphological characteristics (colony color, colony morphology and conidia shape) that 10 observed isolates of *P. oryzae* could be assigned to six morphological groups (PG-I to PG-VI). Various isolates produced ring like, circular, irregular colonies with rough and smooth margins on oatmeal agar media having buff color, grayish black to black color. The colony diameters of different groups were ranged from 67.40 to 82.50 mm (Srivastava *et al*., 2014).

Study of disease reactions on 40 Thai elite rice varieties showed that the pathogenicity of 27 grass infected isolates differed in pathogenicity. Three isolates were found to cause blast symptoms on tested rice varieties. Symptoms on rice leaves were first appeared as small spot and later enlarged up roundish, slightly elongated sporulating spots to narrow or slightly elliptical lesions more than 3 mm long with a brown. The observation in this study accorded by the report of pathogenicity test of *P. grisea* isolated from goose grass, weeping love grass, and rice that varied from no visible symptoms, through isolated discrete lesions with or without brown margins, to coalescing lesions that completely killed the inoculated areas of the leaf (Heath *et al*., 1990).

The results showed that 3 isolates could infect 6, 5 and 2 rice varieties were LPG61102, TAK61105 and PLK61106 respectively, while others were unable to infect any of 40 tested varieties. It has been reported the pathogenicity tests using artificial inoculation, 6 out of 10 *P. oryzae* isolates from rice and 3 isolates from green bristlegrass showed cross-infectivity on green bristlegrass and rice, respectively. The results demonstrated that isolates from green bristlegrass and crabgrass consist of both *P. oryzae* and *P. grisea*, and that *P. oryzae* isolates showed cross-infectivity between rice and green bristlegrass, suggesting host shifts might occur in *P. oryzae* and *P. grisea* (Qi *et al*., 2019).

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References

Borromeo, E. S., Nelson, R. J., Bonman, J. M. and Leung, H. (1993). Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. Journal of Phytopathology 83:393-399.

Heath, M. C., Valent, B., Howard, R. J. and Chumley, F. G. (1990). Interactions of two strains of *Magnaportlze grisecc* with rice, goose grass, and weeping love grass. Canadian Journal of Botany 68:1627-1637.

Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., De Vries, R. P. and Lebrun, M. H. (2014). Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae). Studies in Mycology 79:85-120.

Qi, H., Yang, J., Yin, C., Zhao, J., Ren, X., Jia, S. and Zhang, G. (2019). Analysis of *Pyricularia oryzae* and *Pyricularia grisea* from different hosts based on multilocus phylogeny and pathogenicity associated with host preference in China. Journal of Phytopathology 8:1433-1440.

Ou, S. H. (1985). Rice Diseases. Second ed. Commonwealth Agricultural Bureau, Surry, United Kingdom, pp. 109-201.

Roumen, E., Levy, M. and Notteghem, J. L. (1997). Characterization of the European pathogen population of *Magnaporthe grisea* by DNA finger printing and pathotype analysis. European journal of Plant Pathology 103:363-371.

Saccardo, P. A. (1880). Conspectus generum fungorum Italiae inferorium. Michelia 2:1-38.

Srivastava, D., Shamim, M. D., Kumar, D., Pandey, P., Khan, N. A. and Singh, K. N. (2014). Morphological and molecular characterization of *Pyricularia oryzae* causing blast disease in rice (*Oryza sativa*) from north India. International Journal of Scientific and Research Publications 4:2250-3153.

Urashima, A. S., Igarashi, S. and Kato, H. (1993). Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Disease 77:1211-1216.