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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATIONS OF RICE BLAST FUNGUS, *Magnaporthe oryzae*

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Magnaporthe oryzae is the casual agent of rice blast which causes approximately 30% yield loss annually. The objective of this study was to investigate the effect of media on various morphological characteristics, confirmation of isolates by molecular and phylogenetic study and relationship between morphological characteristics and pathogenicity study. The fungus can be isolated on alternative media. Isolation on oatmeal agar (OMA) showed variation among isolates characteristics. The colonies color slightly varied from brownish black to dark grayish green. Compared to isolates cultured on potato dextrose agar (PDA) they showed darker colours. Sporulation of isolates varied based on the isolates and media. Isolates on OMA showed better sporulation compared to PDA. Radial growth of isolates were different among isolates on each media. Isolate M03 showed the highest amount of sporulation on PDA and OMAwith numbers of 5.43 and 5.57 spore/ml. results shows that highest dry weight is recorded for isolate M03 by the number of 0.57 gram. Molecular characterization results by internal transcribed spacer 1 and 4 showed all isolates belong to *Magnaporthe* spp. and phylogenetic analysis confirmed that isolates belong to *Magnaporthe grisea* deposited in GeneBank with 99% similarity. Pathogenicity results were different based on the isolates and their origin.
Keywords: Magnaporthe, morphology, molecular, phylogenetic, pathogenicity.

INTRODUCTION

The fungus that causes rice blast is called *Magnaporthe oryzae* (formerly *Magnaporthe grisea*). It is an ascomycete because it produces sexual spores (ascospores) in structures called asci, and is classified in the newly erected family *Magnaporthaceae*. The asci are found within specialized structures called perithecia. The mycelium of *M. oryzae* is septate and the nuclei within the mycelium and spores of this fungus are haploid (TeBeest *et al*., 2007).

Rice blast is an important disease economically and biologically but the most serious problem is in term of losses in temperate region and upland conditions. In china whitin last 30 years three outbreaks were occurred with average losses of 2.5 million tons in each period. In India, Japan and Indonesia, the highest occurrence of blast were more than 50%, 42.5% and 70% respectively (Wang and Valnet, 2009). In Malaysia it is reported that almost 1000 ha of rice plantation areas in Kedah state were suffered by rice blast disease annually. The number of cases was reduced in 2004, but increased again in 2009. In 2011, 30 ha of rice fields in Kedah state were affected by disease during the early planting stage which caused the losses of about RM 150000 (New Straits Times, 2012). It is believed that a large quantity of rice (10-30%) which can be enough for feeding 60 million people is blasted each year (Talbot, 2003).

In fungi, the morphological species concept (MSC) is the most common technique of diagnosing species since morphological characteristics of individuals are easily detectable (Taylor *et al*., 2000). For distance conidia and conidiophore morphology are the main characteristics in phylum Ascomycota (Choi *et al*., 2013). *Magnaporthe oryzae* is mainly isolated on PDA. Initial cultures can then be sub-cultured onto PDA or alternative media. Guochang and Shuyuan (2001) reported oatmeal agar (OMA) induced good sporulation. The conidia vary in size and shape, the shape was described as pyriform to obclavate with round base and narrow apex, usually with 2 septate but rarely can have 1-3 septate. The size vary due to different growth condition from 19-23×7-9 µm (Ou, 1985).

One of the quick and simple methods for determining the species composition of fungal communities is based on sequencing particular regions of the fungal genome that has proven a reliable alternative to traditional methods. Ribosomal genes and spacers regions within the fungal genome are good candidates for amplification via the polymerase chain reaction (PCR) since they are comprised of highly conserved tracts with heterogeneous regions in between. The conserved tracts are ideal for universal primer

design that can allow for the amplification and sequencing of heterogeneous regions. Most molecular fungal species identification relies on the amplification and sequencing of the internal transcribed spacer (ITS) region of the fungal genome, which is highly variable among species or even populations of the same species. This region lies between the small subunit (SSU) and the large subunit (LSU) ribosomal RNA (rRNA) genes and contains two noncoding spacer regions (ITS-A and ITS-B) separated by the 5.8S rRNA gene. In Malaysia, study on the biology of the fungus is seriously lacking and not well documented. Therefore, the objectives of this study were conducted by morphological and molecular and pathogenicity studies of *Magnaporthe oryzae* isolates.

MATERIALS AND METHODS

***Isolation of Magnaporthe spp*:** Infected rice leaves, necks, panicles and nodes were collected from rice fields in Selangor, Kelantan, Perak and Penang states during plantation seasons of 2012-2013. Diseased tissues with lesions were cut in small pieces (5×5 mm^2) and were surface sterilized with 1% sodium hypochlorite for 1.5 min followed by 3 washes with sterilized distilled water. In order to use the single spore isolation method, typical disease lesions were placed on moist filter paper in sterile petri dishes and incubated at room temperature for 24 h to induce sporulation. After incubation the lesions were examined under stereo dissecting microscope (E200, Nikon, Japan) to examine spores. A sterilized needle was used to transfer the single spore to potato dextrose agar (PDA) and oatmeal agar (OMA) media. The petri dish was again examined to confirm that correct spore had been transferred. Then the petri dishes were incubated in a glass chamber in dark room at 28±2ºC with 12 hours darkness and 12 hours light until cover the whole petri dishes.

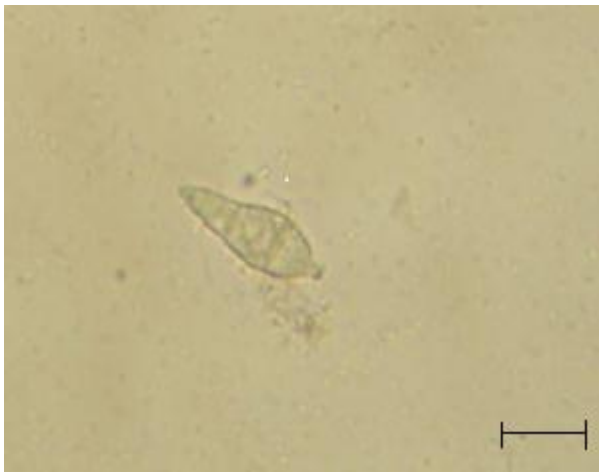


Figure 1. Single spore of *Magnaporthe oryzae* (M03) from leaf tissue on PDA media at 400X magnification.

Confirmation of *Magnaporthe* isolates were carried out by examining the conidia under light microscope. Monthly subcultures were performed to maintain the culture (Hayashi *et al*., 2009). Four states namely Kelantan, Selangor, Penang and Perakwere chosen for sampling. A total of 450 samples were isolated but due to contamination and similarity of lesions with brown spot many of isolates were discarded and only 13 pure *Magnaporthe* were retained.

Morphological characterization

***Radial growth*:** Plugs (5mm) of actively growing cultures of each isolate were placed on PDA and OMA media. The radial growth of different isolates on OMA and PDA were measured daily by average rate of X and Y axis of each isolate from the first day after inculation until maximum growth on the petri dishes. Radial growth of isolates were compared on 10^{th} day.

***Sporulation rate*:** The number of spores was measured using a haemocytometer (Neubaur Brighlined, Germany). The spore suspension was gently mixed and 9µl of spore suspension were dropped on the counting chamber. Chamber was filled slowly and sreadily without any overfill or underfill. Spores in each of $0.1mm^3$ corner squares were counted. Number of spores/ µl was calculated by equation below:

Spores/ml = (n) x 10^4

Where;

n = the average cell count per square of the four corner squares counted.

***Spore induction on PDA*:** *Magnaporthe* isolates were cultured on PDA medium at room temperature for two weeks until the whole surface of the plates was covered with mycelium. Mycelial mats were gently scraped by spatula and plates were placed under wet cheesecloth for two days under continuous light to induce sporulation. An aliquot of 1 ml sterilized distilled water was added into the petri dish and mycelium was scraped with spatula to harvest the spores of *P. oryzae*. The suspensions were then poured into the test tube and were vortexed for 5 seconds. 1 µl of the suspension was drop on the haemocytometer to observe under the microscope in order to count the spores.

***Sporulation on OMA*:** *Magnaporthe* isolates were cultured on OMA media at room temperature for almost two weeks until the petri dishes were covered fully with mycelium. Spores were produced on OMA during the growth without any further application.

***Mycelial dry weight*:** One hundred ml of Potato Dextrose Broth (PDB) was poured in 250 ml Erlenmeyer flask and autoclaved at 121º C and pressure of 1.2 kg/cm^3. Broth was allowed to cool down to room temperature. Isolates were aseptically transferred into each flask. The flasks were incubated using shaker at room temperature for 14 days at 250 rpm. After 14 days of incubation, the mycelial mats of fungal isolates were harvested by filtering on a pre-weighed filter paper and oven dried at 80 ºC, for 12 hrs and then weighed by

2 hrs intervals until constant weight was maintained. The weight of mycelia was determined by deducting the weight of filter paper from the total weight. The dry weight of each isolate was measured in 3 replications (Abiala *et al*., 2010).

Spore size: The spore sizes of 13 *Magnaporthe* isolates were measured using ocular and stage micrometer. A total of 50 spores of each isolate were measured on each media.

***Molecular characterization liquid culture preparation*:** Four plugs (5 mm) of actively growing culture were transferred to 250 ml PDB (Difco™, France) in a conical flask and incubated at room temperature for 14 days on an orbital shaker (PROTECH™, Malaysia) at 250 rpm. Mycelia were harvested by filtering through No.3 Whatman filter paper and immediately frozen in liquid nitrogen and was grinded until fine powder was obtained and stored at -20º C until further use.

***DNA Extraction*:** Around 100 to 200 mg of fungal mycelia was ground in liquid nitrogen using sterilized mortar and pestle. A 500 µl CTAB buffer (pre-warmed at 55°C, 5.0 mg PVP and 25 µl 2-ME) was added to each sample and mixed homogeneously. The tube was then incubated at 65°C for 60 min. Approximately 500 µl of 24:1 mixture of chloroform and isoamyl alcohol was added to each tube, mixed by vortex or inversion and centrifuged at 13,000 rpm for 10 min at room temperature. Upper aqueous phase was transferred slowly into a new microcentrifuge tube. This process was repeated twice. After estimating the volume of aqueous phase, approximately half volume of cold 7.5 M ammonium acetate was added. Around 2/3 volume (using the combined volume of aqueous and added ammonium acetate) of cold (-20°C) isopropanol was added and the tube was inverted gently. To precipitate DNA, tubes were placed in -20°C for 60 min and centrifuged at 13,000 rpm for 20 min. The supernatant was removed and the tube was centrifuged for 1 min and the remaining liquid was removed. The pellet was rinsed with 70% cold ethanol, dried under vacuum, re-suspended in sterile distilled water (SDW) and quantified using a spectrophotometer (Ultra spec 2000 Pharmacia Biotech). The concentration of DNA were adjusted at 530 ng/µl (Murray and Thompson, 1980).

***PCR and data analysis*:** One µl of the internal transcribed spacer (ITS) region 1 and 4 including 5.8 rDNA were amplified in 30 µl reaction on a Bio-Rad thermo cycler (C-100 Gradient, Canada) according to Bussaban *et al*., (2005) using a PCR mastermix (DreamTaq Green Master Mix ,2X, Fermentas™ , Canada) containing DreamTaq Green buffer (2X), 4mM $MgCl_2$ and dNTPs (dATP, dCTP, dGTP and dTTP, 0.4 mM each). The sequences of the primersITS1 (5' TCC GTA GGT GAA CCT GCG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') were used (White *et al.,* 1990). Thermal cycling was initiated by 35 cycles of denaturation at 95°C for 30 Seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 min.

***Gel electrophoresis and sequencing*:** PCR products were electrophoresed on 1% agarose gel prepared with 1X Tris Borate-EDTA(TBE) pH 8.3 and using 1X TBE as running buffer. All agarose gels were processed under constant electric field, in a horizontal configuration. The gel contained FloroSafe DNA Stain (1stBase, Malaysia), and the DNA loading Dye at concentration of 2 µl. The fragments were viewed using an UV transilluminator (GelDoc, BIO-RAD, Canada) and photograph recorded when necessary. The band sizes of DNA of samples were estimated and were compared with DNA marker (1stBase, Malaysia). PCR products were sent to 1stBase Malaysia for purifying and sequencing. Sequences were aligned using Bioedit software (version 7.13) and then deposited in Genebank.

***Phylogenetic analysis*:** DNA sequences were manually edited using Bioedit (version 7.13) and aligned using ClustalW with a gap opening penalty of 10 and gap extension penalty of 0.02 for multiple alignments. For comparison reference sequences of *Magnaporthe oryzae* and out group sequences of *Gaeumannomyces graminis* were obtained from the Genebank database (Hirata *et al*., 2007). Phylogenetic and molecular evaluator analysis of aligned gene sequences were conducted using MEGA6 by maximum likelihood and maximum parsimony methods on combined data sets. A 1000 bootstrap replication was used to assess the stability of clades.

***Pathogenicity test*:** Plants were grown in seedling polythene boxes, and 14 days old rice seedlings were transplanted in each pot containing 5 kg of soil. Inoculation of plants with 13 *Magnaporthe* isolates was performed when the plants were at their 3-5 leaf growth stage to select the most virulent isolate. For this purpose, *Magnaporthe* conidial suspension of each isolate was adjusted to 3×10^5 spores/ml and sprayed on 3-5 leaf stage of rice seedling (Kanzaki *et al*., 2002). Fifteen mL of conidial suspension was sprayed onto rice plants in seedling box. Inoculated rice plants were then placed in dark moist chamber at the temperature of ≤30±2°C with ≥95% (measured by hydrometer) relative humidity for 48 hours. After 4-6 days symptoms of blast disease were recorded on rice leaves. The experiment was carried out as factorial experiment in complete block design with four replications. Disease incidence (DI) and disease severity (DS) was assessed based on the appearance of symptoms of disease starting four days after inoculation.

DI= (number of diseased plants/ total number of plants)×100

Leaf blast severity was evaluated based on visual assessment of lesions caused and scored with a nine grade scale according to IRRI standards (2006) (Table 1). For each replication of seedlings, DS was calculated according to the equation below (Cai *et al*., 2008):

$$DS = [\sum(r\times nr)/(9\times Nr)]\times100$$

Where:

$r \rightarrow$ rating value, according to IRRI (2006)

$n_r \rightarrow$ number of infected leaves with a rating of r

$N_r \rightarrow$ total number of leaves tested (for each replication)

Table 1. Nine grade scale description used for rating rice blast severity (IRRI, 2006)

Grade	Description
0	No lesion observed
1	Small brown specks of pin-point size or larger brown specks without sporulating centre
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area
5	Typical blast lesions infecting 4-10% of the leaf area
6	Typical blast lesions infecting 11-25% of the leaf area
7	Typical blast lesions infecting 26-50% of the leaf area
8	Typical blast lesions infecting 51-75% of the leaf area and many leaves are dead
9	More than 75% leaf area affected

***Data analysis*:** Analysis of Variance (ANOVA) was performed using SAS statistics software package (version 9.3 for windows). Statistical differences among treatments were determined using the protected least significant difference (LSD) test at the 0.05 probability level (Gomez and Gomez ;1984). Sporulation rate data were transformed as $\log_{10}$.

RESULTS AND DISCUSSION

***Morphological characterization Radial growth*:** The results of the growth diameter of 13 isolates of *Magnaporthe* on 10th day on both PDA and OMA media was measured and presented in Table 2. Isolates M06, M07, M08 and M10 showed the rapid growth on PDA and isolates M09 and M11 showed the fast growth on both media.

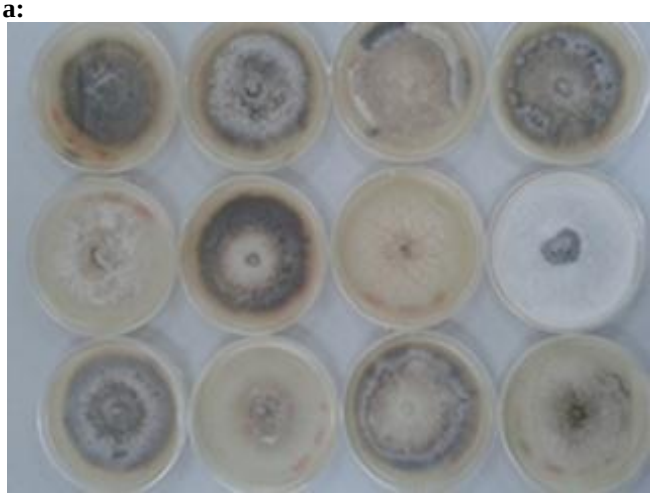
Table 2. Radial growth of 13 *Magnaporthe* isolates on different media.

Isolate	PDA (diameter in cm)*	OMA (diameter in cm)
M01	5.05±0.17cd	5.28±0.08fg
M02	5.00±0.18d	5.00±0.10h
M03	5.05±0.05cd	5.17±0.06gh
M04	5.62±0.25bcd	5.52±0.03def
M05	5.53±0.24bcd	5.57±0.06def;
M06	5.45±0.35bcd	5.57±0.06def
M07	7.32±1.06a	5.40±0.00efg
M08	7.10±0.30a	7.03±0.06b
M09	7.08±0.20a	7.30±0.44a
M10	6.82±0.18a	6.70±0.10c
M11	7.30±0.26a	7.50±0.26a
M12	5.98±0.38b	5.63±0.06de
M13	5.73±0.31bc	5.77±0.15d

*Means with the same letter in common show radial growth which are not significant on PDA and OMA at p <0.05 (Test statistic is LSD).

The radial growth rates greatly vary depending on the growth media and the origin of the sample. The isolates showed significant differences in their growth rate and there were some variation among colony characteristics on these two growth media.

a:



b:

Figure 2. (a) different colony color of *Magnaporthe oryzae* isolates on PDA media. (b) Isolates of *Magnaporthe oryzae* (M03) on PDA culture medium (14 days old)

On PDA, cultures showed a range of colours that vary from greyish brown to light cream (Figure 2a). Colony colours were determined using the colour chart of Rayner (1970). The typical *Magnaporthe* culture (M03) was grayish brown in colour (Figure 2b). The mycelium types also vary in isolates.

They were aerial, effuse on thin hairy mycelium (Table 3). On OMA 13 isolates which were tested had shown darker colours compared to PDA. The colours of isolates were slightly varied from brownish black to dark greyish green. The mycelium type was flat for all isolates except M12 and M13 from Perak which had shown aerial mycelium.

Table 3. The colony characteristics of 13 *Magnaporthe* isolates on PDA and OMA media.

Isolate	Tissue	PDA		OMA	
		Colour	Hyphae	Colour	Hyphae
M01	Leaf	Greyish brown	Aerial	Greyish black	Flat
M02	Leaf	Greyish brown	Effuse	Greyish black	Flat
M03	Panicle	Greyish brown	Aerial	Greyish black	Flat
M04	Leaf	Light grey	Aerial	Grey	Flat
M05	Leaf	Light grey	Aerial	Grey	Flat
M06	Node	Greyish green	Effuse	Greyish black	Flat
M07	Neck	Greyish brown	Effuse	Greyish brown	Flat
M08	Leaf	Greyish green	Effuse	Greyish brown	Flat
M09	Leaf	Greyish brown	Effuse	Greyish black	Flat
M10	Leaf	Brownish cream	Aerial	Light grey	Flat
M11	Node	Greyish green	Aerial	Greyish black	Flat
M12	Neck	Brownish cream	Thin hairy	Greyish black	Aerial
M13	Neck	Light grey	Thin hairy	Greyish green	Aerial

Cultural morphology varies greatly with isolates and the medium. The amount of aerial mycelium varies from very scant to a tick cottony mass. The colour varies from whitish or cream to gray and black due to different media and isolates.

***Sporulation rate*:** The amount of sporulation of isolates had shown significant difference on culture media. On PDA isolates M01, M02 and M03 induced the highest amount of sporulation of 5.37, 5.36, 5.43 spores/ml as $\log_{10}$, respectively.

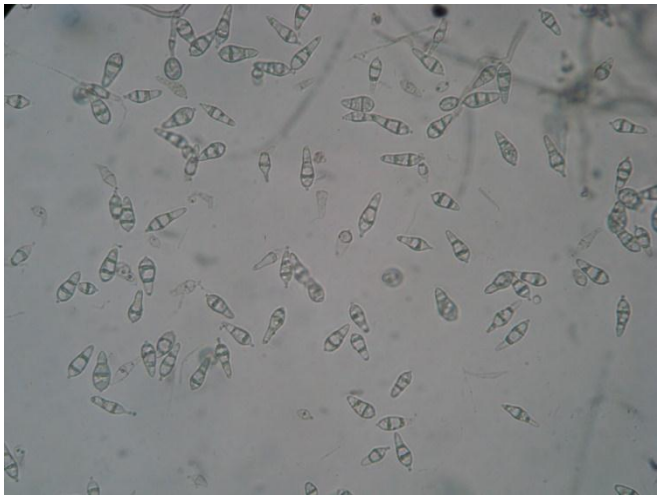


Figure 3. Conidia of *Magnaporthe* under microscope at 400X magnification.

On OMA the highest amount of sporulation was obtained for isolate M03 of 5.57 spores/ml as $\log_{10}$ (Table 4).

Table 4. Amount of sporulation (spore/ml) $\log_{10}$ on PDA and OMA media.

Isolate	Mean sporulation on PDA (spore/ml)	Mean sporulation on OMA (spore/ml)
M01	5.37a*	5.45b
M02	5.36a	5.48b
M03	5.43a	5.57a
M04	5.10de	5.24ef
M05	5.05de	5.24ef
M06	5.21bc	5.30cde
M07	5.20c	5.34cd
M08	5.26bc	5.44b
M09	5.26bc	5.44b
M10	5.11d	5.21fg
M11	5.28b	5.35c
M12	5.99f	5.15g
M13	5.03ef	5.27def

*Means with the same letter in common are not significant difference in the amount of sporulation on culture media at $p < 0.05$ (Test statistic is LSD) Numbers shows the amount of sporulation into $\log_{10}$.

***Mycelia dry weight*:** There is differences between mycelial dry weight of different *Magnaporthe* isolates at $p < 0.05$. Isolates M01, M02, M03 and M08 were observed with the highest weight of 0.57 mg (Table 5).

Table 5. Mycelial dry weight of 13 *Magnaporthe* isolates on Potato Dextrose Broth.

Isolate	Mycelia dry weight (g)
M01	0.5723a*
M02	0.5670a
M03	0.5733a
M04	0.5410bc
M05	0.5380c
M06	0.5430bc
M07	0.5440bc
M08	0.5723a
M09	0.5430bc
M10	0.5446b
M11	0.5463b
M12	0.5400bc
M13	0.5380c

*Means with the same letter in common are not significant in terms of mycelia dry weight among different *Magnaporthe* isolates at $p<0.05$ (Test statistic is LSD).

***Spore size*:** The spore sizes of 13 *Magnaporthe* isolates were measured on both PDA and OMA culture media. There was significant difference between isolates on PDA. Isolate M09 had shown the biggest spore size among isolates on PDA. On OMA isolates M02 and M03 had shown the biggest spore size of 25.60×9.33 µm and 25.67×8.53 µm respectively (Table 6).

Table 6. The spore size of 13 *Magnaporthe* isolates on both PDA and OMA growth. Media.

Isolate	Mean spore size on PDA (µm×µm)	Mean spore size on OMA (µm×µm)
M01	25.33×8.50dfe*	23.83×7.56b
M02	26.50×9.83ab	25.60×9.33a
M03	26.33×9.33bc	25.67×8.53a
M04	21.67×7.83dfe	20.67×7.17d
M05	21.67×7.33f	20.50×6.67d
M06	21.67×8.83cde	20.67×7.83d
M07	23.50×10.17cd	19.83×8.00d
M08	21.33×8.33cde	20.67×7.83d
M09	23.50×10.17a	22.17×9.50c
M10	19.00×7.67ef	18.00×7.00e
M11	19.17×8.67def	16.83×7.17f
M12	18.33×7.33ef	17.56×7.00ef
M13	18.00×7.83def	17.50×7.17ef

*Means with the same letter in common are not significant difference in spore size on culture medium at p <0.05 (Test statistic is LSD).

***Molecular characterization*:** PCR amplification using universal primers amplified a fragment of the expected size (590 bp) from the ITS1 and ITS4 regions. 1kb DNA marker was used as positive control (Figure 4).

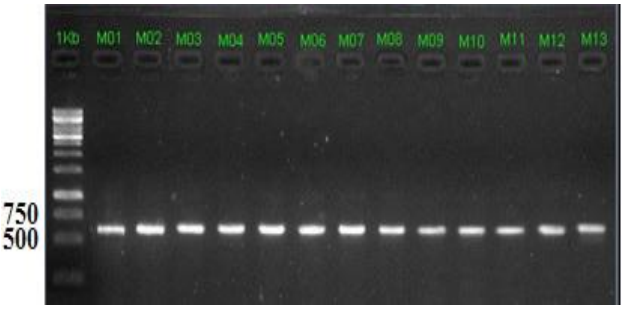


Figure 4. Gel electrophoresis of DNA fragments of 13 *M. oryzae* isolates.

PCR product were purified and sequenced to authenticate the species. The isolates were deposited in GeneBank. Accession number is listed in the Table 7.

***Phylogenetic analysis*:** Phylogenetic analysis of ITS1 and ITS4 genes from putative isolates of *M. oryzae* and isolates obtained from GeneBank constructed 3 different clades. Isolated *M. oryzae* was placed in clade I with *Magnaporthe oryzae*-Ken 54-04 and *Magnaporthe oryzae*-Ken 54-04. Clade II containing *Magnaporthe grisea* and clade III containing *Pyricularia zizaniicola* were obtained from GeneBank. Clade I was supported with high bootstrap value of 99% indicating high level of similarity for this clade. The constructed phylogenetic tree supported identification of molecular characterization which clustered them in the same group as *M. oryzae* (Fig. 5).

Table 7. Thirteen *Magnaporthe* isolates accession number as deposited in GeneBank.

Isolate Code	Genus and Species	GenBank accession no.
M01	*M. oryzae*	KJ766300
M02	*M. oryzae*	KJ766301
M03	*M. oryzae*	KJ782606
M04	*M. oryzae*	KJ782607
M05	*M. oryzae*	KJ782608
M06	*M. oryzae*	KJ782609
M07	*M. oryzae*	KJ782610
M08	*M. oryzae*	KJ782611
M09	*M. oryzae*	KJ850436
M10	*M. oryzae*	KJ850437
M11	*M. oryzae*	KJ850438
M12	*M. oryzae*	KJ850439
M13	*M. oryzae*	KJ850440

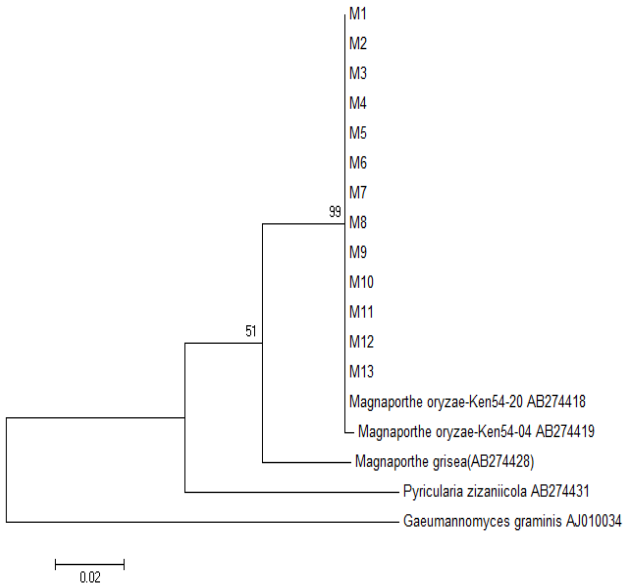


Figure 5. The relationship between the Malaysian *Magnaporthe* isolates and other *Magnaporthe* species based on concatenated ITS gene sequences. Numbers represent bootstrap >50% from 1000 replications heuristic searches. *Gaeumannomyces graminis* was selected as outgroup.

***Pathogenicity test*:** Pathogenicity test results in glasshouse showed that isolates M03 was the most virulent isolate which was obtained from Kelantan. The disease incidence and severity of M03 on seedling of rice plant were 65.56% and 38.64%, respectively. *Magnaporthe* isolates (M12, M13) which originated from Perak showed the lowest disease incidence and severity (Table 8).

Table 8. Pathogenicity test results of 13 *Magnaporthe* isolates in glasshouse.

Isolates	Disease incidence (DI) %*	Disease severity (DS)%
M01	38.43±5.61def	19.96±4.20de
M02	48.15±3.21bc	26.75±1.78bc
M03	65.56±5.09a	38.64±1.50a
M04	43.33±5.77cde	26.75±5.83bc
M05	55.19±5.01b	30.66±2.78b
M06	44.81±5.01bcde	26.54±3.85bc
M07	55.19±5.01b	24.53±2.23cd
M08	55.19±5.01b	18.40±1.67e
M09	46.97±2.62bcd	20.88±1.17de
M10	36.11±2.41ef	16.05±1.07e
M11	46.67±5.77bcd	17.04±0.64e
M12	32.42±6.70f	9.80±3.64f
M13	30.30±5.25f	9.76±2.10f

*Means with the same letter in common are not significantly different at p <0.05 (Test statistic is LSD)

DISCUSSION

Cultural morphology varied greatly with isolates and the medium. The amount of aerial mycelium varied from very scant to a tick cottony mass. The colour varied from whitish or cream to gray and black due to different media and isolates. There was significant difference in the amount of sporulation between two culture media. Sporulation of *Magnaporthe* on OMA media was highly significant in comparison to PDA, therefore, it was confirmed that OMA is an ideal media for this purpose (Vanaraj *et al*., 2013). The amount of sporulation among isolates vary depending on the source of plant tissue which they were isolated (Ou, 1985). Based on the result of previous studies, panicle blast is the most severe type of blast disease, so the isolate M03 with panicle source shows the most amount of sporulation on both medias (Ng *et al.,* 2012; Latif *et al.,* 2011) According to studies, conidia under moist condition were longer than the ones under dry condition. However, variations in widths were not significant. Existence of variability among the isolates of *Pyricularia grisea* with respect to conidial size was well documented (Ou, 1985). It is probable that the different environments such as geographical distribution and ecological conditions under which the various isolates were growing exert an important influence upon the form, size and septation of conidia of *Magnaporthe* isolates produced. The conidia were found to show variations in septation, ranging from one to three septations. The majority of the conidia had three septations. Although the pattern of septation of six isolates of *Magnaporthe* was similar in terms of dominance in frequency distribution of conidia with 3 septation, they were found to differ in the presence septation in some groups. Conidia with one septation were rarely observed in all isolates whereas conidia with two septations were observed in all isolates. Similar variations in septation on *Magnaporthe* conidia was also reported by Gashaw, Alemu, and Tesfaye (2014).

Magnaporthe oryzae influenced significantly on the growth of rice plants and produced typical rice blast disease symptoms on inoculated plants. Isolate M03 showed the highest amount of sporulation, mycelial dry weight and spore size. It can be suggested, there is positive relation between above mentioned characteristics and disease severity. In the parsimony and distance analysis all isolates were sorted into one clade with two magnaporthe oryzae isolates obtained from Genebank with high bootstrap support (MP=99%), while the *M. grisea* was seprated in clade II with low bootstap support (MP=51%). Based on the similarity of discussed isolated the phylogenetic tree supported the results of morphological analysis (Bussaban *et al.,* 2005).

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