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Review: Rice Blast Disease

Saleh Ahmed Shahriar^{1*}, Abdullah All Imtiaz², Md. Belal Hossain³,
Asmaul Husna¹ and Most. Nurjahan Khatun Eaty⁴

¹School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

²Institute of Seed Technology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

³Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

⁴Faculty of Agriculture, International University of Business Agriculture and Technology, Dhaka, Bangladesh.

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ABSTRACT

Rice blast caused by *Magnaporthe grisea* is the major damaging disease in nearly all rice growing nations. Economically relevance with 60 percent of total population of world depending on rice as the main source of calories, may have destructive effects of the disease, however, this pathogen has developed into a pioneering model system for researching host-pathogen interactions. The disease outbreak depends on the weather and climatic conditions of the various regions. The disease's occurrence and symptoms vary from country to country. Susceptible cultivars cause huge rice production loss in yield. The principal cause of resistance breakdown in rice against rice blast disease is pathogenic variability. During sexual hybridization, pathogenic changes may provide evidence of pathogenic variation found at the asexual stage of the fungus. The virulent pathotypes cause severe disease incidence. Only through pathogenicity research the pathotypes can be determined using a collection of different rice varieties that are usually different carrying various resistance genes. Rice breeders now have a number of resistant genes however, most of the breeding programs emphasized upon monogenic resistance. Genetic heterogeneity of *M. grisea* should be taken into account when screening blast resistant rice genotypes through morphological

*Corresponding author: E-mail: shahriar777.ss@gmail.com;

analysis, pathogenicity and molecular characterization. Knowledge on the virulence of the rice blast and host resistant is essential for managing the disease. Cultivation of resistant varieties with chemical control is highly effective against blast pathogens.

Keywords: *Oryza sativa* L; *Magnaporthe grisea*; morphology; cultural characters; molecular characterization; pathogenicity.

1. INTRODUCTION

Rice is very common worldwide cultivated cereal food crops. Rice is infected with several pathogenic species and diseases. Among them blast of rice disease is the most significant and devastating disease of rice [1]. *Magnaporthe grisea* pathogen (Anamorph *Pyricularia grisea* Sacc. synonym *Pyricularia oryzae* Cav.) causes the disease. Based on location and environmental conditions, the disease incidence as well as severity of rice blast varies annually [2]. Rice is grown in warm or cool subtropical humid areas. The tropical humid climates in Asia are very conducive to the epidemics of rice blast disease. Rice blast development is favored by a number of factors such as high relative humidity (above 80 percent), low temperature (15°C-26°C), cloudy weather, more wet or rainy days, longer durations of dew, sluggish wind movement, availability of collateral hosts and excessive doses of nitrogen fertilizers [3,4]. During the epidemic years, the disease causes huge yield losses of up to 100 per cent [5,6]. The disease is most evident when the pathogen affects the collar, blades of the leaf, necks and panicles [7]. First appearance of lesions or spokes as minutes of brown specks on leaf tissue and gradually growing spindle shaped [2]. The center is grayish with brown margin. The lesions may extend and thus eventually coalesce the entire leaf into killings. Even so, *M. grisea* has been reported to have high pathogenic variability in the host range and specificity of the varieties. The principal cause of resistance breakdown in rice against rice blast disease is pathogenic variability [8]. The pathogenic heterogeneity degree of *M. grisea* isolates are isolated from the rice varieties. Rice blast disease can be divided into different pathotypes based on the pattern of infection found on a sample of genotypes of rice differentials [9]. However, resistant varieties may sometimes become ineffective owing to evolutionary changes in the pathogen population. So, understanding pathogenic variation of *M. grisea* is critical in overcoming the constraints that many rice breeding techniques face. This pathogen heterogeneity is due to changes in chromosome

numbers or genomic rearrangements [10]. Similarly, parasexual recombination was identified as being one of the means of variation in *M. grisea* [11]. Further understanding of pathogenic changes during sexual hybridization may provide evidence of pathogenic variation observed at the fungus asexual stage. Virulence experiments using differentials blast host are labor intensive as well as complicated by the inoculation methods and conditions of the environment [12]. Different molecular approaches may have alternative techniques in this regard for characterizing blast pathogen strains [13,14]. Molecular researches are currently effective strategies in the detection and characterization of *M. grisea*. The use of DNA techniques such as polymerase chain reaction (PCR) is however the most appropriate approach to pathogen detection [15]. PCR is an effective technique for distinguishing between closely related strains. This research aimed at identifying, characterizing and discovering pathogenic variant of *M. grisea* using the rice differentials and PCR techniques. Fingerprinting of genome has a significant role to play in further characterizing the structure of fungi population and investigating their heterogeneity [16]. The present study was carried out to understand in details of following objectives: Morphology, cultural characters, molecular characterization and pathogenicity of rice blast disease.

2. OCCURRENCE AND DISTRIBUTION OF RICE BLAST DISEASE

Two cultivated rice species are *Oryza sativa* L (Asian rice) and *Oryza glaberrima* S (African rice) [17]. *Oryza glaberrima* is abundantly cultivated in various agro-ecological zones in West Africa but is largely prohibited with greater agronomic performances of high-yielding *Oryza sativa* cultivars [18]. Moreover, cultivars of *Oryza sativa* are mostly not adequately suited to different biotic and abiotic conditions in Africa. It has been observed that *Oryza glaberrima* has several useful features such as moderate to high levels of blast resistance [17], *Rice yellow mottle virus* [19], rice gall midges, insect pests [20] and nematodes [21]. The variety was also recorded

to be tolerant to abiotic stresses such as acidity, iron toxicity, drought and competition from weeds [22]. Rice blast is one of the most damaging rice diseases of its widespread and destructive nature, making yield losses up to 60-65% in vulnerable rice varieties [23]. The fungus may infect any above portion of rice plants, including roots and seeds. It also revealed of systemic movement of the pathogen from seed to seedlings [24]. *Magnaporthe grisea* fungal growth and conidial development are maximum at 28°C, moderate at 23°C and minimum at 15°C and growth was suppressed at temperature of greater than 37°C. Mycelial growth increased with pH increased ranging from 3.5-6.5 that subsequently decreased. The fungus showed highest mycelial growth at pH 6.5 and lowest growth at pH 3.5 [25]. In a field condition, moderately affected by infection, around 50% of production may be lost. Rice blast alone is calculated to demolish enough rice production every year to feed more than 60 million people [26]. Rice blast disease is spread out all continents where the rice is grown in about 85 countries, in both low land and upland environments. Rice blast occurs wherever rice is grown but the disease takes place at extremely variable severities depending upon climate as well as cropping patterns. Environments with regular and extended dew times and mild daytime temperatures are more conducive for explosion [27,11]. Rice blast was recorded as severe problem in Northern Territory [28], Brazil [29], Australia [30], Sri Lanka [31], Egypt [32], Colombia [33], South Korea [34], Philippines [35], Japan and China [36]. It was reported first as fever disease of rice in China by Soon Ying-shin in 1637 [29], in Japan reported as Imochi-byo by Tsuchiya in 1704. It was reported as brusone by Astolifi in Italy in the year of 1828 and in India it was first reported in Tamil Nadu in 1913 [37]. The disease makes yield in loss up to 1-100% in Japan [38], 70% in China [39], 21-37% in Indonesia [40], 30-100% in Bangladesh, and 30-50% in South America and other Southeast Asian countries [41]. Frequent breakdown of resistance resulting significant losses in yield ranging from 20-100% in some areas of Japan. South Korea with temperate climate is another country where blast disease makes huge yield losses every year. During the blast epidemic in a year of 1984-1985 almost 20% of the rice area was seriously destroyed [42]. The disease has an immense significant in temperate, tropical, subtropical Asia, Latin America and Africa and distributed throughout the world in about 85 countries [43]. Various reports from Nutsugah

[44], Twumasi [45,46], identified the rice blast disease as a serious threat to Ghana's rice production. This pathogen is the key constraint for production in West Africa, the largest area of African production, with yield losses varying from 3-77 percent. The fungus can infect plants in both upland and lowland rice production systems, at all stages of growth and development. Low land rice produced in Asia's temperate and subtropical climate is highly susceptible to the pathogen, while tropical upland areas are only susceptible to irrigation [47,44]. The disease incidence increased every year in Malaysia, affecting approximately 4033 ha of paddy fields in 2005 during disease outbreak. Based on these results, although the area affected was below 5 percent of the rice area planted, estimated yield loss from panicle blast was as high as 50-70% [41]. A survey conducted that several rice fields in Kuala Muda, Yan, and Kota Setar in Kedah states were affected with panicle blast during the year 2010/2011 main rice-growing season. The infection occurred on a rice variety, called MR-219 that was considered immune to rice blast. The infection led to panicles rotting or grains breaking or hollow hulls, resulting in huge yield losses [40,41]. The incidence and severity of rice blast disease was observed during the seasons of Boro (irrigated ecosystem) and Transplantated Aman (rain-fed ecosystem) in ten agro-ecological zones (AEZs) of Bangladesh. The incidence and severity of the disease was higher in irrigated ecosystems (Boro season) (21.19%) than in rain-fed ecosystems (Transplantated Aman season) (11.98%) regardless of locations (AEZs) [2]. Rice blast is correlated negatively with temperature and incidence. This indicated that the incidence of the disease increases with temperature declines. Relative humidity is positively correlated with rice blast suggesting a rise in the incidence of disease as the moisture increases. Rainfall has also been correlated positively with disease incidence [48]. The prevalence and spreading of rice blast in Jammu and Kashmir reported an incidence of 25% disease and 15% severity, and the incidence increased from transplantation to initiation of panicles [49]. A survey conducted in Kashmir's temperate districts showed that the severity of leaf blast ranging from 3.7% to 41.3%. Maximum neck blast was recorded at Kulgam (7.3%), followed by Khudwani (5.4%) and Larnoo (3.8%) regions of Anantanag district. In each district with a mean range of 0.4-4.8 per cent, the most damaging step of neck blast severity was found [1]. Survey observed the effect of blast disease on rice plants over 45 regions of India

and found the higher Percent Disease Index (PDI) at dough stage (30.45 per cent) followed by booting stage (29.77 per cent) and tillering stage (15.4 per cent) in lower land of rice growing areas [50]. Paddy fields were heavily exploding and grain yield decreased by 76 percent when rice blast infection occurred immediately after the flowering of rice plants at Tamil Nadu in India [51,52]. Rice blast disease is a big issue in the river belts of Penna and Godavari in India's Andhra Pradesh. The blast fungus may attack over 50 other grass species. It causes disease on the leaves, nodes, and panicles at the seedling and adult plant stages of nearest paddy fields. The yield relationship with blast incidence and found a significant reduction in yield showing a 4% loss due to the incidence of disease in 4% [37].

3. NATURE AND SYMPTOMS OF RICE BLAST DISEASE

The climate with regular and extended dew periods and a cool daytime temperature is highly favorable for disease prevalence [53]. Symptoms showing on leaves can differ depending upon the environmental conditions, the plant age and the resistance level of host plants. On susceptible rice cultivars, lesions can firstly appear as gray-green and water-soaking with a deep green border expanding quickly to a few centimeters in length, sometimes becoming soft-colored tanning with necrotic borders. The lesions frequently remain short in size (1 to 2 mm) and gray to deep brown color on resistant cultivars [54]. Symptoms of collar node infection consist of general area necrosis when the two tissues come together. Collar infections may kill the whole leaf and can spread to and around the sheath a few millimeters later, when the plant is damaged in the collar region [29]. The fungus may attack all of the ground portions of rice plant at various stages of growth: the leaf, collar, node, internode, base or neck, and various parts of panicle, and often the sheath of the leaf [55]. *M. grisea* infects and generates lesions on the following plant parts (leaf, neck, collar and panicle) and causes leaf blast, neck blast, collar blast and panicle blast respectively [56]. It begins in the middle with a dark border a traditional blast lesion on the rice leaf and is spindle-shaped [57]. The fungus is most prevalent on the leaves and causes blasting of the leaves during vegetative growth phase, or on the necks and panicles during a period of reproductive stage [12]. Systemic transmission of the fungus takes place between seeds and seedlings. *M. grisea* infects

and creates lesions to all rice plant organs, and while the pathogen attacks the younger leaves, violet lesions can be found transforming into a spindle formation with a gray center and violet to brown terminal. Brown spots only present on the older leaves and resistant cultivar plants. In susceptible younger leaves, spots coalesce and make leaves to wither, especially at the seedling as well as tillering phase [58]. In leaf blast the primary lesions are found as white to gray-green with darker borders. Older lesions appeared as white gray, encircled by a red brown end and shaped as diamonds (wide center pointing towards either end). The size of the lesion is usually 1-1.5 cm in length and 0.3-0.5 cm in width. The lesions assemble and may kill the whole leaf under favorable conditions. In case of collar rot, the lesions present at the copulation of the leaf blade and the leaf sheath and can also spoil the entire leaf [56]. Leaf blast fungus cause severe necrosis of leaves and hinder the filling of grains, resulting in reduced quantity and weight of grains. This induces partial sterility to complete when the last node is attacked [57]. Lesions on the leaves are usually spindle-shaped, width center and pointing towards either end. Generally, larger lesions form a diamond shape including a grayish center and brown border. Under favorable conditions, lesions on the infected leaves expand rapidly and tend to coalesce, resulting in complete leaf necrosis from a distance giving burnt appearance [59]. Leaf blast reduces the net photosynthetic rate of individual leaves to much greater than the visible fraction of the diseased leaves [60]. Neck blast is the most damaging stage and may occur without significant damaging of the leaves [6]. The signs are more extreme in case of neck blast marked by the infection at the base of the panicle and it starts rotting [7]. Neck blast infection produces triangular purplish lesions, expanding lesions on both sides of the neck node, symptoms that are very harmful to the development of grain. Younger nodes attacked from the neck create white panicles in color. Infected panicles appear as white and are unfulfilled in part or in whole. The whitehead symptoms can be easily confused with a stem borer attack resulting in a white and dead panic as well [12]. The neck blast infects the panicle causing seed failure to fill, or causing the whole panicle to fall over as it rots. The lesions are often grayish brown discoloration of panicle branches which may break at the lesions over time. Neck blast infection results in development of triangular purplish lesions accompanied by expanding on either side of the neck. The panicles become white when young

Table 1. Per cent yield loss of rice production in different countries caused by rice blast disease

Country	Yield loss	References
Japan	1-100%	[39]
Japan (some areas)	20-100%	[42]
China	70%	[39], [41]
Indonesia	21-37%	[41]
Bangladesh	30-100%	[38], [40]
South America and other Southeast Asian countries	30-50%	[38], [40]
Malaysia	50-70%	[41]
West Africa	3-77%	[44]
India (Tamil Nadu)	76%	[52]

necks are infected and later infection caused incomplete grain filling and poor grain quality [58].

4. BIOLOGY OF RICE BLAST PATHOGEN

M. grisea's mycelium consists of septate, uninucleate, branched hyphae. Moreover, the hypha becomes brown as the fungus gets older. In general, pathogen development is comparatively stronger on the upper surface making the spot darker on the upper side. Conidiophores are relatively darker in plain, seventh, basal portion [36]. At the apex of the conidiophores, *M. grisea* formed their conidia, which are normally three cells and found plentifully in lesions [61]. The conidial cells are produced for several hours following exposure to extend relative humidity, and exposed under gusty conditions. After the emergence of the lesion, the average production rate of conidia is 3 to 8 days and its each day production usually peaks between late night and 25 days [12]. The conidial cells are existent in the air of tropics during the whole year [62]. Under favorable conditions, the conidia sporulate in lesions of susceptible rice varieties and an individual leaf lesion with many conidiophores may discharge about 20 thousands conidia every night up to next 20 days [63]. Throughout daytime, conidia are stuck and no sporulation occurs less than 89 percent of RH. Optimum temperature requires for sporulation ranging 25-29°C and rises with a relative humidity over 92 percent [12]. New lesions of blast emerge at optimum temperatures within 4 to 5 days [62]. For infection, moisture is needed on the surface of the leaf. Optimum temperature requires 25-29°C for conidia germination and 16-24°C for appressorium formation [12]. Prolonged wetness of the leaves, night temperature about 20°C and the use of high nitrogen fertilizers favor the growth of rice

blast fungus [62]. Within 21 days, fungus produces the sexual fruiting bodies called perithecia. Ascospores are produced in asci which are found in specialized formation, perithecia. The fungus is a heterothallic carrying bipolar mating system, consists of two types of mating (MAT1-1 and MAT1-2) and sexual reproduction occurs between those two opposite mating types [9,64]. However, sexual reproduction is absent or rarely found in nature; some isolates from several grass hosts (*Eleusine* spp.) have been recorded as fertile strains [65]. Almost all the blast isolates found in the rice field are males and therefore they cannot cross with each other, however many grass isolates are hermaphrodites [12]. There is a high possibility among the isolates can cross and produce fertile strains from rice, and from other grasses. Such fertile strains enhance *M. grisea* genotypical variability where progeny can have new capacity to infect various rice cultivars [66].

5. DISEASE CYCLE OF RICE BLAST

Rice blast fungus can infect all above portions of rice plants including leaves, stems, neck, nodes, and panicles at all stages of growth and development due to its polycyclic nature. When airborne conidia land on rice plants, these adhere to the surface through the sticky mucilage produced during hydration from an apex compartment of conidium tip [62,67,68]. Conidia germinate when enough humidity is present on the host plant surfaces. Germ tube emerges from conidium's tapering end and grows over the plant surface. The germ tube is swollen and enlarged and to form an appressorium after sometime. Fungus requires hard surface to form appressoria structure [62,64,68]. Appressoria contains chitin and melanin like molecules in host cell wall and the presence of glycerol enhances turgor pressure to allow penetration peg

produced from the appressoria to enter the cuticle and cell wall of rice plant [62],69,70]. The appressoria passes via stomata into the rice plant. The blast fungal hyphae expand into the tissues of plants and ultimately develops lesions. Plant tissue invasion and colonization is intended by the fungal hyphae which invades the plasma membrane as well as epidermal cells. Specialized feeding structures or feeding hyphae are formed during early tissue invasion to help

colonize the tissues and obtain nutrients from living plant tissues. The hyphae move into various plant cells through plasmodesmata [68]. The blast lesions are evident within 3 to 4 days of infection [62,68]. The blast fungus sporulates quickly under high humidity and releases conidia in plenty. The conidia are usually transmitted by wind or rain splash to neighboring rice plants, starting another disease cycle [12,29].

Table 2. Symptoms caused by rice blast disease

Infected plant parts	Blast symptoms	References
Leaves	Lesions gray-green, water-soaking with green border, soft-colored tanning with necrotic borders.	[54]
Leaves	On younger leaves violet lesions, spindle formation with a gray center and violet to brown terminal, on older leaves brown spots.	[58]
Leaves	Primary lesions are white to gray-green with darker borders, older lesions appeared as white gray, encircled by a red brown end and shaped as diamonds	[56]
Leaves	Lesions on the leaves are usually spindle-shaped, larger lesions form a diamond shape including a grayish center and brown border.	[59]
Neck	Neck blast marked by the infection at the base of the panicle and it starts rotting	[7]
Neck	Triangular purplish lesions, expanding lesions on both sides of the neck node, attacked younger nodes create white panicles in color. Infected panicles appear as white and are unfulfilled in part or in whole.	[12]
Neck	The lesions are often grayish brown discoloration of panicle branches, triangular purplish lesions accompanied by expanding on either side of the neck. The panicles become white when young necks are infected.	[58]

Table 3. Morphological characteristics of rice blast pathogen

Pathogen characters	Size/shape/color	References
Colony appearance	68.40 to 83.50 mm in diameter	[73]
Colony appearance	Ring shape colonies with irregular surface and soft margins, grayish black in color	[74]
Colony appearance	Average length 21.3 to 28.5 μm and average width 7.4 to 14.8 μm	[76]
Conidia	Pyriiform shape, narrowed tip, rounded base, hyaline to light olive, 18-23 x 8-10 μm	[72]
Conidia	Conidial shape are pyriiform (pear-shaped) with rounded at the bottom and narrowed on the part of pointing or blunt end.	[73]
Conidia	15-32 μm in length and 6-9 μm width	[75]
Conidia	16-25 μm x 5-8 μm (Average, 17.3 x 5.4 μm)	[77]
Conidia	Length 17.6 to 24.0 μm and width from 8.0 to 9.6 μm	[37]
Chlamydo spores	5-12 μm in diameter, thick-walled	[72]

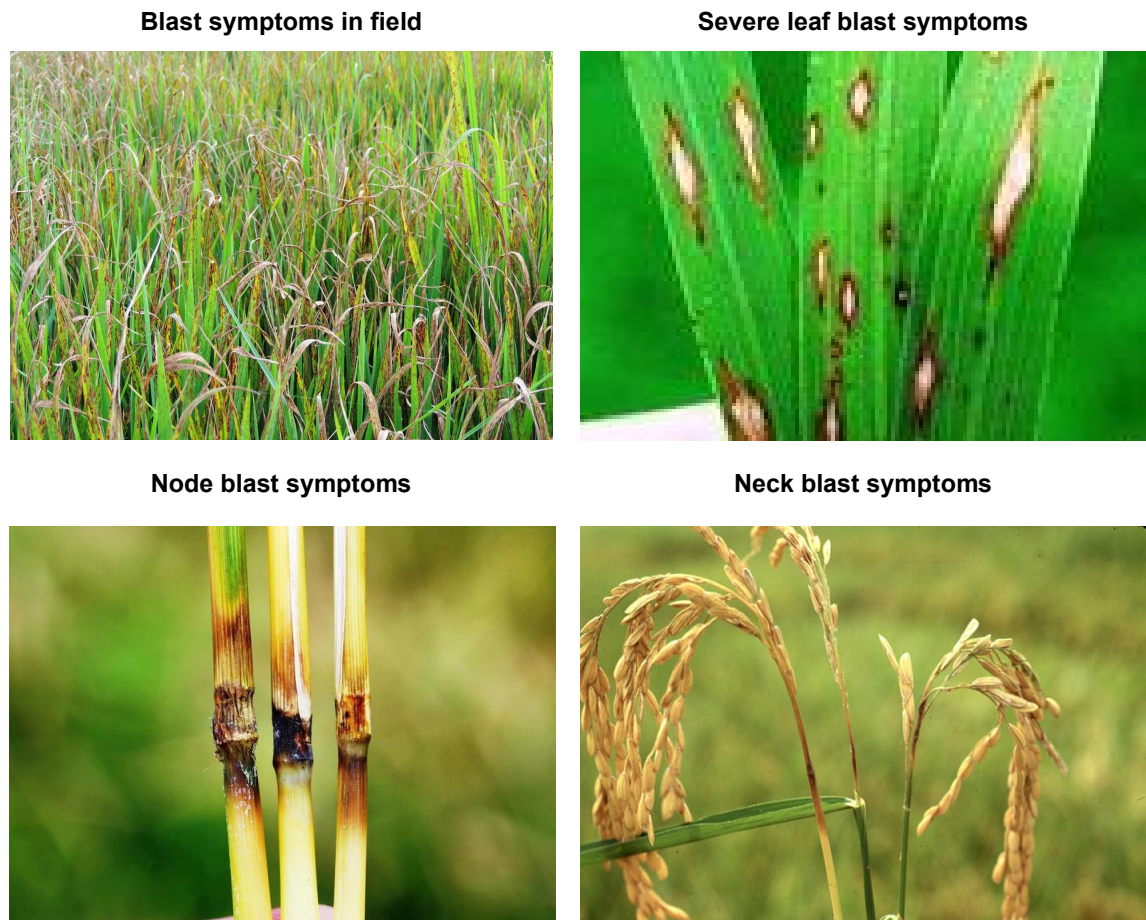


Fig. 1. Symptoms of rice blast disease

Source: Google

6. MORPHOLOGY OF RICE BLAST PATHOGEN

Magnaporthe oryzae's morphological variation collected from different rice and grass hosts disclosed that isolates showing rapid vegetative growth like as gray green or gray white developed large numbers of spores than those with lower vegetative growth (e.g., submerged growth patterns). Isolates from grass hosts showed an irregular morphology of the spore that was longer, cylindrical, and pyriform [71]. Isolates are grayish cultures, single or fascicular conidiophores, simple, rarely branched and showing sympodious growth. Conidia shaped pyriform, narrowed tip, rounded base, three septates, sometimes one or two septations, hyaline to light olive, 18-23 x 8-10 μm , with an evident protruding radical hilum at the tip of the conidiophore. Chlamydospores, mostly produced in culture, 5-12 μm in diameter, thick-walled [72].

The colony diameters of various isolates ranged from 68.40 to 83.50 mm, and the conidial shape of the various isolates was pyriform (pear-shaped) with rounded at the bottom and narrowed on the part of pointing or blunt end [73]. Blast fungi isolates developed ring shape colonies with irregular surface and smooth borders, grayish black in color [74]. The morphology of *M. grisea* spores measuring 15-32 μm in length and 6-9 μm in width. Usually 22-27 x 7-9 μm with short basal appendage, other dimensions such as basal appendage 1.3-1.9 (average 1.6 μm) μm in width, basal cell 4.7-11.6 (average 7.8 μm), middle cell 1.9-11.4 (average 6.6 μm), apical cell 7-13 (average 7 μm) in length [75]. The average isolate length ranging from 21.3 to 28.5 μm and average width ranged from 7.4 to 14.8 μm [76]. Mycelium was first colored in crops with hyaline, then modified to olive, 1.2-5.3 μm width, septate, branching. The spore sizes were 16-25 x 5-8 μm (Average 17.3 x 5.4 μm).

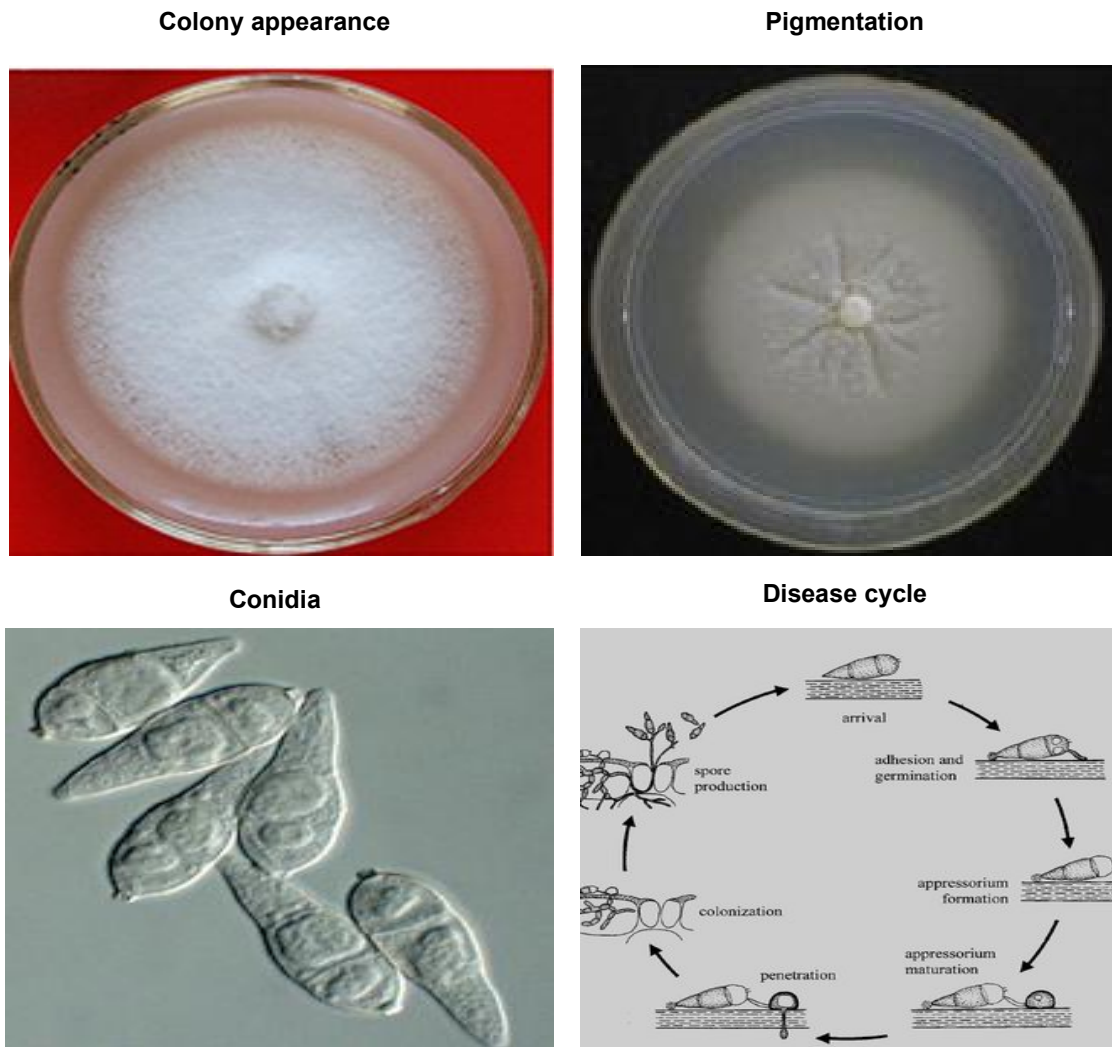


Fig. 2. Different characteristics of rice blast pathogen

Source: Google

Mostly 2 celled conidia have been found from rice grain media and 3 celled conidia have been found in infected leaf samples [77]. The dimensions of conidia formed by *M. grisea* varied in length from 17.6 to 24.0 μm and width from 8.0 to 9.6 μm [37].

7. CULTURAL CHARACTERS OF RICE BLAST PATHOGEN

Cultural characters of all *Magnaporthe oryzae* monoconidial isolates were filed by raising them at 28°C for 15 days on a PDA medium. Cultural characters include the color of the fungal mycelium and its radial development (cm). *M. grisea* spores of various isolates were collected from a culture plate mounted on a clean slide in

lactophenol. Spores were measured using a precalibrated ocular micrometer under high power objective (40x). The average spore size was then determined, and the spores shape was recorded. Snapshots were taken to demonstrate the pathogen's characteristic spore morphology [74]. Blast cultures on PDA media supported the maximum mycelial growth followed after 7 days of incubation by Richard's Agar medium. Sporulation of *M. grisea* was recorded in PDA medium and Richard's Agar medium after 15 days of incubation. Moreover, the Czapek-Dox medium was found to be ineffective for both vegetative growth as well as test pathogen sporulation [78]. PDA culture medium could provide the best vegetative growth medium for *M. grisea*, whatever the light situation.

Nevertheless, *M. grisea* could sporulate either continuously or at intervals when light was supplied. A combination of 16/8 hr light/darkness cycles and the addition of rice materials to cultivated media may lead to better sporulation of *M. grisea* [21]. The colony of all rice blast isolates was generally buff with excellent growth on OMA (Oat meal agar), grayish black along medium growth on seed-host extract+2 percent sucrose agar, increased mycelial growth together smooth colony border on PDA and raised mycelium with concentric ring pattern on Richard's agar medium [79]. Culture of various isolates of the colonies of *M. grisea* present as white on OMA, rice polish medium and malt extract agar, gray on PDA medium and white gray on rice agar medium [74]. *M. grisea* isolated from samples of the infected rice leaves, necks and nodes, were allowed to grow on oat meal agar (OMA) with biotin and thiamine (B & T). Cultures were then purified by dilution process and single spores were allowed to grow and multiplied at 25⁰ C on OMA + B & T [80]. Highest colony diameter of *M. grisea* from rice isolates were found on malt extract agar and Leonin agar [20]. Among different non-synthetic media, PDA medium supported maximum radial growth (86.00 mm), next was seed-host extract + 2 per cent sucrose agar medium (80.36 mm) followed by oat meal agar (76.00 mm) [77]. Higher sporulation on the culture medium of wheat meals was observed in alternating light-dark regime [81].

8. MOLECULAR CHARACTERIZATION OF RICE BLAST PATHOGEN

8.1 Genomic DNA Extraction

Total Genomic DNA of *M. grisea* from the mycelial mats are extracted with modified CTAB system [82]. Gel electrophoresis and Nanodrop spectrophotometer were used to measure the concentration of fungal genomic DNA. Mortar and pestle were used to macerate about 100 mg of the dried mycelial mats. The contents were transferred to microfuge tubes and vortexed for 2 mins and incubated for half an hour at 65⁰ C, followed by addition of a solution of 1.7 M potassium acetate [83]. After incubation, the upper aqueous lift was then transferred to a new microfuge tube and re-extracted with an equal volume of chloroform and isoamyl (24:1) and, after adding 1/10th volume of 3 M sodium acetate, precipitated with chilled ethanol at 10 thousand rpm for 10 minutes. Upper aqueous phase (300 µl) was mixed with 5 M NaCl 0.5 volume and 2 volume of ice-cool isopropanol,

and overnight incubated at a temperature -20°C. The content was centrifuged at a temperature 4°C for 10 minutes, the DNA pellet was air dried and dissolved in Tris-EDTA buffer 50 µl and stored at a temperature of -70°C with adjusting pH 8.5. The genomic DNA was examined in 0.8 per cent agarose gel electrophoresis and the sample DNA concentrations were calculated using Nanodrop Fluoro spectrometer [83].

8.2 PCR Amplification

PCR amplification of ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') region [84] and Pot2 transposon region of *M. grisea* using pfh2a (5'-CGTCACACGTTCTTCAACC-3') and pfh2b (5'-CGTTTCACGCTTCTCCG-3') [85] as forward and reverse primers, respectively were performed. PCR reaction was formed in a mixture of 20 µl containing ~50 ng of total DNA, 10 µl of Takara master mix (2X concentration) and 20 pmol of forward primers and reverse primers each. The reaction was performed in thermocycler (master cycle in Eppendorf) [85]. The PCR products were analyzed in 1.5 per cent agarose gels together with 50 bp DNA ladder. Using gel documentation system the gels were exposed under UV after electrophoretic run. The desired size band was cut using sterile scalped blade, and the elution of DNA was performed as per standard protocol. The PCR system for region amplification comprised of a primary denaturation of 4 minutes at temperature 94°C followed by 40 cycles of 2 minutes of denaturation at temperature 94°C, 45 s of annealing at 53°C, 2 min of extension at 72°C and last extension at 72°C for 10 minutes. For Pot 2 transposon, the PCR system comprised of a primary denaturation of 2 minutes at 94°C followed by 30 cycles of denaturation of 45 s at 94°C, annealing of 45 s at 55⁰ C and extension of 45 s at 72°C. The last extension had been done at 72°C for 10 min. In 1.2% agarose containing ethidium bromide at 80 V for 1 hr, amplified products were isolated by gel electrophoresis and recorded in a documentation unit [83].

8.3 Random Amplified Polymorphic DNA (RAPD) Analysis

Magnaporthe grisea isolates were tested to estimate their genetic variability by RAPD analysis using different random primers. Few primers produced easily scorable and consistent banding patterns. The generated fingerprints

were evaluated for overall clearness of the banding pattern. The primers showed polymorphism and consistently produced 2 to 7 bands of 0.3kb to 2kb although majority was below 1kb. All primers were polymorphic and having polymorphism information content (PIC) values ranging from 0.54 to 0.90 [74]. Dendrogram constructed based on Jaccard's similarity coefficient using the marker data from *Magnaporthe grisea* isolates with UPGMA analysis separated into two major groups A and B at 0.53 of similarity coefficient. Maximum similarity among ten blast fungus isolates based on RAPD profile were found in between Blast H5 and Blast H13 (80% of similarity) followed by in between Blast B6 and Blast B4 (75% of similarity) whereas minimum similarity were found in between Blast H3 and Blast H1 (35% of similarity) followed by in between Blast H3 and Blast B6 (43% of similarity) [74].

8.4 Mapping Survey Using Micro-satellite Markers (SSR)

A subset of 57 SSR primers from a total of 400 polymorphic primer pairs was surveyed on 220 F₂ individuals to establish their segregation pattern. Among them, 52 SSR primer pairs produced unambiguous amplified products. Maximum number of markers were scored on chromosome 7 (10 primers) followed by chromosome 1 (9 primers) [86]. Multiple copy fragments were observed in some of the primers. Only a single polymorphic fragment was scored among the multiple fragments. Out of 52 marker loci, 44 (84.61%) fit into the expected segregation ratio of 1:2:1 based on χ^2 -test at 0.05% probability value. Out of 8 markers which deviated from the Mendelian segregation ratio, 5 (9.61%) exhibited segregation distortion towards Moroberekan and 3 (5.77%) skewed towards White Ponni. The overall allele frequency for the 44 loci showed an overabundance of heterozygotic alleles [86].

9. PATHOGENICITY

Sasaki first reported the presence of strains of *M. grisea* with differential pathogenicity [87]. Inoculation of *M. grisea* on rice leaves the variable pathogenicity was observed. Symptoms appear on the leaves first as pinhole spots and later extend, elongated necrotic spots to narrow or slightly elliptical lesions longer than 3 mm with a brown margin surrounded by ash colored dead surfaces [87]. In pots two to three seeds have

been sown. *M. oryzae*'s spore suspension (5×10^4 conidia per ml) was combined with Tween 20 (0.02 percent) and sprayed on the rice seedlings, two weeks old, using a hand sprayer before runoff. For one week, the inoculated plants were incubated at temperature 25°C and 90 per cent relative humidity in the growth chamber. Diseased leaves were collected and the pathogens were re-isolated, purified and stored on PDA plates at 5°C [69,88]. *Magnaporthe grisea* isolated from two different weed hosts namely *Digitaria ciliaris* and *Digitaria marginata*, thus pathogenicity was verified to rice plants on cross inoculation. The pathotype of weed hosts was found to be similar to the pathotype (IC-12) which infects rice plants by inoculating blast differentials [89]. Pathogenicity test showed that after 7th days of second inoculation the usual symptoms of the disease formed on the leaves and increased by up to 60 days. Initially, the symptoms appeared as white to grayish specks between 7 to 12 days after first inoculation along the leaf margins. Later they were elliptical spots that were elongated and almost diamond-shaped at pointing ends. Some lesions with brown to reddish-brown borders in the middle are necrotic. Few spots collapse, forming large lesions within 20-25 days of inoculation. The fungus developed elongated, grayish to black lesions on the plant's neck, causing the plants to split at the point of the neck within 25 days of second inoculation. Disease also appeared as a brown diamond shaped lesions on rice seeds [90]. Virulence diversity assay may disclose the existence of undetected races on a various set of cultivars. Nonetheless, the Japanese differentials used in this analysis for *M. grisea* isolates were found to be effective because the reactions were either heavy susceptible (type 4 lesions) or fully resistant (type 0 lesions) [57].

10. RECOMMENDATIONS

Remarkable progress should be undertaken in identifying and tagging of blast resistance genes with different molecular markers. Almost all the genes identified to date are resistant for leaf blast. However, neck blast is highly significant. Therefore, attempts should be taken to identify and tag genes for resistance against neck blast. Breeding for neck blast resistance will then become feasible. Biological control should be introduced for environment friendly rice production. As female strain of blast pathogen remains near to rice field in weed/grass, proper weeding should be ensured during rice production. Rice cultivation should be

manipulated with advance and modern cropping system. Crop rotation could be one of the main techniques for reducing the occurrence of disease. As adapted to climate change, new varieties should be released.

11. CONCLUSION

Pathogenic variation between strains plays an important role in dynamic of rice blast disease and thus in the success of integrated disease control, particularly for breeding of resistant rice varieties. Nonetheless, the results based on the current study, the cause of rice blast disease should be considered both characterization and pathogenic variation of *M. grisea* strains when screening the rice germplasm against *M. grisea*. Genetic variability among *M. grisea* pathotypes should be taken into consideration when using *M. grisea* for blast resistance screening of rice genotypes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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