# MORPHOLOGICAL VARIABILITY IN RICE BLAST PATHOGEN *PYRICULARIA ORYZAE* (COOKE) SACC.

**A THESIS**

**BY**

**PAPRY AKTHER**

**STUDENT ID: MS-190805**



**A Thesis (Course No. PLP-6104) Submitted to the Agrotechnology Discipline in Partial Fulfillment of the Requirements for the Degree of Master of Science in Plant Pathology**



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# Morphological variability in rice blast pathogen *Pyricularia oryzae* (cooke) sacc.

**By**

**PAPRY AKTHER**

**STUDENT ID: MS-190805**

**Approved as to Style and Contents**

**By**

Sabiha

**Prof. Dr. Mst. Sabiha Sultana**
**(Supervisor)**

**Prof. Md. Rejaul Islam**
**(Co-Supervisor)**

**(Prof. Dr. Sarder Safiqul Islam)**
**Chairman, Examination Committee**
**And Head, Agrotechnology Discipline**

**MARCH 2022**

**DEDICATED**

**TO**

**MY BELOVED PARENTS**

## ABSTRACT

Rice is a staple food crop for majority of human population worldwide. Significant grain yield losses are reported due to blast disease caused by *Pyricularia oryzae* across all rice growing areas of the world. Though, presently available blast management strategies reduce disease significantly but blast epidemics are still common, thereby causing devastating yield losses. The present study was aimed at determining the variability among the *Pyricularia oryzae* isolates. Leaf blast samples were collected from South-Western regions of Bangladesh the causal pathogen was isolated following tissue planting method in the Plant Pathology Laboratory of Agrotechnology Discipline, Khulna University, and Khulna, during September 2020 to September 2021. Morphological variability was determined based on nine morphological components like as front mycelial color, reverse mycelial color, growth behavior, length of conidia (µm), width of conidia (µm), conidial color, radial growth at 12 days (mm) after incuation, radial growth at 14 days (mm) after incuation and radial growth at 16 days (mm) after incuation. Seven types of mycelial colors were recorded. These were- Light brown, Deep brown, Light gray, Olivaceous grey, Grey, Deep grey and Black. Two types of growth behavior- Smooth and Rough were present in *P. oryzae* isolates. Two types of conidial color- Blackish and Transparent were also observed among the 19 isolates. Cluster analysis considering nine morphological components showed five distinct groups. Three isolates were placed in each cluster I and III, cluster II contains seven isolates, cluster IV contained five isolates and cluster V contained only one isolate (PO-11). PO-11 showed distinct morphological variations among the 19 isolates, which was originated from AEZ-11. PCA biplot demonstrated a high range of variation and showed with high negative correlation between two groups of variable. One consisted of conidial length, conidial breadth and growth behavior and another consists of reverse mycelial colony color, front mycelial colony color, conidial color, radial growth at 12 days, 14 days and 16 days after incubation. Among the nine morphological components, front and reverse mycelial color, growth behavior and conidial length played more than 80% role in generating variation among the nine tested components.

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**The Author**

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# CHAPTER I

## INTRODUCTION

Rice (*Oryza sativa*) is the most important crop and the main source of food grain for more than half of the world population, especially in South Asia, Southeast Asia and Latin America. In these regions, it represents a high-value commodity crop. Rice is the source of subsistence for more than one third of human population. It is the main staple food in the Asia and the Pacific region, providing 21% of global human per capita energy and 15% per capita protein (Akter et al., 2019). Rice is cultivated in 114 countries across the world. The plant is native to Tropical and Subtropical Southern Asia and Southeastern Africa. More than 90% of the world's rice is grown and consumed in Asia (Kole, 2006).

Rice is the staple food of Bangladeshi people and it constituted about 85% of the total food grain production. It covers about 76.86% of the total cultivable land in the country. The yield of rice in Bangladesh is 3.53t/ha (BBS, 2020).

The average per hectare production of rice in Bangladesh is extremely low as compared to other rice growing countries of the world due to drought, lack of resistant/ tolerance against diseases and pests, inadequate plant population and low nutrients status of soils, natural disaster, flood, wrong treatment etc. (Linscombe, 2006).

Rice is known to be attacked by many pests and diseases which cause huge annual losses worldwide. Among fungal diseases, rice blast caused by *P. oryzae* is of significant economic importance. Outbreaks of rice blast is a serious and recurrent problem in all rice growing regions of the world. About 28-36% average loss due to blast has been reported and in certain areas yield losses could be as high as 80-100% (Hossain et al., 2017).

The incidence of rice blast was recorded on Boro season and Transplanted Aman all over Bangladesh. BRRI dhan28, BRRI dhan29, BRRI dhan50, BRRI dhan61 and BRRI dhan63 are marked as blast susceptible varieties. The disease has the potential to destroy 70% of crop yields in worst-case scenarios, and they cause substantial losses for farmers in Bangladesh (Rayhanul et al., 2019).

Rice blast fungus can attack the rice plant at any growth stage and can cause severe leaf necrosis and impede grain filling, resulting in decreased grain number and weight. Rice blast pathogen infect all the above ground parts of rice plants at different growth stages, i.e., leaf,

collar, nodes, internodes, base or neck and other parts like panicle and leaf sheath (Ram et al., 2007). The drizzle brings down the spores (seeds of fungi able to grow as new fungi) on the leaf, node or on panicles. As sooner as a spore drops on the rice plant it starts growing to produce conidiophores (a fungal hypha) and conidia (asexually produced spore) within 3-4 days (Rahman and Uddin, 2017).

Morphology of *Pyricularia oryzae* has determined based on colony diameter, color and shape of conidia under in vitro conditions. Several studies on differential response of *Pyricularia oryzae* isolates obtained from rice, finger millet on different media were reported. A high morphological variability among isolates of *P. oryzae* has been reported from Telangana and Andhra Pradesh during kharif (Yashaswini et al., 2017). Detailed studies of morphological structure of blast pathogen are used to examine taxonomy of the pathogen and epidemiological aspects of disease spread, which may be useful in developing resistant cultivars in breeding programs (Mahto et al., 2012).

Frequent and prolonged dew periods in the morning along with lower night temperature than day time is the most favorable for blast inoculation (Castilla et al., 2009). Continuous rains and average night temperatures between 18-25°C during the flowering stage of the crop followed by sunny, hot and humid days are the favorable climatic condition of this disease. The prolonged duration of the wetting period with 30°C occurs highest blast intensity (Chumley and Valent, 1989). Temperature, moisture, sunshine, humidity and wind speed play a major role in the infection and development of the disease (Couch and Kohn, 2002).

Morphological variations are one of the criteria to understand the nature of virulence *P. oryzae* (Couch and Kohn, 2002). Analysis of morphological characteristics are very much important to maintain *P. oryzae* in laboratory conditions to sustain its viability, to get high sporulation and to maintain the pathogen for further experiments. This is ultimately required for carrying out investigations on assessment of pathogen virulence and genetic variation (Khadka et al., 2012).

Morphological variability of *Pyricularia oryzae* can help in developing disease management strategies of rice blast. Morphological variability of the rice blast pathogen has not been studied in the southwestern region of Bangladesh.

**Objectives**

With the above facts the present piece of research work was under taken to fulfill the following objectives:

i. To isolate and identify the rice blast pathogen from the AEZ of south western region of Bangladesh

ii. To determine the morphological variability among the isolates of *Pyricularia oryzae*

# CHAPTER II

# REVIEW LITERATURE

## 2.1. Rice blast disease

Rice blast, caused by *Pyricularia oryzae*, was first found in California in 1996. This fungus has invaded all rice growing areas. Virulent genotypes regularly emerge, causing rapid resistance breakdowns. However, the world-wide genetic subdivision of *Pyricularia oryzae* populations on rice and its past history of invasion have never been elucidated (Bevitori and Ghini, 2015).

The incidence of rice blast was recorded on Boro season and Transplanted Aman in all over country. The government of Bangladesh is taking initiative to the extension of the Aus coverage. It means rice is grown with intensive care throughout the year. Thus the popular varieties are getting susceptible to some pest and diseases. Even the pests are getting the opportunities to change their races for better adaptation (Biswas, 2017).

## 2.2. *Pyricularia oryzae* the causal organism of rice blast

The fungus *Pyricularia oryzae* is the causal agent of rice blast disease. The perfect stage of *Pyricularia oryzae* was earlier named as *Ceratosphaeria grisea (*Hebert, 1971). Later Yaegashi and Nishihara (1976) suggested the genus *Magnaporthe.* Yaegashi and Udagawa (1978) finally proposed *M. grisea* as a perfect stage of *Pyricularia oryzae* instead of *Ceratosphaeria grisea*. *M. grisea* is an ascomycete fungus. It is an extremely effective plant pathogen as it can reproduce both sexually and asexually to produce specialized infectious structures known as appressoria that infect aerial tissues and hyphae that can infect root tissues (Hebert, 1971).

Outbreaks of rice blast are a recurrent problem and it is extremely difficult to control in all rice growing regions of the world including Bangladesh (Khan et al., 2014). Bangladesh has experienced several epidemic outbreak of rice blast disease since 1980 (Shahajahan et al., 1994). Khan et al. (2014a) reported that most of the popular cultivar of riceincluding aromatic varieties, in both irrigated and rain feed low lands were highly susceptible to blast disease.

## 2.3. Distribution of Rice Blast disease and Host range

Environments with regular and extended dew times and mild daytime temperatures are more conducive for explosion. The disease makes yield in loss up to 1-100% in Bangladesh, India, Indonesia and other Southeast Asian countries Rice blast was recorded as severe

Source: https://ekrishi24.blogspot.com/2021/10/rice-blast-disease_14.html

Figure 1. Symptoms of rice blast

**2.5. Significance of blast disease of rice**

The fungus *Pyricularia oryzae* attacks at all stages of the crop and symptoms appear on leaves and nodes (Shahjahan, 1994). The pathogen may infect all the above ground parts of a rice plant at different growth stages: leaf, collar, node, internode, base, or neck, and other parts of the panicle, and sometimes the leaf sheath. The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and its rotting (Bonman et al., 1989). Recent reports have shown that the fungus has the capacity to infect plant roots also (Dean, 1997; Hamer et al., 1988).

Leaf blast lesions reduce the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction. Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu et al., 2005).

The neck blast infects the panicle causing failure of the seeds to fill or causing the entire panicle to fall over as it is rotted. Infection of the necks can be very destructive and directly reduces the economic value of the product (Zhu et al., 2005; Srinivasprasad et al., 2011).

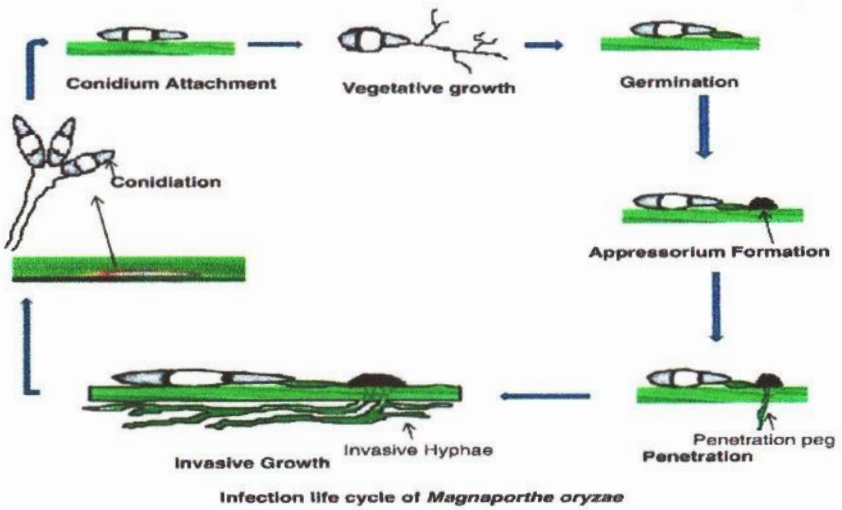
When young necks are infected, the panicles become white in colour and later infection caused incomplete grain filling and poor grain quality (Hajimo, 2001).

## 2.6. 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 from an apex compartment of conidium tip (Chumley and Valent, 1989).

Conidia germinate when enough humidity is present on the host plant surfaces(Fig.2). Germ tube emerges from conidium's tapering end and grows over the plant surface. The germ tube is swollened and enlarged and to form an appressorium after sometimes. Fungus requires hard surface to form appressoria structure (Hamer et al., 1988).

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. The appressoria passes via stomata into the rice plant (Chumley and Valent, 1989).




Source: https://link.springer.com/chapter/10.1007/978-981-13-6043-5_5

Figure 2. Disease cycle of ice blast (*Pyricularia oryzae)*

The fungal hypha expands 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. The blast lesions are evident within 3 to 4 days of infection (Hamer et al., 1988).

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 (Hamer et al., 1988).

### 2.7. Yield loss of rice due to blast disease

*Pyricularia oryzae* causes rice blast disease in rice cultivation areas worldwide. Annual rice losses caused by this fungus during 90's had been estimated at 35% of the worldwide production (Oerke and Dehne, 2004).

The disease causes yield losses from between 1- 100% in Japan, 70% in China, 21-37% in Bali Indonesia (Suprapta and Khalimi, 2012), and 30-50% in South America and Southeast Asia (Baker et al., 1997).

Blast disease caused by *Pyricularia oryzae* upsets production statistics of rice in Pakistan (Jia et al., 2000). In Pakistan during the last two decades, rice blast is mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and place like Gaggoo Mandi (Arshad et al., 2008).

In West Africa, the largest area of African production, this pathogen is the main constraint to production with yield losses ranging from 3-77% (Nutsugah et al., 2008).

Heavy yield losses have been reported in many rice growing countries. For example 75, 50 and 40 percent grain loss may occur in India (Padmanabhan, 1965), Philippines (Ou, 1985) and Nigeria (Awodera and Esuruoso, 1975).

### 2.8. Morphology of *Pyricularia oryzae*

*Pyricularia 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 are grayish cultures, single or fascicular conidiophores, simple, rarely branched and showing sympodious growth (Hawksworth, 1990).

The colony diameters of various isolates ranged from 68.40 to 83.50 mm. Blast fungi isolates developed ring shape colonies with irregular surface and smooth borders, grayish black

in color (Gashaw et al.,2014). Cultures are grayish in color, conidiophores single or in fascicles, simple or rarely branched, show sympodial growth (Hawksworth, 1990).

Conidia are pyriform in shape and hyaline in color, produced acrogenously, one after another. Conidia are three celled, the middle cell being much wider and darker, and end cell germinates giving out germ tube. Conidia are rarely two celled or four celled. Conidia formed singly at the tip of the conidiophores at points arising sympodially and in succession, pyriform to obclavate, narrowed toward tip, rounded at the base, three celled rarely one or two celled, hyaline to pale olive, 19-23 × 7-9 µm, with a distinct protruding basal hilum (Hawksworth, 1990).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 (Mijan, 2000).

The mycelium consists of septet, uninucleate, branched hypha. However, as the fungus gets older, the hypha become brown. Generally, growth of the pathogen is relatively more on upper surface making the spot more dark on upper side. Conidiophores are simple, septate, basal portion being relatively darker (Hawksworth, 1990).

Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown. Chlamydospores often produced in culture, thick-walled, 5-12 µm diameter. Fungus produce sexual fruiting bodies called perithecia within 21 days. Perithecia are flask-shaped that carry asci containing ascospores, the products of meiosis. Ascospores are arranged as unordered octads or as larger populations of randomly selected ascospores (Nicholas and Talbot, 2003).

### 2.9. Morphological variability of *Pyricularia oryzae*

Vanaraj et al. (2013) cultured different isolates of *Pyricularia oryzae* on oat meal, rice extract agar, maize extract agar and grass extract agar. Spore induction was hastened on maize stem pieces than on rice and. Spore density on maize was 3420/ml of water as against 500/ml on grass. When spores of 11 isolates of *P. oryzae* were compared, conidia of the isolate from grass.

Onofeghara et al. (1973) found that colony characters developed by each isolate are not dependent on the medium on which it is growing—a pointer to the fact that such characters may be genetically controlled. Appresoria produced *in vivo* were considerably larger than those produced *in vitro*. On the basis of appresorial types, the isolates were found to fall into two physiological races—smooth-walled and rough-walled.

# CHAPTER III

## MATERIALS AND METHODS

The research work was carried out to determine the morphological variation among the isolates of *P. oryzae*. The materials used and methodologies followed in this experiment are discussed below.

### 3.1 Experimental site and period

The experiment was conducted in the Plant Pathology laboratory of Agrotechnology Discipline, Khulna University, Khulna during the period from September 2020- September 2021.

### 3.2 Collection of diseased rice leaf sample

For analysis of variability in aspect of morphology of *Pyricularia oryzae*, typical leaf blast diseased leaves/neck of rice were collected from South-Western rice growing regions of Bangladesh during March, 2020 to September, 2020 (Table 1 and Fig 3).

Figure 3. Collected diseased samples

Table 1. Blast disease leaf sample collection site with designation and pathogenic reaction

| AEZ No. | Zones | Location | No of isolates | Isolate Designation | Pathogenic reaction |
|---|---|---|---|---|---|
| AEZ-11 | High Ganges River Floodplain | Bagherpara-Jeshore, Sharsha-Jeshore | 2 | PO-2, PO-11 | + |
| AEZ-12 | Low High Ganges River Floodplain | Lohagara-Narail, | 1 | PO-1 | + |
| AEZ-13 | Low Ganges River Floodplain | Satkhira Sadar, Dumuria-Khulna, Samnagar-Satkhira, Batiaghata-Khulna, Debatta-Khulna, Terokhada-Satkhira, Morrelganj-Khulna, Rupsha-Khulna, Tala-Satkhira, Chitalmari-Bagerhat, Dasmina-Patuakhali, Mollahat-Bagerhat, Banaripara-Barishal, Boalmari-Faridpur, Asasuni-Satkhira, Kathalia-Jhalokathi | 16 | PO-3, PO-4, PO-5, PO-6, PO-7, PO-8, PO-9, PO-10, PO-12, PO-13, PO-14, PO-15, PO-16, PO-17, PO-18, PO-19 | + |

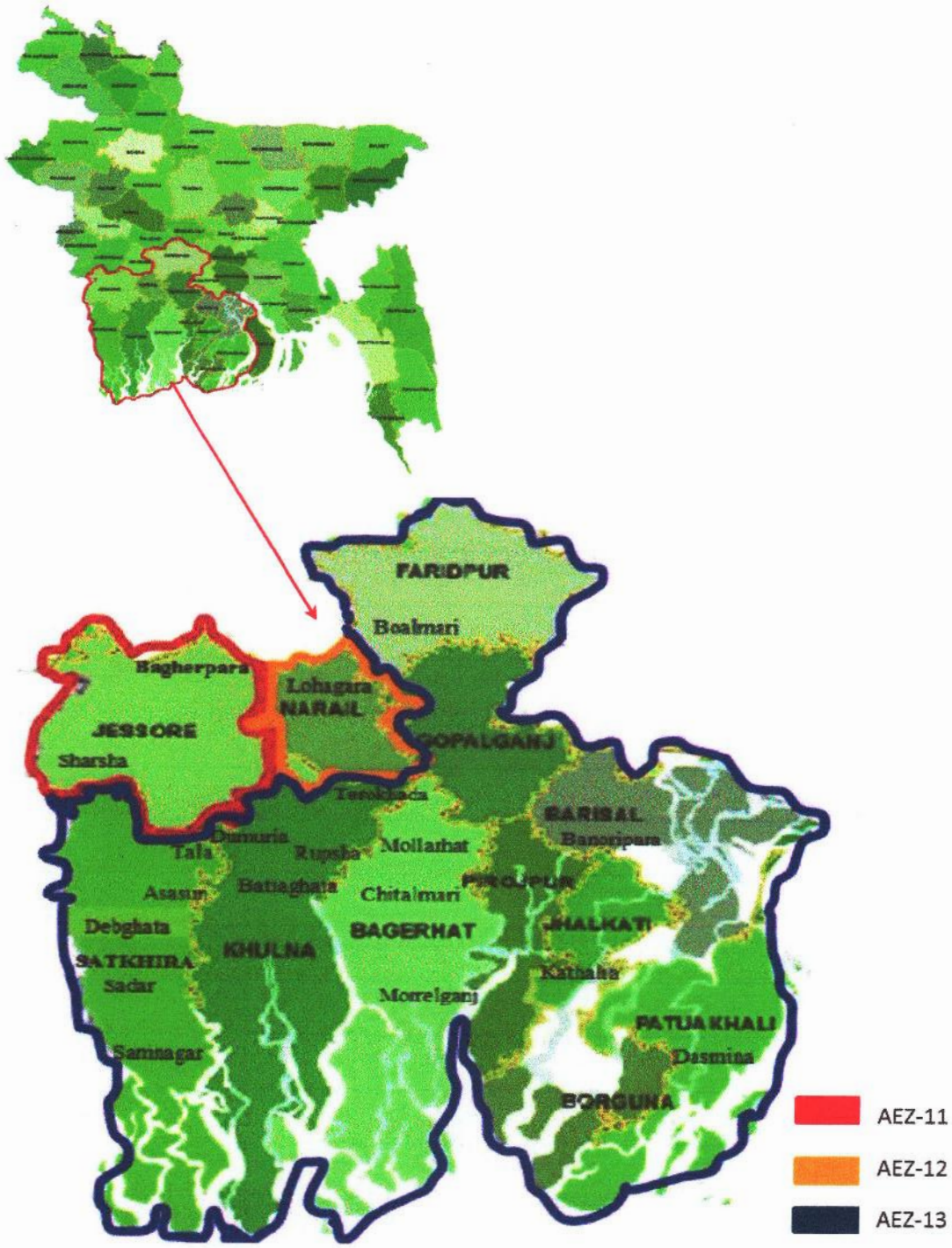



Figure 4. Map of disease sample collection area

### 3.3 Isolation Procedure of *Pyricularia oryzae*

#### 3.3.1 Preparation of Potato Sucrose Agar (PSA) Medium

At first 200g potato was cut into slice and then boiled in 1000 ml water. After boiling, it was sieved. Exactly 200ml potato extract along with 20g sucrose was mixed with 800ml of distilled water. After few minutes, 20g agar was mixed with it while microwaving the mixture and then sterilized in autoclave at 121°C temperature under 1.1 Kg/cm$^2$ pressure for about 30 minutes.

#### 3.3.2 Pathogen isolation procedure

The plant parts such as leaves, neck were used for isolation of the pathogen. Diseased samples were cut into small pieces around the infected area including the edge of the lesion (1-2cm) and subjected to surface sterilization with 1% sodium hypochlorite. Further these samples were kept inside laminar air flow on moist blotting paper to enhance sporulation for 2-3days.

After incubation, these infected plant pieces were examined under electrical microscope to confirm the typical shape of *P. oryzae* spores. Single spore was picked up with a glass needle and incubated on PSA media for about one week.

Multiplication was conducted on Laminar Air Flow. 5mm diameter mycelial discs of *Pyricularia oryzae* were taken from 7 days old pre-cultured petri dishes and inoculated to PSA (Potato Sucrose Agar) plates with 3 replications under aseptic conditions and incubated at 25°C for 16 days. Radial growths of the fungus were measured on 12$^{th}$, 14$^{th}$ and 16th days of incubation.

Brushing into the 16 days old mycelia was done to irritate mycelia and to sporulate and kept under light for 24 hours. Every time clean brushes were used to avoid any kind of contamination.

The plates were arranged in a growth chamber following complete randomized design (CRD) with three replications.

### 3.4 Collection of data

Mycelial characters were recorded through visual observation after 12 days of incubation and conidial characters were recorded under microscopic observation at 40× magnification after 16 days of incubation. Radial growth of colony was recorded using scale. Conidial length and width was measured using Zeiss version 2.0 computer program at 10µm unit scale.

To observe mycelial characters, regular observation was done after incubation and data on the parameters were recorded:

- Reverse mycelial color
- Front mycelial color
- Colony growth behavior
- Radial growth at 12,14 and 16 days after incubation (mm)

Conidial characters were recorded after 16 days of incubation. The characters were:

- Length of conidia (µm)
- Width of conidia (µm)
- Color of conidia

### 3.5 Design of experiment and data analysis

The petri dishes arranged in a growth chamber following Completely Randomized Design with three replications. Variations among the isolates based on morphological characters were analyzed following multivariate analysis: descriptive statistics, correlation matrix, Principal Component Analysis (PCA), and cluster analysis was performed following Agglomerative Clustering Method by using STAR (Statistical Tool for Agricultural Research, version-2, IRRI, Losbanos, Philippines). Cluster analysis and PCA was performed to identify accession groups and studied components of variability which may contribute in variation.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Morphological variation of *Pyricularia oryzae*

### 4.1.1 Descriptive statistics

Mean value of nine components of morphological variability such as front color, reverse color, growth behavior, length of conidia (μm), width of conidia (μm), radial growth at 12 days (mm), radial growth at 14 days (mm), radial growth at 16 days (mm) and conidial color were 3.84, 5.89, 1.05, 6.81 μm, 2.25 μm, 6.82 mm, 7.41 mm, 7.82 mm and 1.32 respectively (Table 2). From the tabulated value, mean all of the components were greater than the standard error. The data also revealed that highest coefficient of variation was observed among conidial color and lowest coefficient of variation was observed among radial growth at 12 days of mycelia. That means conidial color are more variable component among the nine tested component of *P. oryzae* isolates.

Table 2. Range, mean, standard error and coefficient of variation of different components for morphological variability among the *Pyricularia oryzae*

| Variable | Minimum | Maximum | Mean ± SE | CV (%) |
|---|---|---|---|---|
| Front color | 2.00 | 6.00 | 3.84 ±0.36 | 27.86 |
| Reverse color | 3.00 | 8.00 | 5.89 ±0.47 | 23.94 |
| Growth behaviour | 1.00 | 2.00 | 1.05 ±0.08 | 21.90 |
| Conidia length | 5.69 | 7.78 | 6.81 ±0.23 | 9.99 |
| Conidia breadth | 1.79 | 2.66 | 2.25 ±0.07 | 8.89 |
| Radial at 12 | 5.57 | 7.60 | 6.82 ±0.19 | 8.36 |
| Radial at 14 | 5.93 | 8.23 | 7.41 ±0.22 | 9.04 |
| Radial at 16 | 6.47 | 8.65 | 7.82 ±0.23 | 8.70 |
| Conidial Color | 1.00 | 2.00 | 1.32±0.16 | 36.36 |

SE – Standard error and CV – Coefficient of Variation

### 4.1.2 Different morphological groups among the isolates of *Pyricularia oryzae*

Cluster analysis was done considering nine morphological components which \showed five distinct groups Cluster I, II, III, IV and V (Figure 5). Cluster I contains three isolates, cluster II contains seven isolates, cluster III contains three, cluster IV contains five isolates and only one isolates belonging in cluster five (Table 3).

Seven types of mycelial colony colors were observed among the 19 isolates of *P. oryzae* (Figure 6). They were- a) Light brown, b) Deep brown, c) Light gray, d) Olivaceous grey, e) Grey, f) Deep grey and g) Black (Figure 6, Figure 7 and Appendix 1). *Pyricularia oryzae* were showed smooth and rough growth behavior (Figure 8 and Appendix 1). Blackish and transparent colored conidia were also observed among the 19 isolates (Figure 10 and Appendix 2).

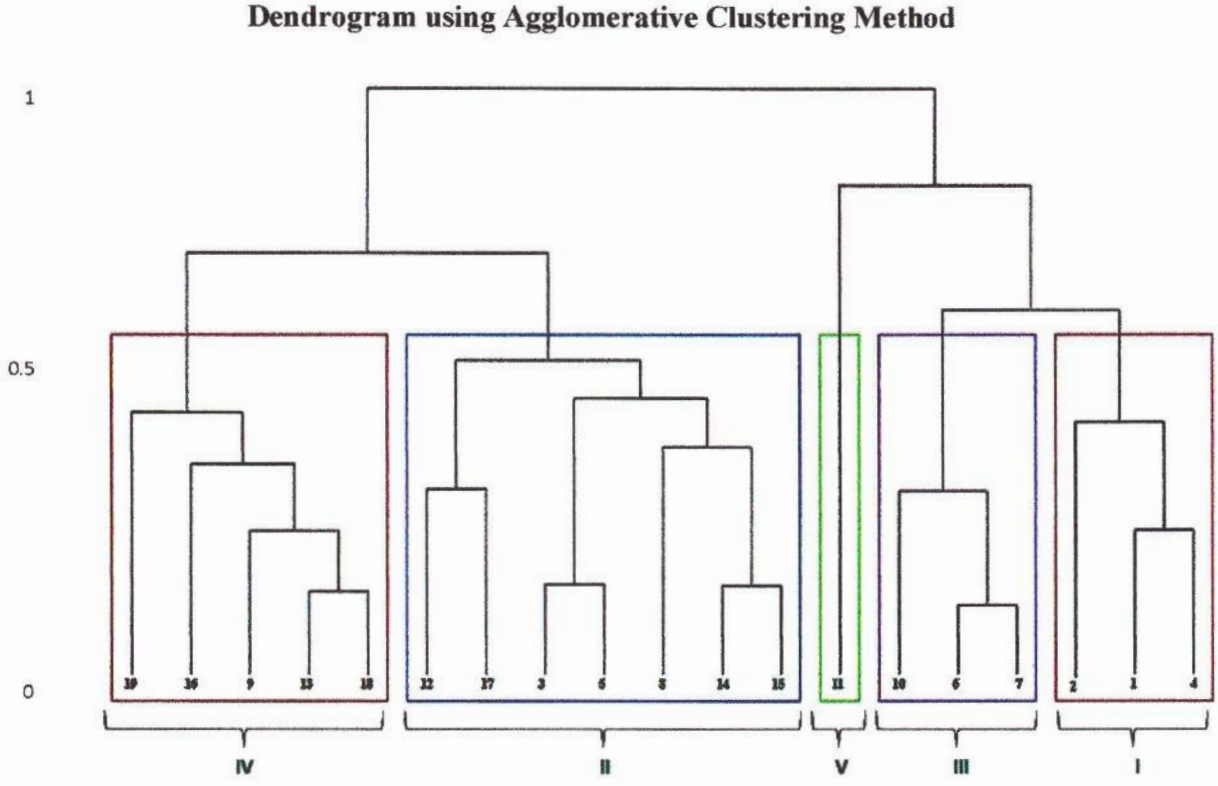



Figure 5. Dendrogram constructed with the data obtained from studied nine morphological components of *Pyricularia oryzae*

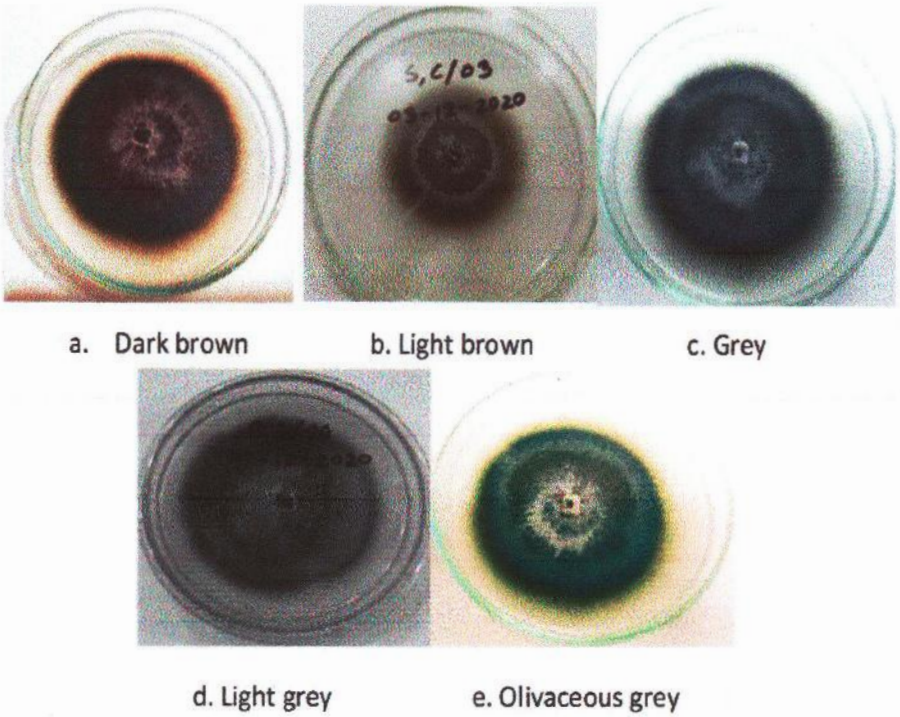



Figure 6. Front myceial color of *Pyricularia oryzae* isolates

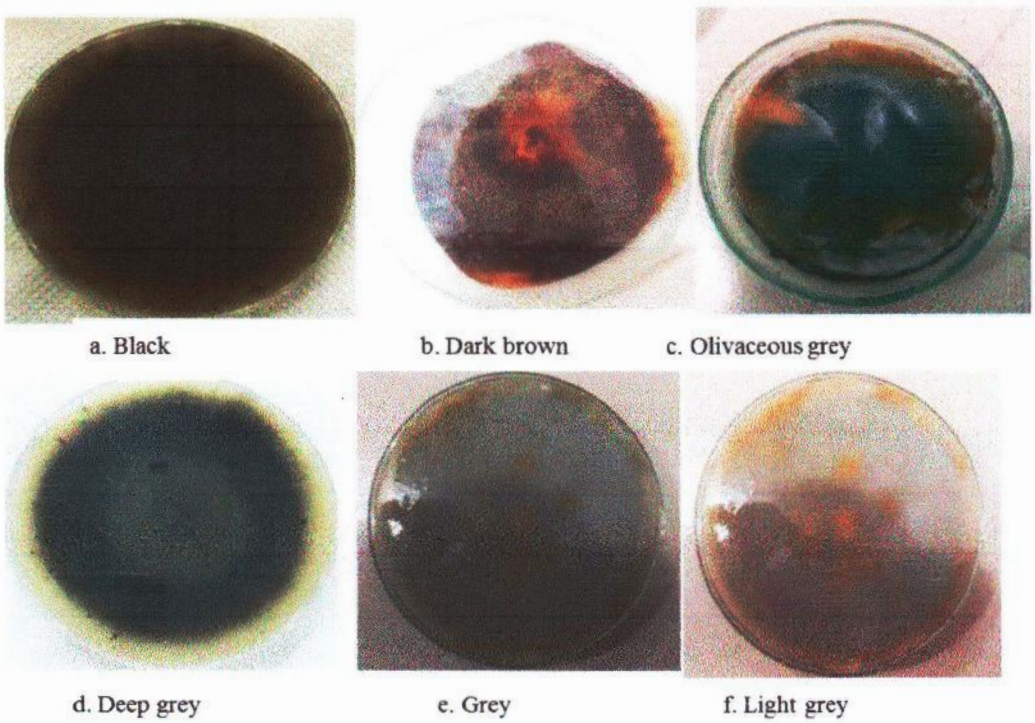
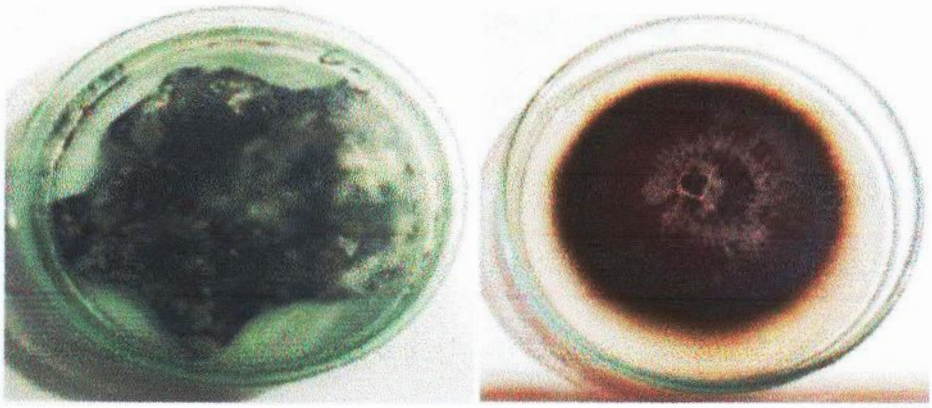



Figure 7. Reverse myceial color of *Pyricularia oryzae* isolates



a. Rough b. Smooth

Figure 8. Growth behaviour of *Pyricularia oryzae* isolates

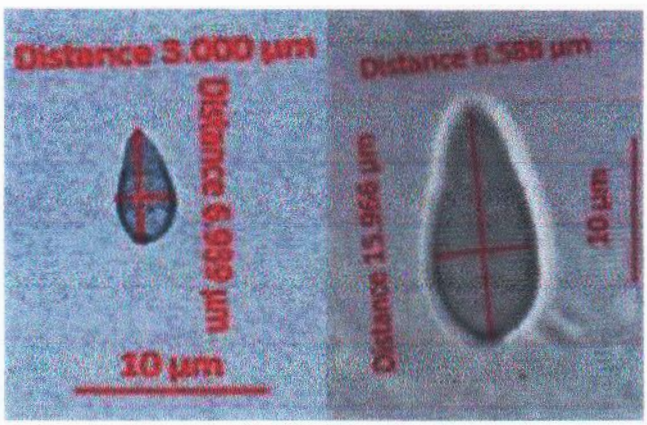
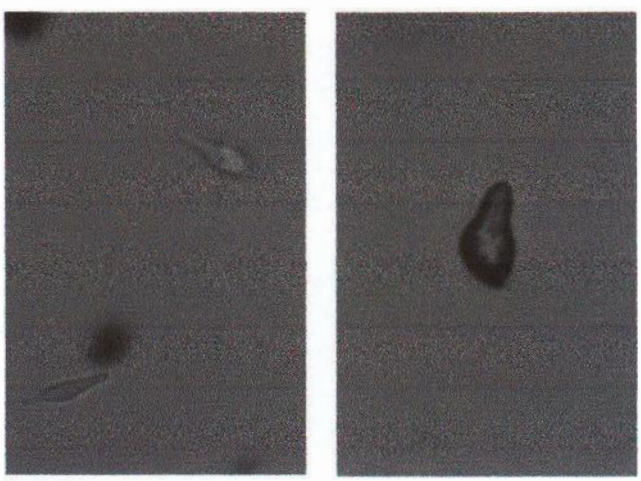





Figure 9. Shape and size of *Pyricularia oryzae* spore



a. Transparent b. Blackish

Figure 10. Spore colours of *Pyricularia oryzae*

Table 3. Grouping of nineteen isolates of under *Pyricularia oryzae* different cluster based on nine morphological components

| Cluster No. | *Pyricularia oryzae* isolates | Number of members in each cluster |
|---|---|---|
| I | PO-1, PO-2, PO-4 | 3 |
| II | PO-3, PO-5, PO-8, PO-12, PO-14, PO-15, PO-17 | 7 |
| III | PO-6, PO-7, PO-10 | 3 |
| IV | PO-9, PO-13, PO-16, PO-18, PO-19 | 5 |
| V | PO-11 | 1 |

### 4.1.3 Correlation among the morphological components

The correlation among the components of morphological variability represents the relationship among the components. Among the components some of them showed positive, some of them showed negative and some of them showed non-significant relationship between the components. Higher and positive correlation was observed between radial growth at 14 days and radial growth at 16 days. Lower and positive correlation was observed between mycelial front color and radial growth at 14 days after inoculation and also lower and positive correlation was observed between conidial length and breadth. Growth behavior and conidial color represented non-significant correlation among the all components. In case of mycelial front colony color, it showed significant and positive correlation between reverse mycelial color and radial growth at 14 days. On the other hand, reverse mycelial color and conidial breadth showed non-significant correlation among all components. Conidial length showed non-significant correlation among all components except conidial breadth (Table 4).

Table 4. Pearson correlation between the nine morphological components of *Pyricularia oryzae*

| | FC | RC | GB | CL | CB | R12 | R14 | R16 | CC |
|---|---|---|---|---|---|---|---|---|---|
| FC | | | | | | | | | |
| RC | 0.653** | | | | | | | | |
| GB | -0.418$^{NS}$ | -0.325$^{NS}$ | | | | | | | |
| CL | -0.509* | -0.171$^{NS}$ | 0.072$^{NS}$ | | | | | | |
| CB | -0.154$^{NS}$ | -0.037$^{NS}$ | 0.090$^{NS}$ | 0.469* | | | | | |
| R12 | 0.437$^{NS}$ | 0.112$^{NS}$ | -0.423$^{NS}$ | -0.183$^{NS}$ | -0.255$^{NS}$ | | | | |
| R14 | 0.469* | 0.046$^{NS}$ | -0.083$^{NS}$ | -0.306$^{NS}$ | -0.196$^{NS}$ | 0.835** | | | |
| R16 | 0.415$^{NS}$ | 0.005$^{NS}$ | -0.052$^{NS}$ | -0.260$^{NS}$ | -0.252$^{NS}$ | 0.802** | 0.9701** | | |
| CC | 0.321$^{NS}$ | 0.135$^{NS}$ | -0.160$^{NS}$ | -0.393$^{NS}$ | -0.281$^{NS}$ | 0.300$^{NS}$ | 0.350$^{NS}$ | 0.264$^{NS}$ | |

FC-Front color, RC-Reverse color, GB-Growth behavior, CL-Length of conidia (µm), CB-Breadth of conidia (µm), R12-Radial growth at 12 days (mm), R14-Radial growth at 14 days (mm), R16-Radial growth at 16 days (mm) and CC-conidial color.

"- Significance @ 0.01,*- Significance @ 0.05 and NS – Non-significance

### 4.1.4 Contribution of components in morphological variation

Principal component analysis (PCA) was done to identify the extent of contribution each of the components to observe variations percentages among the 19 isolates of *P. oryzae* (Table 5 and Figure 11). Each component is linked with an eigen value which represents the amount of variation. Mycelial color, growth behavior and conidial size accounted for 90% of the total variation. These components greatly generated variation in morphology. Contributions of components are presented in Table 5.

Table 5. Estimation of percentages of variation of eleven morphological components of *Pyricularia Oryzae*

| Variable | *Eigen* value | Variation (%) | Cumulative variation (%) |
|---|---|---|---|
| Front color | 3.71 | 41.27 | 41.27 |
| Reverse color | 1.64 | 18.27 | 59.55 |
| Growth behaviour | 1.30 | 14.39 | 73.94 |
| Conidia length | 0.85 | 9.42 | 83.36 |
| Conidia breadth | 0.71 | 7.88 | 91.24 |
| Radial at 12 | 0.50 | 5.51 | 96.75 |
| Radial at 14 | 0.17 | 1.93 | 98.68 |
| Radial at 16 | 0.10 | 1.14 | 99.82 |
| Conidial Color | 0.02 | 0.18 | 100.00 |

Extraction method: Principal Component Analysis

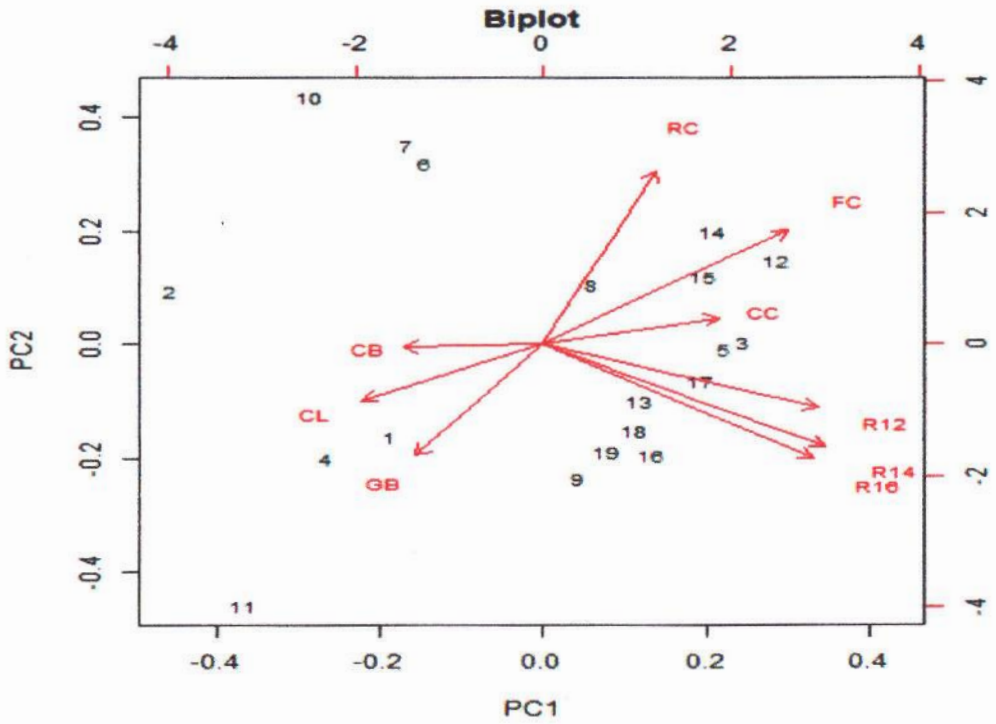



Figure 11. PCA Biplot constructed with the data obtained from studied nine morphological components of *Pyricularia oryzae*

PC1 and PC2 strongly correlated variables considering among the tested components. In addition to the observations, the biplot represented high negative correlation between two groups of variable (One consists of conidial length, conidial breadth and growth behavior and another consists of reverse mycelial colony color, front mycelial colony color, conidial color, radial growth at 12 days, radial growth at 14 days and radial growth at 16 days). The data also revealed a high range of variation between reverse mycelial colony color and radial growth at 14 days; front mycelial colony color and radial growth at 16 days respectively. PCA plot also disclosed strongly negative correlation be present between conidial color and conidial breadth.

### 4.2 Discussion

In the present experiment, multivariate analysis was performed for observing morphological variability based on nine morphological components. After 16 days of incubation on potato sucrose agar media, isolates of *P. oryzae* showed great diversity in their growth. The results of the present investigation indicate existence of variation in terms of mycelial color, texture, radial growth, conidial size, mycelial color and growth behaviour among the isolates.

Considering the cultural variability eight distinct mycelial front color and reverse color were observed like as Light brown, Deep brown, Light gray, Olivaceous grey, Grey, Deep grey and Black. Longya et al. (2020) found colony color varied from white to grey and some strains were unable to produce melanin pigment, while most showed a range of melanin production capabilities. Studies on the morphological characteristics of different rice blast strains in Thailand showed differences in mycelial color, morphology and conidial shape.

In Pearson correlation, it was observed that among nine morphological components some of them showed positive, some of them showed negative and some of them showed insignificant relationship between the components. Higher correlation was observed among reverse mycelial color, front mycelial color, radial growth at 14 days and radial growth at 16 days. Growth behavior and conidial color represented insignificant correlation among the all components. Jaiswal et al. (2007) also demonstrated higher correlation between the virulence and the morphological characters of wheat blast pathogen. Some researcher found that virulence and aggressiveness is strongly correlated with morphological traits like as colony color, conidial color and spore production (Thompson et al., 2011).

The present experiment exposed two types of colony growth behavior (Smooth and Rough) and one type of mycelial elevation (flat). Yashaswini et al. (2017) observed eleven isolates having greyish white colonies with rough texture growth behaviour and nine isolates having greyish white colonies with smooth texture growth behavior among twenty isolates. Longya et al. (2020) found two types of elevation (Flat and fluffy) among *Pyricularia oryzae*.

One type of conidia shape (pyriform) and two types of conidial color (Blackish and transparent) were recorded in the present study. Sonah et al. (2009) found most conidia were pyriform shaped and one strain (RBR55001) produced oval conidia which was different from other strains. Yashaswini et al. (2017) found almost all conidia were pyriform having hyaline to pale olive color.

In the present experiment, all the isolates collected from AEZ-11, AEZ-12 and AEZ-13 (Table 1) and cluster analysis showed five distinct groups (Cluster I, II, III, IV and V)based on nine morphological characteristics. The cluster I, II, III, IV and V consisted of 3, 7, 3, 5 and 1 number of isolates; respectively. The only one isolate, PO-11 belongs to cluster V and is derived from AEZ-11. PO-11 was more distinct among the 19 isolates of *Pyricularia oryzae*. Yashaswini et al. (2017) reported that twenty isolates of *Pyricularia oryzae* isolates were

grouped into three Clusters (I, II and III) on the basis of their colony texture and mycelia color.

Principal component analysis (PCA) revealed that mycelial and conidial characters contributed in variability. Among nine morphological components mycelial color, growth behavior and conidial size play for 90% role in generating variation. The data also revealed a high range of variation between reverse mycelial colony color and radial growth at 14 days; front mycelial colony color and radial growth at 16 days.

# CHAPTER V

# SUMMARY AND CONCLUSION

## 5.1 Summary

The experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna. during the period of September 2020- September 2021 to determine the morphological variation among the isolates of *Pyricularia oryzae*. Nineteen isolates of *Pyricularia oryzae* were collected from South-Western region of Bangladesh. Experiment on morphological variability was conducted following Complete Randomized Design (CRD) with three replications.

Morphological variability among *P. oryzae* isolates were observed by considering front mycelial color, reverse mycelial color, growth behavior, length of conidia (µm), width of conidia (µm), radial growth at 12 days (mm), radial growth at 14 days (mm), radial growth at 16 days (mm) and conidial color. Multivariate analysis was done to observe morphological variation.

Mean all of the components were greater than the standard error. Highest coefficient of variation was observed among conidial color (36.36%) and lowest coefficient of variation was observed among Radial growth at 12 days of mycelia (8.36%). That means conidial color helps to create more variability.

Five distinct clusters were found from cluster analysis based on morphological characters. The cluster I, II, III, IV and V consisted of 3, 7, 3, 5 and 1 number of isolates. Eight mycelial colony colors, two types of growth behaviors, one type of mycelial elevation, one type of conidial shape and two types of conidial colors were also found.

Principal component analysis (PCA) and correlation matrix revealed reverse mycelial colony color, radial growth at 14 days, front mycelial colony color and radial growth at 16 contributed in variability. Among the nine morphological components, front color, reverse color, growth behaviour and conidial length play for more than 80% role in generating variation. PCA biplot shows that a high range of variation took place between reverse mycelial color and radial growth at 12 days; front mycelial color and radial growth 16 days respectively.

## 5.2 Conclusion

The followings are the salient findings of the present research:

i. Highest coefficient of variation was observed in conidial color (36.36%) and lowest coefficient of variation was observed in radial growth at 12 days of mycelia (8.36%).

ii. Five distinct clusters are found based on nine morphological components.

iii. PO-11 isolate from cluster V showed distinct morphological variation among the 19 isolates.

iv. Seven mycelial colony colors, two types of growth behaviors, two types of conidial colors and one type of conidial shape were also found.

v. Among the nine morphological components, Front color, Reverse color, Growth behavior and Conidial length play for more than 80% role in generating variation among all other components.

vi. PCA biplot demonstrated a high range of variation took place between Reverse mycelial colony color and R14; Front mycelial colony color and R16 days respectively.

vii. Highly significant correlation was found between R12 to R14, R14 to R16 and Front mycelial color to Reverse mycelial color.

Based on findings of the present study it may be concluded that distinct variations exists among the isolates of *P. oryzae* collected from different AEZs of South-Western rice growing regions of Bangladesh as determined based on nine morphological characters.

### 5.3 Recommendations

The present piece of research is concentrated only in South Western regions of Bangladesh, which doesn't reflect the scenario of the variation of rice blast pathogen in whole country. Working with the sample from all over the country and continuing upto 2-3 years will be fruitful and effective in practical point of view.

## CHAPTER 6

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# APPENDICES

**Appendix 1. Mean mycelial characteristics of *Pyricularia oryzae* isolates**

| Isolate No. | Front mycelial color | Reverse mycelial color | Growth behaviour | Radial growth at 12 days (mm) | Radial growth at 14 days (mm) | Radial growth at 16 days (mm) |
|---|---|---|---|---|---|---|
| **PO-1** | Deep brown | Deep brown | Smooth | 6.83 | 6.85 | 7.25 |
| **PO-2** | Light brown | Light grey | Smooth | 5.57 | 5.93 | 6.47 |
| **PO-3** | Light grey | Grey | Smooth | 7.13 | 7.67 | 8.32 |
| **PO-4** | Light brown | Light grey | Smooth | 6.60 | 6.97 | 7.45 |
| **PO-5** | Light grey | Grey | Smooth | 7.07 | 7.97 | 8.17 |
| **PO-6** | Light grey | Black | Smooth | 6.63 | 6.57 | 7.08 |
| **PO-7** | Light grey | Black | Smooth | 6.47 | 6.50 | 6.87 |
| **PO-8** | Olivaceous grey | Grey | Smooth | 6.52 | 7.50 | 7.85 |
| **PO-9** | Light grey | Olivaceous grey | Smooth | 7.37 | 7.88 | 8.35 |
| **PO-10** | Light grey | Black | Smooth | 5.83 | 6.42 | 6.63 |
| **PO-11** | Light brown | Light grey | Rough | 5.82 | 7.18 | 7.67 |
| **PO-12** | Grey | Deep grey | Smooth | 7.07 | 7.95 | 8.53 |
| **PO-13** | Light grey | Grey | Smooth | 7.45 | 7.87 | 8.15 |
| **PO-14** | Olivaceous grey | Deep grey | Smooth | 6.90 | 7.62 | 8.05 |
| **PO-15** | Olivaceous grey | Grey | Smooth | 7.20 | 7.78 | 7.75 |
| **PO-16** | Deep brown | Grey | Smooth | 7.60 | 7.95 | 8.30 |
| **PO-17** | Light grey | Grey | Smooth | 7.20 | 7.98 | 8.35 |
| **PO-18** | Light grey | Grey | Smooth | 7.18 | 7.98 | 8.62 |
| **PO-19** | Light grey | Grey | Smooth | 7.20 | 8.23 | 8.65 |

**Appendix 2. Mean conidial characteristics of *Pyricularia oryzae* isolates**

| Isolate No. | Cinidial length(µm) | Conidial breadth(µm) | Conidial color |
|---|---|---|---|
| **PO-1** | 6.91 | 2.27 | Blackish |
| **PO-2** | 6.95 | 2.24 | Blackish |
| **PO-3** | 6.09 | 1.79 | Transparent |
| **PO-4** | 7.47 | 2.46 | Blackish |
| **PO-5** | 6.11 | 2.00 | Transparent |
| **PO-6** | 7.21 | 2.32 | Blackish |
| **PO-7** | 7.37 | 2.17 | Blackish |
| **PO-8** | 7.34 | 2.26 | Transparent |
| **PO-9** | 7.78 | 2.34 | Blackish |
| **PO-10** | 7.10 | 2.55 | Blackish |
| **PO-11** | 7.01 | 2.33 | Blackish |
| **PO-12** | 5.69 | 2.12 | Blackish |
| **PO-13** | 6.90 | 2.15 | Blackish |
| **PO-14** | 5.77 | 2.31 | Transparent |
| **PO-15** | 5.75 | 2.41 | Transparent |
| **PO-16** | 7.51 | 2.26 | Transparent |
| **PO-17** | 6.00 | 1.94 | Blackish |
| **PO-18** | 7.16 | 2.25 | Blackish |
| **PO-19** | 7.25 | 2.66 | Blackish |