Int. J. Phytopathol. 12 (02) 2023. 141-152 DOI: 10.33687/phytopath.012.02.4686

Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

UNCOVERING THE MORPHOLOGICAL AND GENETICAL HETEROGENEITY OF *PYRICULARIA ORYZAE* (COOKE) SACC. IN SOUTHWESTERN REGION OF BANGLADESH

Sabiha Sultana, Joyanti Ray, Papry Akther, Debproshad Mondal, Chhoa Mondal
Agrotechnology Discipline, Khulna University, Khulna-9208, Bangladesh.

ARTICLE INFO

Article History
Received: March 11, 2023
Revised: July 11, 2023
Accepted: August 13, 2023

Keywords
Blast
Bangladesh
Genetical heterogeneity
Morphological heterogeneity
Pyricularia oryzae
RAPD

ABSTRACT

Significant yield losses are reported due to blast disease caused by *Pyricularia oryzae* across all rice growing areas of the world. Though, management strategies reduce disease significantly but blast epidemics are still common. This research was aimed to determine the variability among the 19 *Pyricularia oryzae* isolates of South-Western regions of Bangladesh. Morphological variability was determined based on nine morphological components like front mycelial color, reverse mycelial color, growth behavior, conidial length (µm), conidial width (µm), conidial color, radial growth at 12 days (mm), 14 days (mm) and 16 days (mm) after incubation. Cluster analysis considering nine morphological components showed five distinct groups. Front and reverse mycelial color, growth behavior and conidial length played more than 80% role in generating variation among the nine tested components. Higher genetic variation was also detected among the 19 isolates of *P. oryzae.* Here, larger numbers of amplified DNA fragments was formed by Primers UCB-155 which showed distinct polymorphism. Between two clusters, Cluster II consists of 18 isolates that were originated from different origin but having maximum 94% genetical similarity. Cluster I contain only one isolates PO-16. From pair wise distance matrix, maximum distance (80%) was found between PO-16 and PO-03 isolates and minimum distance (6%) was obtained between the isolates PO-11 and PO-12. Results of this experiment revealed that, higher genetic diversity among isolates of *P. oryzae* existed in the south western region of Bangladesh but there has no direct relation with genetic diversity and geographical origin.

Corresponding Author: Joyanti Ray
Email: joyaku2005@yahoo.com

INTRODUCTION

Pyricularia oryzae, a fungus that causes rice blast disease causes severe economic loss in Bangladesh during the Boro and Transplanted Aman seasons. 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) as all of the above-ground portion of rice plants, including the leaf, collar, nodes, internodes, neck, and panicle are infected by these fungi at various growth stages (Ram *et al.*, 2007).

Based on the colony diameter, conidial color, and form under *in vitro* conditions, *Pyricularia oryzae*'s morphology has been identified. Morphological studies of blast pathogen are used to examine taxonomy of the pathogen and epidemiological features of the disease spread, which may be helpful in creating resistant cultivars through breeding programs (Mahto *et al.*, 2012). Resistant rice variety can control disease initially however it lost its resistancy due to the emergence of a new race of *P. oryzae* (Utami *et al.*, 2006). Genetic

variation in pathotype and structure of this pathogen population is strongly influenced by the geographical conditions of a region (Taheri and Irannejad, 2014).

High genetic variability and the capacity to produce multilocus data from the genome are requirements for excellent genetic markers in the majority of genetic variation investigations (Jabbarzadeh *et al.*, 2010). RAPD is practical since it may be used to populations for which no unique markers have been produced. Second, it's a more cost-effective technique, doesn't involve complicated procedures of DNA sequencing, needs only a simple laboratory with minimum instruments and machines to perform polymerase chain reaction (PCR). Because of its greater stability, potency, and precision compared to morphology, RAPD is extremely significant (Guo *et al.*, 2001). Study on morphological and genetical variability of *Pyricularia oryzae* in the southwestern region of Bangladesh can help in developing blast management strategies for that region. Thus, the present study was undertaken to assess the morphological and molecular variability among the isolates of *Pyricularia oryzae* using Random Amplified Polymorphic DNA (RAPD) marker.

MATERIALS AND METHODS

Collection of leaf sample

Typical leaf blast diseased leaves/necks of rice were collected from south-western rice growing parts of Bangladesh (Table 1).

Pathogen isolation, morphological and molecular identification

Samples that were infected were divided into small pieces, including the lesion's margin (1-2 cm), and surface sterilized with 1% sodium hypochlorite. These samples were then set in inside laminar air flow on moist blotting paper for 2-3days to enhance sporulation. After incubation, these plant pieces were examined under light 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. Morphological descriptions given by (Barnett and Hunter, 1972) were followed to identify *Pyricularia oryzae* conidia.

Table 1. Blast diseased 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	+

Molecular identification based on internal transcribed spacers (ITS) and ITS rDNA sequence analysis

Isolates of *Pyricularia oryzae* were grown in 100 ml potato dextrose broth. Approximately 20 g of mycelia were harvested and ground in liquid nitrogen and DNA was extracted using DNA purification kit (Invitrogen).

ITS (internal transcribed spacers) regions were amplified following polymerase chain reactions (PCR). PCR reaction were carried out in a 25 µl volume using universal eukaryotic forward primers ITS5-GGAAGTAAAAGTCGTAACAAGG and reverse primer ITS4- TCCTCCGCTTATTGATATGC and 50 ng μl^{-1} of DNA for each isolate. Dideoxynucleotide chain termination method was performed for DNA sequencing of PCR products (Sanger *et al.*, 1977). The National Centre for Biotechnology Information (National Institutes of Health, Bethesda, USA) internet server was used to

conduct the rDNA homology searches using the Basic Local Alignment Search Tool (BLAST) program. Sequences and accession numbers for compared isolates were retrieved from the GenBank database.

Determination of morphological variability among the nineteen isolates of *Pyricularia oryzae*

Mycelial characteristics were recorded through visual observation after 12 days of incubation and conidial characters were recorded under light microscope (Carl Zeiss Microscope affiliated with Zen Blue 2.0 software) at 40× magnification light microscopic objective after 16 days of incubation. The radial growth of the colony, conidial length and width was measured using Zeiss version 2.0 computer program at 10µm unit scale. To observe mycelial features, regular observation was done after incubation and data on the reverse mycelial color, front mycelial color, colony growth behavior, and radial growth at 12, 14 and 16 days after incubation (mm) were recorded. Conidial length (µm), conidial width (µm), and color of conidia were recorded only after 16 days of incubation.

Experimental design and data analysis of morphological components

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

Resolve genetical variability among the nineteen isolates of *Pyricularia oryzae*

Nineteen Isolates of *Pyricularia oryzae* were grown in 100 ml potato dextrose broth. About 20 g of mycelia was taken out and powdered in liquid nitrogen, and DNA was extracted using an Invitrogen DNA purification kit. NanoDrop Spectrophotometer (Thermo Fisher Scientific, Germany) was used to quantify purified DNA at a wave length of 260 nm. The final concentration of the template DNA for PCR was adjusted to 50 ng $(\mu L)^{-1}$ and stored at -20°C.TAE buffer preparation was prepared following (Brown and Lorenz, 2016; Rebecca *et al.*, 2011).

PCR amplification and gel electrophoresis

Polymerase Chain Reaction was performed in a 0.2 ml thin-walled PCR tube. Amplifications were performed in thermos cycler (Biometra 4, Germany) using the following protocols: 94°C for 4 minutes, followed by 40 cycles of 94°C for 30 seconds, 30°C for 1 minute and final extension at 72°C for 5 minutes, then cooled down to 8°C. Amplified products were separated by electrophoresis on 1% agarose gels with TAE running buffer and stained with ethidium bromide (at 0.5 µg mL^{-1}).

PCR product analysis

The name of the primer and the size of the band amplified were used to identify RAPD fragments. Only clear-cut segments were given points as potential loci with presence and absence of two alleles. The genetic relationship among the nineteen isolates was analyzed from the combined 0/1 matrix data of random primer profiles by the Phylogenetic Analysis Using Pasimony (PAUP) version 4.0 computer program and the fragments size were estimated following standard 1 kb DNA marker. The matrix was analyzed using pair-wise distance coefficient. The dendrogram was formed following Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973) using pair-wise distance matrix. Data were also used to cluster analysis by neighbor-joining (NJ) method (Saitou and Nei, 1987) to estimate the evolutionary relationship among the isolates.

Calculation of polymorphism percentage

It was calculated by dividing the number of polymorphic bands by the total number of bands and multiplied by 100.

$$\text{Polymorphism } \% = \frac{\text{No. of polymorphic bands}}{\text{Total number of bands}} \times 100$$

RESULTS

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 1). 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 V (Figure 1).

Seven types of mycelial colony colors were observed among the 19 isolates of *P. oryzae* (Figure 2 and 3). They were- a) Light brown, b) Deep brown, c) Light gray, d) Olivaceous grey, e) Grey, f) Deep grey and g) Black

(Figure 2 and 3). *Pyricularia oryzae* were showed smooth and rough growth behavior (Figure 4). Blackish and transparent colored conidia were also observed among the 19 isolates (Figure 4).

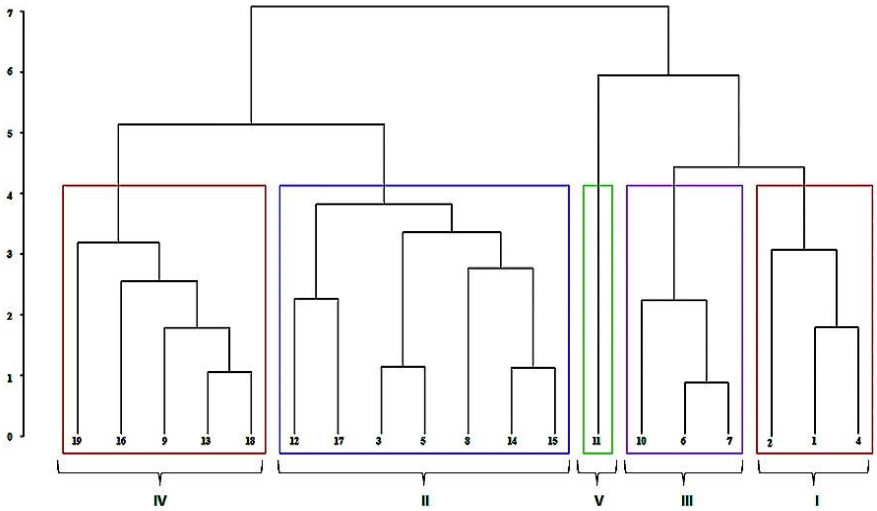

Figure 1. Dendrogram constructed with the data obtained from the studied nine morphological components of *P. oryzae*.

Figure 2. Variation in front mycelial color of *Pyricularia oryzae* isolates.

Correlation among the morphological components

Relationship among different morphological components can be described by correlation. Among the components some of those showed positive, some of them showed negative and some of those showed non-significant relationship between the components. Higher positive correlation was observed between radial growth at 14 and 16 days. But lower positive correlation was experienced between mycelial front color and radial growth at 14 days after inoculation and also lower positive correlation was observed between conidial length and breadth. Growth behavior and conidial color represented non-significant correlation among all the components. In case of mycelial front colony color, there was significant 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 the components except conidial breadth (Table 2).

Figure 3. Variation in reverse mycelial color of *Pyricularia oryzae* isolates.

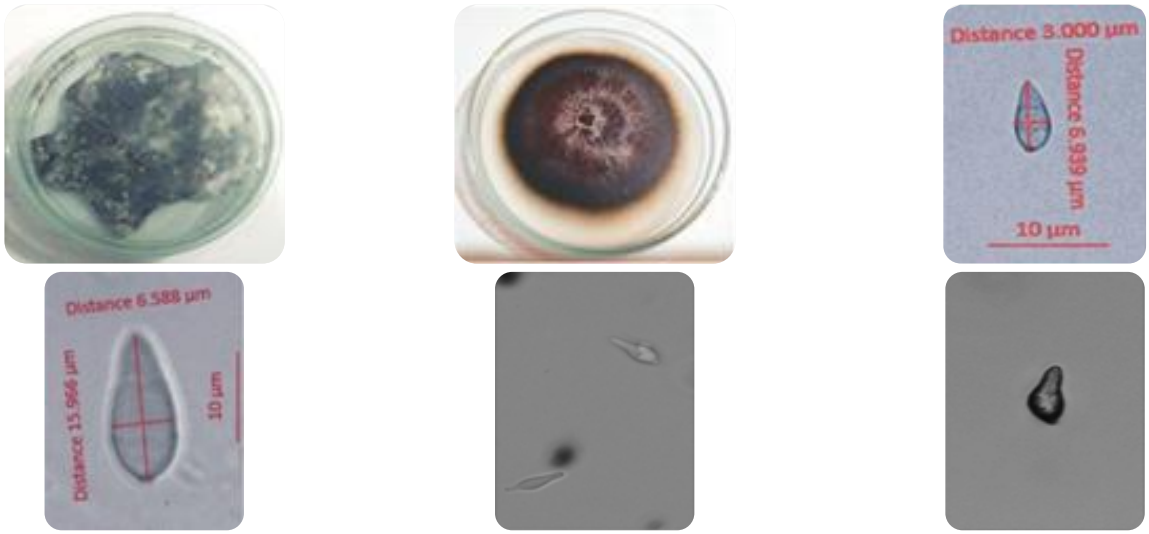

Figure 4. A) Photograph of mycelial growth behavior; B) shape and size of conidia and C) spore colour of *Pyricularia oryzae* isolates

Table 2. Pearson correlation between the nine morphological components of *Pyricularia oryzae* isolates.

Morphological Components	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

Contribution of components in morphological variation

Principal component analysis (PCA) was done to identify the extent of contribution of each of the components to observe variations percentages among the 19 isolates of *P. oryzae* (Table 3 and Figure 5). Each component was 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 variations in the morphology. Contributions of components are presented in Table 3.

Table 3. Estimation of percentages of variation of the eleven morphological components of *P. oryzae* isolates tested.

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

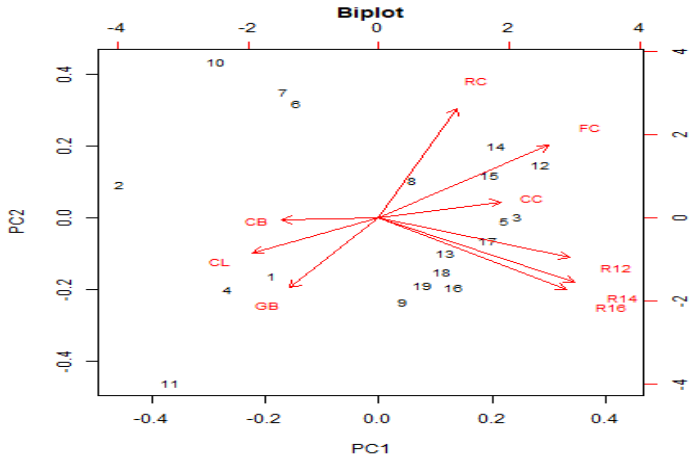

Figure 5. PCA biplot constructed with the data obtained from the studied nine morphological components of *Pyricularia oryzae* isolates.

Dendrogram using the Agglomerative Clustering Method

PC1 and PC2 were 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, 14 and 16 days). The data also revealed a high range of variation between the 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 which was present between the conidial color and the conidial breadth.

Genetical Characterization of *Pyricularia oryzae* through Randomly Amplified Polymorphic DNA (RAPD) Marker

DNA fingerprints of *Pyricularia oryzae* through RAPD

Genetic variation was detected among the 19 isolates of *Pyricularia oryzae* using RAPD markers. Tested 10 primers resulted in amplification of distinct and reproducible bands in the present investigation. The amplified maximum number of DNA fragments was 87. Primers UCB-155 generated greater numbers of

amplified fragments and gave distinct amplification. Fragments present in at least one and maximum 29 isolates (5-90% occurrence) were considered polymorphic, while fragments present in at least 30 isolates (95% occurrence) were monomorphic (Muller et al., 2005). This criterion established a total of 87.17% as being polymorphic fragments (Table 4). The PCR amplified products using RAPD primers are shown in the Figure 6. It is noteworthy to mention that most of the primers produced >80% polymorphic fragments.

Table 4. Primers used for RAPD analyses of 19 isolates of *Pyricularia oryzae* and number of amplified DNA fragments.

S. No.	Primer code	Primer Sequence (5′-3′)	Maximum amplified bands No.	Polymorphic bands No.	Monomorphic bands No.	Polymorphism (%)
1	OPA-02	TGCCGAGCTG	11	10	1	90.91
2	OPA-03	AGTCAGCCAC	5	4	1	80.00
3	OPA-12	TCGGCGATAG	7	6	1	85.71
4	OPA-14	TCTGTGCTGG	7	7	0	100.00
5	OPA-18	AGGTGACCGT	6	5	1	83.33
6	OPB-05	TGCGCCCTTC	13	12	1	92.31
7	UBC-155	CTGGCGGCTG	14	13	1	92.86
8	OPF-04	GGTGATCAGG	10	8	2	80.00
9	OPH-09	TGTAGCTGGG	5	5	0	100.00
10	UBC-173	CAGGCGGCGT	9	6	3	66.67
	Total		87	76	11	87.18

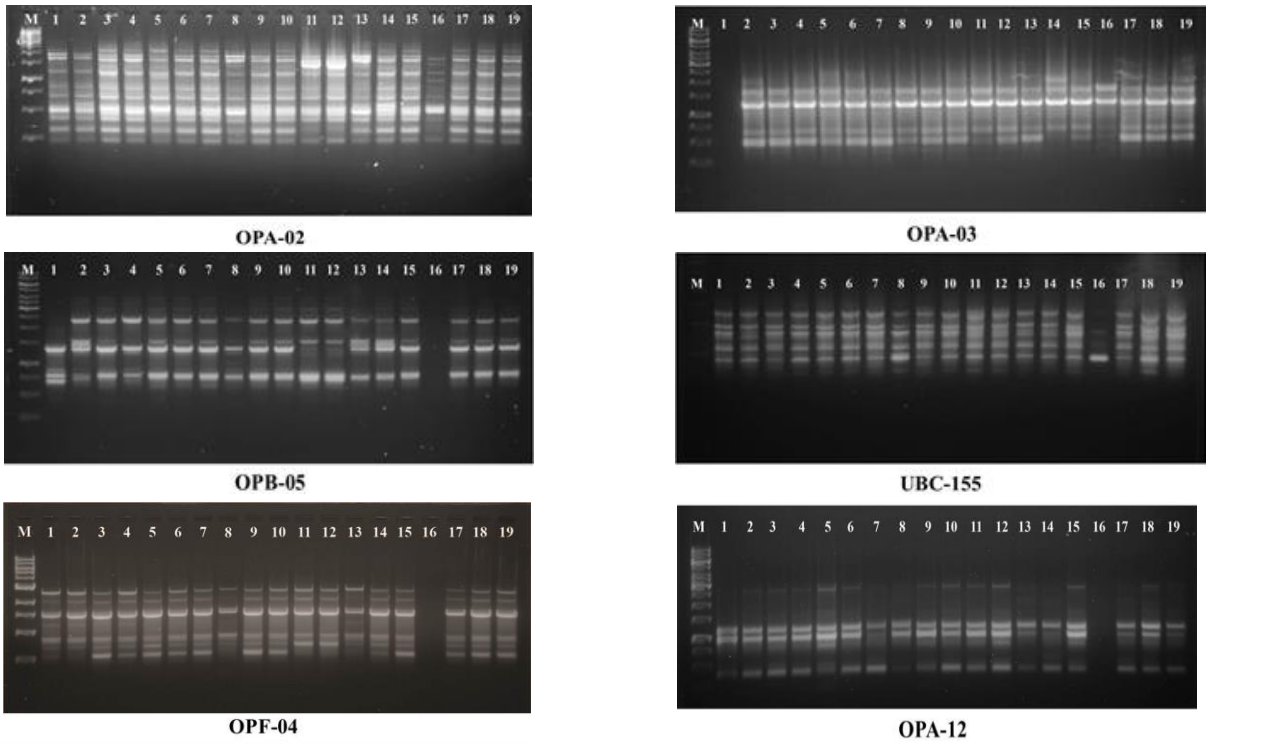

Figure 6. RAPD profile of 19 isolates of *Pyricularia oryzae* generated by using OPA-2, OPA-3, OPB-5, UCB-155, OPF-4 and OPA-12 primer; M- Kilo base molecular weight ladder.

Cluster analysis

The dendrogram was constructed with the data generated from the amplifications of the 19 isolates from 10 primers, which exhibited two very distinct clusters (Figure 7). The cluster II consisted of 18 isolates, cluster I contained only one isolates (PO-16). The cluster II was further subdivided into two sub-clusters. The cluster IIA contained 16 isolates but the cluster IIB contains only

two isolates.

Pair wise genetic similarity

Analyzing the matrix constructed with the amplification data, there was coefficient of similarity between the pairs, the maximum similarity was 94% between the isolates PO-11 and PO-12; which belonged to AEZ-11 and the AEZ-13 but the minimum pair wise similarity was calculated only 2% between the isolates PO-16 and PO-03 originating from the AEZ-13 (Table 5).

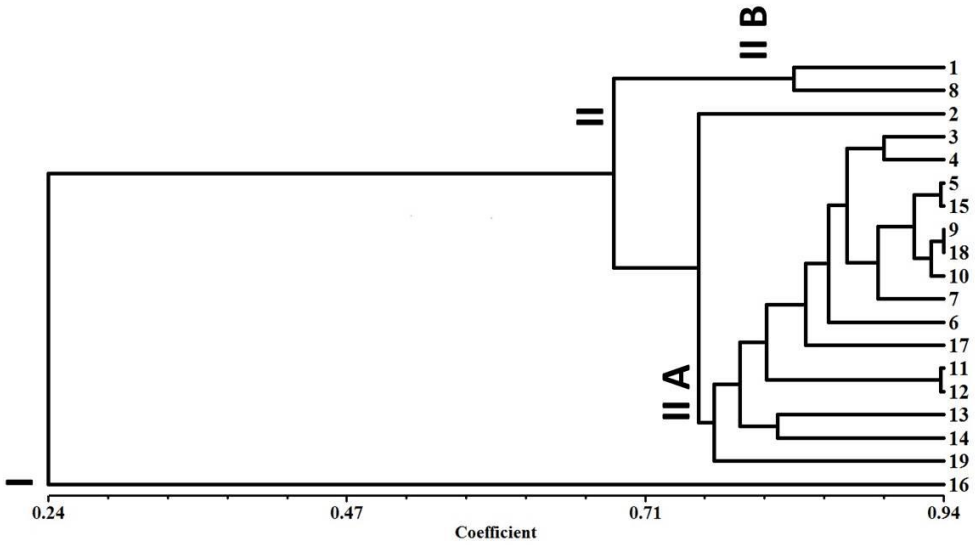

Figure 7. Dendrogram constructed based on combined data obtained from using 10 primers in RAPD analysis of 19 isolates of *P. oryzae.*

DISCUSSION

Pyricularia oryzae had predominantly a sexual, haploid, and heterocaryotic reproduction and had virulent diversity, due to extensive genetic exchange by nuclear fusion, somatic hybridization and consequently chromosomal rearrangement for the haploidization. Plant pathogens diversity has been revolutionized by molecular techniques particularly the PCR technique has helped to study population structure and genetic diversity (Banerjee *et al.*, 2014).

In the present experiment, multivariate analysis was performed for observing the morphological variability based on the nine morphological components. After 16 days of incubation on the PSA media, isolates of *P. oryzae* showed great diversity in their growth.

Considering the mycelial colony color, five front colors and six reverse colors were observed. 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 due to variation in melanin production capabilities. Higher correlation was observed among reverse mycelial color, front mycelial color, radial growth at 14 days and 16 days after incubation. Growth behavior and conidial color represented insignificant correlation among all the components. Jaiswal *et al.* (2007) also demonstrated higher correlation between the virulence and the morphological traits of wheat blast pathogen. Some researchers found that virulence and aggressiveness was 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*.

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

Table 5. Pair wise genetic similarity of 19 isolates of *Pyricularia oryzae* based on DNA polymorphism.

Isolate No.	PO1	PO2	PO3	PO4	PO5	PO6	PO7	PO8	PO9	PO10	PO11	PO12	PO13	PO14	PO15	PO16	PO17	PO18	PO19
PO1	-																		
PO2	0.67	-																	
PO3	0.73	0.74	-																
PO4	0.75	0.76	0.89	-															
PO5	0.74	0.78	0.84	0.86	-														
PO6	0.71	0.76	0.82	0.80	0.89	-													
PO7	0.66	0.70	0.83	0.84	0.86	0.81	-												
PO8	0.82	o.67	0.70	0.75	0.70	0.68	0.63	-											
PO9	0.67	0.78	0.84	0.86	0.92	0.86	0.90	0.68	-										
PO10	0.71	0.78	0.92	0.90	0.92	0.86	0.90	0.68	0.92	-									
PO11	0.64	0.78	0.77	0.78	0.81	0.78	0.80	0.60	0.84	0.81	-								
PO12	0.62	0.73	0.75	0.74	0.83	0.80	0.81	0.59	0.83	0.83	0.94	-							
PO13	0.66	0.75	0.74	0.79	0.74	0.79	0.76	0.70	0.75	0.75	0.74	0.69	-						
PO14	0.70	0.74	0.73	0.78	0.80	0.82	0.83	0.66	0.81	0.81	0.77	0.79	0.81	-					
PO15	0.72	0.76	0.86	0.88	0.94	0.88	0.88	0.69	0.94	0.90	0.86	0.84	0.76	0.82	-				
PO16	0.28	0.25	0.21	0.24	0.22	0.24	0.21	0.32	0.22	0.22	0.22	0.22	0.26	0.23	0.22	-			
PO17	0.66	0.70	0.80	0.85	0.80	0.78	0.86	0.69	0.88	084	0.73	0.72	0.80	0.80	0.82	0.22	-		
PO18	0.73	0.77	0.86	0.88	0.90	0.88	0.88	0.66	0.94	0.94	0.83	0.81	0.76	0.86	0.92	0.21	0.86	-	
PO19	0.67	0.65	0.71	0.72	0.78	0.79	0.80	0.63	0.82	0.75	0.68	0.70	0.71	0.78	0.80	0.25	0.77	0.80	-

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 belonged to cluster V derived from High Ganges River Floodplain (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* were grouped into three Clusters (I, II and III) on the bases of their colony texture and mycelia color.

Principal component analysis (PCA) revealed that the mycelial and conidial traits contributed in variability. Among the nine morphological components, mycelial color, growth behavior and conidial size played for 90% role in generating variation. The data also revealed a high range of variation among reverse mycelial colony color and radial growth at 14 days; front mycelial colony color and radial growth at 16 days. Relationship among the formations, dispersal, and infection behavior of spores and environmental factors have been reported by Suzuki (1974). Different environmental factors, such as temperature, pH, moisture, degree of aeration, and amount and type of nutrients affected fungal morphology (Gaddeyya *et al.*, 2012). Furthermore, mycovirus infection in *P. oryzae* causes morphological changes, including changes in their growth on agar, melanin biosynthesis, and formation of conidia and appressoria (Moriyama *et al.*, 2018).

In this experiment, higher genetic variation was also detected among the isolates of *Pyricularia oryzae.* HereThe UCB-155 primers produced more amplified DNA fragments and displayed clear polymorphism. These results showed that random PCR primers might be used to research, characterise, and evaluate intra-specific polymorphisms among Pyricularia oryzae isolates (Muller *et al.*, 2005). Powell *et al.* (1996) reported that the level of polymorphism revealed the degree of genome coverage. The molecular profiles generated by RAPD markers showed the presence of some common fragments in all the isolates.

In this experiment 1.0 kb size DNA fragment was found in all tested isolates using 10 different primers, So it is assumed that this fragment might be the virulent identifying band of *Pyricularia oryzae*. However the common amplification product might be useful in generating diagnostic marker (Bala and Yogesh, 2015). After cluster analysis two distinct clusters were observed. Cluster II consisted of 18 isolates that are originated from different origin but having maximum 94% genetical similarity. But the cluster I contained only one isolates PO-16. However, our results also agree with the earlier findings of Leisova-Svobodova *et al.* (2012). From this experiment, most genetically diverse isolated that means 80% genetical distance was observed between PO-16 and PO-03 isolates were belonged the same AEZ-13. But these regions of Bangladesh are belonged to Eastern Gangetic Plains (EGP) of South Asia (Natarajan and Prathapar, 2013). However, proven information is that Eastern Gangetic Plains (EGP) of South Asia is the hot spot of blast disease of rice (Sharma *et al.*, 2007). Gene-environment interactions may be attributed for the genetic diversity. The diversity found in this study's isolates of *Pyricularia oryzae* using RAPD primers may be caused by a variety of variables, including population dynamics, gene flow, mutations, and the multinuclear character of conidia, which might result in the phenomena of high polymorphism (Mann *et al.*, 2014).

From pair wise distance matrix, the maximum distance (80%) was found between PO-16 and PO-03 isolates while the minimum distance (6%) was obtained between the isolates PO-11 and PO-12. But these genetically most closely related isolates were originated from High Ganges River Floodplain (AEZ-11) and Ganges Tidal Floodplain (AEZ-13). That means, geographical diversity was not only responsible for generating genetical distance among the isolates. Aggarwal *et al.* (2009) and Frazzon *et al.* (2002) also observed a lack of relation between genetic similarity and geographic origin. Aggarwal *et al.* (2010) also observed high polymorphism among *Pyricularia oryzae* isolates. The genetic variation in *P. oryzae* in natural conditions might be due to genome adaptation by which it overcomes host resistant. Sexual mating and parasexual recombination are two main mechanisms for genome adaptation in fungi (Noguchi, 2011), but sexual reproduction in rice blast fungus has not been observed in the field and sexual spores have been produced only in laboratory settings. Therefore, parasexual recombination could most likely be the major mechanism for the exchange of DNA fragments (Chuma *et al.*, 2011) that causes genetic variation in the rice blast population. But the variability was independent of their geographic origin due to genetic diversity of a population, which was not established based on a single characteristic, such as a location, rather it might be affected by many other factors.

CONCLUSION

Morphological and genetic studies revealed that *Pyricularia oryzae* was a highly diverse pathogen. Experimented 19 isolates of *P. oryzae* were grouped into five distinct clusters morphologically by multivariate analysis and two distinct clusters were found by RAPD analysis. Among the nine morphological components, front color, reverse color, growth behavior and conidial length played for more than 80% role in generating variation among all other components. The primer UCB-155 generated greater numbers of amplified DNA fragments and showed distinct polymorphism. The results showed that, higher genetic diversity existed in south western region of Bangladeshi isolates of *Pyricularia oryzae*, but there had no direct relation with the genetic diversity and the geographical origin as well.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

REFERENCE

Aggarwal, R., V. Singh, M. Gurjar, S. Gupta and P. Srinivas. 2009. Intraspecific variations in Indian isolates of *Bipolaris sorokiniana* infecting wheat based on morphological, pathogenic and molecular characters. Indian Phytopathol, 62: 449-60.

Aggarwal, R., V. B. Singh, R. Shukla, M. S. Gurjar, S. Gupta and T. R. Sharma. 2010. URP-based DNA fingerprinting of *Bipolaris sorokiniana* isolates causing spot blotch of wheat. Journal of Phytopathology, 158: 210-16.

Bala, A. and V. Yogesh. 2015. Molecular characterization of *Bipolaris sorokiniana* populations from winter cereals. International Journal of Plant Protection,

8: 331-37.

Banerjee, S., R. Poswal, S. Gupta, S. Sharma, B. Bashyal and R. Aggarwal. 2014. Molecular characterization of *Bipolaris* spp. using universal rice primer (URP) markers. Indian Phytopathology, 67: 49-54.

Barnett, H. L. and B. B. Hunter. 1972. Illustrated genera of imperfect fungi. CAB International.

Brown, S. D. and A. Lorenz. 2016. Single-step marker switching in *Schizosaccharomyces pombe* using a lithium acetate transformation protocol. Bio-protocol, 6: e2075-e75.

Chuma, I., C. Isobe, Y. Hotta, K. Ibaragi, N. Futamata, M. Kusaba, K. Yoshida, R. Terauchi, Y. Fujita and H. Nakayashiki. 2011. Multiple translocation of the *AVR-Pita* effector gene among chromosomes of the rice blast fungus *Magnaporthe oryzae* and related species. PLoS pathogens, 7: e1002147.

Frazzon, A. P. G., A. T. S. Matsumura and S. T. Van Der Sand. 2002. Morphological characterization and genetic analysis of *Drechslera teres* isolates. Genetics and Molecular Biology, 25: 235-41.

Gaddeyya, G., P. S. Niharika, P. Bharathi and P. R. Kumar. 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Advances in Applied Science Research, 3: 2020-26.

Guo, L. D., K. D. Hyde and E. C. Liew. 2001. Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. Molecular phylogenetics and evolution, 20: 1-13.

Jabbarzadeh, Z., M. Khosh-Khui, H. Salehi and A. Saberivand. 2010. Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. African Journal of Biotechnology, 9: 6091-95.

Jaiswal, S., Sweta, L. Prasad, S. Sharma, S. Kumar, R. Prasad, S. Pandey, R. Chand and A. Joshi. 2007. Identification of molecular marker and aggressiveness for different groups of *Bipolaris sorokiniana* isolates causing spot blotch disease in wheat (*Triticum aestivum* L.). Current Microbiology, 55: 135-41.

Leisova-Svobodova, L., V. Minarikova, L. Kucera and S. Pereyra. 2012. Structure of the *Cochliobolus sativus* population variability. Plant pathology, 61: 709-18.

Longya, A., S. Talumphai and C. Jantasuriyarat. 2020. Morphological characterization and genetic diversity of rice blast fungus, *Pyricularia oryzae*, from Thailand using ISSR and SRAP markers. Journal of Fungi, 6: 38-51.

Mahto, B. N., S. Gurung, A. Nepal and T. B. Adhikari. 2012. Morphological, pathological and genetic variations among isolates of *Cochliobolus sativus* from Nepal. European journal of plant pathology, 133: 405-17.

Mann, M. B., C. C. Spadari, T. Feltrin, A. P. G. Frazzon, J. C. Germani and S. T. Sand. 2014. Genetic variability of *Bipolaris sorokiniana* isolates using URP-PCR. Tropical Plant Pathology, 39: 163-71.

Moriyama, H., S.-i. Urayama, T. Higashiura, T. M. Le and K. Komatsu. 2018. Chrysoviruses in *Magnaporthe oryzae*. Viruses, 10: 697-703.

Muller, D., C. M. Judd and V. Y. Yzerbyt. 2005. When moderation is mediated and mediation is moderated. Journal of personality and social psychology, 89: 852-56.

Natarajan, R. and S. A. Prathapar. 2013. Hydrogeology of the eastern Ganges Basin: An overview IWMI Working Paper 157. International Water Management Institute.

Noguchi, M. T. 2011. Parasexual recombination in *Magnaporthe oryzae*. Japan Agricultural Research Quarterly, 45: 39-45.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-38.

Ram, T., N. Majumder, B. Mishra, M. Ansari and G. Padmavathi. 2007. Introgression of broad-spectrum blast resistance gene (s) into cultivated rice (*Oryza sativa* ssp. indica) from wild rice *O. rufipogon*. Current Science, 92: 225-30.

Rayhanul, M., F. Aminuzzaman, M. Chowdhury, L. Laila and M. Ahmed. 2019. Survey on rice blast in some selected area of Bangladesh and in vitro evaluation of fungicides against *Pyricularia oryzae*. Bangladesh Journal of Plant Pathology, 35: 59-64.

Rebecca, L., B. Zothansanga Singh, G. Gurusubramanian and N. Senthil. 2011. DNA finger printing of *Bacillus thuringiensis* based on repetitive DNA sequences using ERIC-PCR. Sci Vis, 11: 147-54.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4: 406-25.

Sanger, F., S. Nicklen and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. proceedings of the national academy of sciences, 74: 5463-67.

Sharma, R. C., G. Ortiz-Ferrara, J. Crossa, M. Bhatta, M. Sufian, J. Shoran, A. Joshi, R. Chand, G. Singh and R. Ortiz. 2007. Wheat grain yield and stability assessed through regional trials in the Eastern Gangetic Plains of South Asia. Euphytica, 157: 457-64.

Sneath, P. H. and R. R. Sokal. 1973. Numerical taxonomy. The principles and practice of numerical classification. WF Freeman & Co.: San Francisco.

Sonah, H., R. Deshmukh, S. Parida, S. Chand and A. Kotasthane. 2009. Morphological and genetic variation among different isolates of *Magnaporthe grisea* collected from Chhattisgarh. Indian Phytopathology, 62: 469-78.

Suzuki, H. 1974. Studies on the behavior of the rice blast fungus spore and the application for forecasting method of the rice blast disease. Japanese Journal of Phytopathology, 40: 165-67.

Taheri, P. and A. Irannejad. 2014. Genetic structure of various *Magnaporthe oryzae* populations in Iran and Uruguay. Australasian Plant Pathology, 43: 287-97.

Thompson, D. S., P. L. Carlisle and D. Kadosh. 2011. Coevolution of morphology and virulence in *Candida* species. Eukaryotic cell, 10: 1173-82.

Utami, D. W., H. Aswidinnoor, S. Moeljopawiro, I. Hanarida and R. Reflinur. 2006. Inheritance of blast resistance (*Pyricularia grisea* Sacc.) on interspecific crossing between IR64 and *Oryza rufipogon* Griff. HAYATI Journal of Biosciences, 13: 107-07.

Yashaswini, C., B. Pushpavati and M. S. Madhav. 2017. Morphological and molecular variability among rice blast pathogen (*Magnaporthe oryzae*) isolates in southern India. Environment and Ecology, 35: 3015-22.