MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF TEN PLANT PATHOGENIC FUNGI

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Abstract

Ten pathogenic fungi of Deuteromycetes were isolated from seven angiospermic hosts such as pointed gourd, tomato, rice, wheat, maize, chickpea and jute. Morphological characterization and molecular analyses were performed for accurate identification of the isolated pathogenic fungi. The sequence results obtained using the ITS1 and ITS4 primers were compared with NCBI GenBank and BOL database using BLAST analysis. *Fusarium chlamydosporum* and *Penicillium pinophilum* are recorded first time from Bangladesh.

Introduction

Plants are extremely important for human and animals. Nowadays, diseases have becoming a worldwide problem and highly concerning factor. Plant diseases are caused by fungi, bacteria, viruses, etc. Fungi represent the major pathogenic micro-organisms that infect plants, causing huge economical loss. They constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases. Fungal attack can be found on leaves, petioles, stems, pods and seeds of different plants and can cause severe damages in the field as well as in storage condition. Fungal diseases are also important constraints in grain legume productions (Njambere *et al.,* 2010).

Cereal crops such as rice, wheat, maize; pulses such as chickpea; vegetable such as pointed gourd and fiber yielding plant such as jute suffer from various kinds of diseases. But correct identification up to species level of a plant pathogenic fungus is important for the development of effective disease control management, quarantine purposes and as a basis for making decisions to protect agricultural crops as well as other natural resources from fungal pathogens (Rossman and Palm-Hernandez, 2008). The identification, taxonomy and epidemiological analyses of fungal pathogens are increasingly dependent on modern molecular techniques, based on PCR amplification of conserved regions of the genome and sequencing of the resulting PCR products (Haynes *et al.*, 1996; Sandhu *et al.*, 1995; Makimura *et al.*, 1994). Morphological identification of fungal pathogens is very common but information on molecular identification of fungal pathogens is rare in Bangladesh. So, the aim of the study was to investigate the morphological and molecular identification of pathogens associated with some selected cereal crops, pulses, vegetable as well as fiber yielding plant.

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Materials and Methods

Diseased plant samples such as rice, wheat, maize, chickpea (Bangladesh Agriculture Research Institute, Joydebpur, Gazipur), pointed gourd, tomato (Local markets, Dhaka) and jute (Bangladesh Jute Research Institute, Manikganj and Farmgate) were collected and used for the present investigation.

Isolation and morphological identification of fungi

Pathogens associated with selected samples were isolated with following "Tissue planting method" on PDA medium (CAB, 1968). Morphological structures of the pathogens were depicted with the help of Camera Lucida. Identities of the fungal isolates were determined following the standard literature (Thom and Raper, 1945; Raper and Thom, 1949; Gilman, 1967; Booth, 1971; Ellis, 1971,1976; Barnett and Hunter, 1972; Sutton, 1980; Ellis and Ellis, 1997).

Molecular characterization of fungi

Molecular identification was done following Islam and Mukherjee (2011) with some modification.

DNA extraction

Fungal mycelia were harvested by scraping the surface of 15 days old cultures with a sterile spatula from the test tube. One gm fungal mycelia were taken in 1.5 ml eppendorf tube and placed in liquid nitrogen. The mycelium was immediately grinded with a homogenizer machine to get fine powder. 750 µl of lysis buffers were added in each eppendorf tube and stir with a vortex to get homogenous mixture. The tubes were transferred to 65°C preheated water bath for 30 minutes. The samples were taken from the water bath and cooled down to room temperature. 700 µl of chloroform: phenol (1:1) mixture was added and mixed gently. The samples were centrifuged at 12,000 rpm for 5 minutes. The aqueous phase was transferred into fresh eppendrof tube and again mixed with 700 µl of chloroform: phenol and centrifuged at 12,000 rpm for 5 minutes. The aqueous phase was transferred to new eppendorf tubes and 70 µl of NaOAc was added. Top off the eppendorf tube with 300 µl isopropanol, gently inverted several times. DNA 'ropes' precipitate was seen here. The samples were centrifuged at 13,000 rpm for 10 minutes to form DNA pellet. The supernatant was discarded and the pellet was washed with 70% ethanol. The pellets were air dried and dissolved in 100 µl TE buffer mixed with RNAse A (final concentration 10 mg/ml). The samples were incubated for 30 minutes at 37 °C. 10 µl NaOAc was added to the tubes and top off the eppendorf tube with 750 µl absolute ethanol and mixed gently. The samples were centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded completely, washed with 70% ethanol, air dried the pellet and dissolved in 100 µl TE buffer. The DNA was allowed to dissolve overnight at 4 °C.

PCR amplification

Molecular identification of the isolates was performed using the internal transcribed spacer (ITS) regions. PCR amplification was conducted using the ITS1 (5'-TCCGTAGGTGAACCT GCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers for the ITS regions. The PCR was carried out in 0.2 ml PCR tube with 25 reaction volume containing 2.0 µl Template DNA, 12.5 µl Master mix, 1.0 µl Forward Primer, 1.0µl Reverse Primer and 8.5 µl MilliQ H_2O. Reaction mixture was vortexed and centrifuged in a microcentrifuge. The PCR was initiated by an initial denaturation step at 94°C for 5 minutes following 30 cycles of 94, 54 and 72°C each for 30 sec, with a final extension step of 5 min at 72°C and ended with 4°C. PCR amplified products were stored in – 20°C freezer for analysis by resolving in 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide. Agarose gel electrophoresis was conducted in

1× TAE buffer at 90 Volts and 300 mA for 40 minutes. One molecular weight marker 1kb DNA ladder was electrophoresed alongside the ITS reactions. DNA bands were photographed by a Gel Documentation system (model: DI-HD, UK).

Sequencing analysis

PCR amplified products were purified by alcohol precipitation and sequenced through automated sequencer in Centre for Advanced Research in Sciences (CARS), University of Dhaka. To identify the genus and species of the isolates, the sequences were analyzed using the BLAST program (http://blast.ncbi.nlm.nih.gov) of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA) as well as BOL database.

Results and Discussion

Morphological identification

Ten pathogens *viz.*, *Aspergillus flavus* (jute seed)*, Bipolaris oryzae* (rice seed)*, Bipolaris sorokiniana* (wheat leaves)*, Colletotrichum gloeosporioides* (jute stem)*, Corynespora cassicola* (tomato leaves)*, Fusarium* sp. (maize seed)*, F. oxysporum* (chickpea seed)*, F. solani* (rice seed)*, Penicillium* sp. and *Trichoderma* sp. (pointed gourd) were identified morphologically (Fig. 1).

Key morphological features of the isolated fungi are given below:

1.   *Aspergillus flavus* Link, Mag. der Ges. Naturf. Freunde Berlin **3**(1): 16 (1809).   (**Fig. 1A**)

Colonies effuse, greenish. Mycelium well-developed, septate, profusely branched and hyaline. Cells multinucleate, conidiophores long. Sclerotium present. Conidia catenulate, dry, usually globose, smooth, green in color.

Specimen examined: Isolated from the seeds of accession No. A-3047 of *Corchorus capsularis*, Bangladesh Jute Research Institute, Dhaka, MA Mamun 4, 23 September 2014.

2.   *Bipolaris oryzae* (Breda de Haan) Shoemaker, Canadian J. Bot. **37**(5): 883(1959).   (**Fig. 1B**)

Colonies greenish grey, reverse light grey, olivaceous with brownish tinge. Mycelium fluffy, aerial, cottony, septate. Conidiophores septate, solitary or in small groups; straight or flexous, sometimes geniculate; simple; pale to mid-brown; bearing conidia at the end and on sides. Conidia olivaceous brown, obclavate, fusiform, straight or curved, prominent hilum with basal scar, 5-9 septate, 39.56-101.89 × 11.96-16.10 μm.

Specimen examined: Isolated from the seeds of *Oryza sativa*, Bangladesh Rice Research Institute, Joydebpur, Gazipur, P Chawdhury 192, 2 January 2015.

3.   *Bipolaris sorokiniana* (Sacc.) Shoemaker, Canadian J. Bot. **37**(5): 883(1959).   (**Fig. 1C**)

Colonies olivaceous brown to very dark becoming generally lighter towards the periphery, margin mostly smooth, sometimes wavy with easily recognizable dark band, large number of conidia usually present in the centre, sometimes entire colony covered by black shiny conidia making the colony black and shiny. Conidiophores brown, short, erect, in most cases single, bearing 1-6 conidia. Ellipsoid, dark brown, mostly straight or slightly curved, wall thick but less towards the ends, broadest in the middle, ends rounded, scar clear within the basal cell. Terminal portion of the end cells sub-hyaline, 68.0-98.6 × 17.0-23.8 μ, 6-9 pseudoseptate.

Specimen examined: Isolated from seeds of *Triticum aestivum*, Bangladesh Agriculture Research Institute, Joydebpur, Gazipur, MS Momtaz 178, 27 December 2014.

4. ***Colletotrichum gloeosporioides*** (Penz.) Sacc., Fung. Agrum. **2**: 6 (1882).   (**Fig. 1D**)

Colonies effuse, greyish, reverse greyish black. Acervuli black, sub-epidermal but later the epidermis are ruptured and expose them. Hyphae septate, hyaline. Conidia hyaline, straight, obtuse at the apex, 11.2−25.2 × 3.6−5.0 µm.

Specimen examined: Isolated from infected stem of *Corchorus capsularis* L., Bangladesh Jute Research Institute, Manikgonj, S Hosen 22, 25 August 2014.

5. ***Corynespora cassiicola*** (Berk. & Curt.) Wei, Mycol. Pap. **34**: 5 (1950).   (**Fig. 1E**)

Colonies effuse, greyish, thinly hairy. Mycelium mostly immersed; stroma absent. Conidiophores pale to mid brown, with up to 9 successive cylindrical proliferations. Conidia solitary or in chains of 2-6, very variable in shape. Obclavate to cylindrical, straight or curved, sub-hyaline to rather pale olivaceous brown or brown, smooth, with 4−20 pseudosepta, 48.6-131.4 × 12-23 µm.

Specimen examined: Isolated from *Lycopersicon esculentum*, Ananda Bazar, S Shamsi 2878, 6 February 2015.

6. ***Fusarium*** Link, Mag. der Ges. Naturf. Fre. Berlin **3**(1): 10 (1809).   (**Fig. 1F**)

Colonies white in early stage and later changed to violet-purple. Monophialides and polyphialides with conidia in chain formation at the head. Macroconidia 1-3 septa, a slightly curved apical cell and a scarcely developed base cell. Microconidia oval form, with a flat base and no septa. Chlamydospores absent.

Specimen examined: Isolated from the seeds of *Zea mays*, BARI, Joydebpur, Gazipur, U Sadia 5, 7 August 2014.

7. ***Fusarium oxysporum*** Schltdl., Flora Berol., Pars secunda: Cryptogamia: 106 (1824)

(**Fig. 1G**)

Colonies whitish, cottony, with purple tinge at maturity. Mycelium hyaline. Microconidia borne on simple phialide arising laterally on hyphae. Microconidia hyaline, oval-ellipsoid cylindrical, 5-13 × 2.3-4 µm. Macroconidia hyaline, straight to curved, 3-5 septate, 25-60 × 3-6 µm.

Specimen examined: Isolated from the seeds of *Oryza sativa*, BARI, Joydebpur, Gazipur, P Chawdhury 165, 10 December 2014.

8**. *Fusarium solani*** (Mart.) Sacc., Michelia **2**(7): 296 (1881).   (**Fig. 1H**)

Colonies whitish, cottony. Mycelium striate, sparse to dense floccose, at maturity brownish vinaceous pigmentation observed. Microconidia developed from lateral conidiophores. Phialides narrow slightly towards apex, 40-75 × 2-3.5 µm. Microconidia oval shaped, 0-1 septate, 8-17 × 2.5-4.5 µm. Macroconidia hyaline, canoe shaped, 0-7 septate, 36-54 × 4.5-6 µm.

Specimen examined: Isolated from the seeds of *Oryza sativa*, BARI, Joydebpur, Gazipur, P Chawdhury 192, 18 February 2014.

9. ***Penicillium*** Link, Mag. der Ges. Naturf. Freunde Berlin **3**(1): 16 (1809).   (**Fig. 1I**)

Colonies typically exhibits certain striking characteristics. These include color and color changes, floccose, broadly spreading and wrinkled. Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex to form a brush like conidia bearing structures called phialides. Conidia pale green, 1-celled, mostly globose or ovoid, produced basipetally.

Specimen examined: Isolated from *Trichosanthes dioica*, Ananda Bazar, Dhaka, AA Islam 08, 10 June 2014.

10. **Trichoderma** Pers., Neu. Mag. für die Botanik **1**: 92 (1794). (**Fig. 1J**)

Colonies green. Conidiophores hyaline, upright, much branched, not verticillate; phialides, single or in groups. Conidia hyaline, 1-celled, ovoid, borne in small terminal clusters; usually easily recognized by its rapid growth and green patches or cushions of conidia.

Specimen examined: Isolated from *Trichosanthes dioica*, Hatirpool Bazar, Dhaka, AA Islam 09, 18 August 2014.
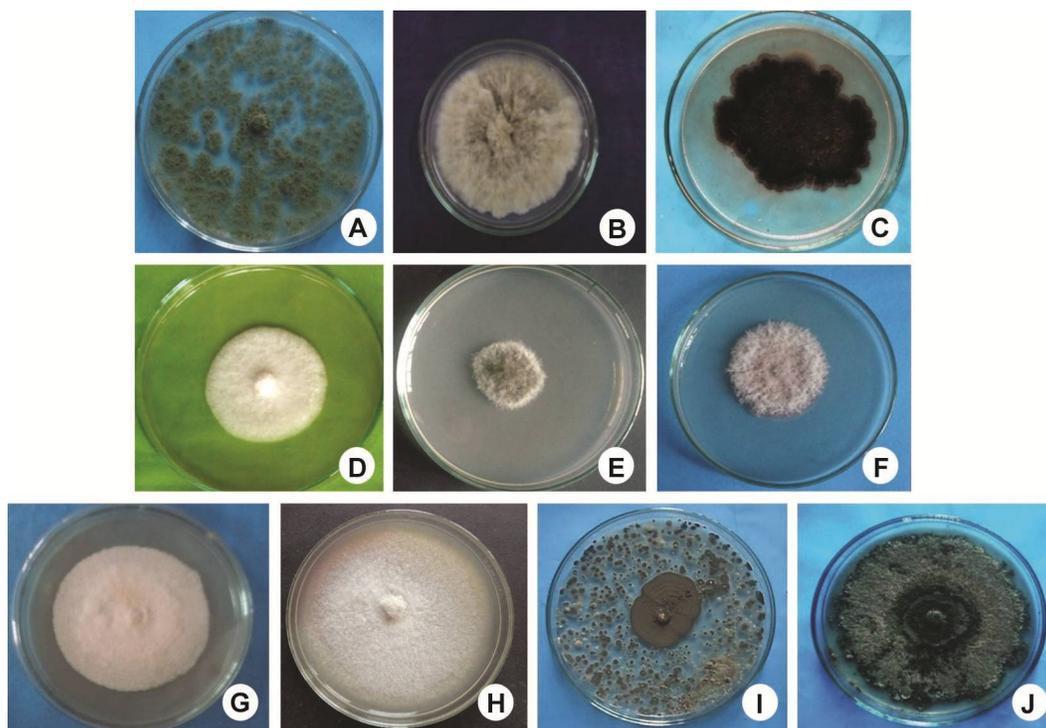

Fig. 1. Colonies of fungal isolates: A. *Aspergillus flavus,* B. *Bipolaris oryzae,* C. *B. sorokiniana,* D. *Colletotrichum gloeosporioides,* E. *Corynespora cassiicola,* F. *Fusarium* sp., G. *Fusarium oxysporum,* H. *Fusarium solani,* I. *Penicillium* sp. and J. *Trichoderma* sp.

Molecular identification

In the present investigation, it was difficult to identify some fungal species such as *Fusarium*, *Penicillium*, *Trichoderma*, etc. based on the morphological features. Therefore, molecular characterization of the fungal species was conducted for proper identification using sequence analysis of ITS region.

Ten isolates were identified by analyzing ITS regions sequences using the ITS1 and ITS4 as forward and reverse primers. In order to confirm at the genomic sequence level, PCR amplified bands (~600 bp) from ten samples were subjected to automated sequencing followed by BLAST analysis (Fig. 2).

Table 1. Per cent identities of nucleotide of selected fungi reported worldwide.

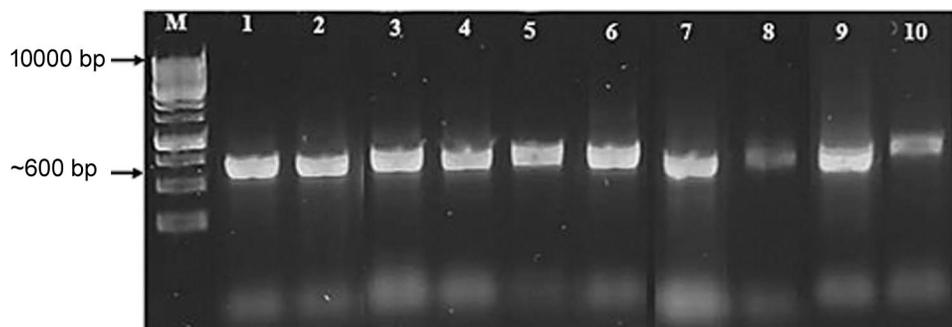| Isolates No.	Length (base pair)	Accession No.	Description	Max score	Total score	Query coverage (%)	E-value	Identity
1	560	KM030311.1	*Bipolaris sorokiniana* strain NRRL 62783 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	953	953	95%	0.0	99%
2	530	KR259525.1	*Colletotrichum gloeosporioides* isolate PAK16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	939	939	100%	0.0	99%
3	560	GU373642.1	*Bipolaris oryzae* isolate Bo9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	926	926	94%	0.0	98%
4	520	KF751873.1	*Fusarium oxysporum* strain MJ-23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	905	905	98%	0.0	98%
5	530	JN541214.	*Corynespora cassiicola* isolate E9807C 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	905	905	96%	0.0	98%
6	540	GU566197.1	*Penicillium pinophilum* strain A26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	939	939	95%	0.0	99%
7	560	LN846705.1	*Trichoderma harzianum* genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate T30	880	880	96%	0.0	96%
8	600	KP641161.1	*Fusarium chlamydosporum* internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	398	398	81%	8e-107	82%
9	560	KF918567.1	*Fusarium solani* strain DE25 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	514	514	71%	7e-142	90%
10	570	LN482572.1	*Aspergillus flavus* genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, strain TUHT176	848	848	96%	0.0	94%

Fig. 2. Gel electrophoresis of the PCR product of 10 fungal isolates performed by ITS1(F) and ITS4 (R) primers and showing ~600 bp amplification.

Analysis of the nucleotide sequences of the amplified fragments allowed the identification of the isolates at the species level. ITS1 and ITS4 primers depicted the identities of the isolated fungi more than 90% sequence similarity except the isolate number 8 which showed 82% sequence similarity (Table 1).

ITS sequences of ten samples were analyzed through NCBI-BLAST program database search system. Results obtained from the BLAST database showed that 99% nucleotide identities with *Bipolaris sorokiniana* strain NRRL 62783, *Colletotrichum gloeosporioides* isolate PAK16 and *Penicillium pinophilum* strain A26; 98% nucleotide identities with *B. oryzae* isolate Bo9, *Corynespora cassicola* isolate E9807C and *Fusarium oxysporum* strain MJ-23; 96% nucleotide identities with *Trichoderma harzianum* genomic DNA sequence; 94% nucleotide identities with *Aspergillus flavus* genomic DNA; 90% nucleotide identities with *Fusarium solani* strain DE25 and 82% nucleotide identities with *Fusarium chlamydosporum* internal transcribed spacer 1.

Table 2. Comparison between morphological and molecular identification of ten fungal isolates.

Isolates No.	Morphological identification	Molecular identification
1	*Bipolaris sorokiniana*	*Bipolaris sorokiniana* strain NRRL 62783
2	*Colletotrichum gloeosporioides*	*Colletotrichum gloeosporioides* isolate PAK16
3	*Bipolaris oryzae*	*Bipolaris oryzae* isolate Bo9
4	*Fusarium oxysporum*	*Fusarium oxysporum* strain MJ-23
5	*Corynespora cassiicola*	*Corynespora cassiicola* isolate E9807C
6	*Penicillium* sp.	*Penicillium pinophilum* strain A26
7	*Trichoderma* sp.	*Trichoderma harzianum* genomic DNA
8	*Fusarium* sp.	*Fusarium chlamydosporum*
9	*Fusarium solani*	*Fusarium solani* strain DE25
10	*Aspergillus flavus*	*Aspergillus flavus* genomic DNA

Comparison between the morphological and molecular identification is presented in Table 2. Out of ten fungal isolates, *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. were not be able to identify morphologically up to species level. But with the help of molecular technique the above three fungi identified as species level such as *Fusarium chlamydosporium*, *Penicillium pinophilum* and *Trichoderma harzianum*.

From the perusal of literature, it is revealed that *Fusarium chlamydosporum* and *Penicillium pinophilum* has not been reported in any relevant literature in Bangladesh (Shamsi *et al.*, 2017, 2018; Siddiqui *et al.,* 2007). Among the isolated fungi, *Fusarium chlamydosporum* and *Penicillium pinophilum* are new record for Bangladesh. Hence, *Fusarium chlamydosporum* and *Penicillium pinophilum* are reported here first time from Bangladesh.

The present investigation suggests that barcode based molecular technique is more accurate, rapid and reliable means of fungal identification. ITS-based molecular techniques may be a significant complement to traditional mycological detection by culture, which is becoming increasingly important in clinical mycology as well as plant pathology.

References

Barnett, H.L. and Hunter, B.B. 1972. Illustrated Genera of Imperfect Fungi. Burgess Pub. Co. U. S. A. pp. III +241.

Booth, C. 1971. The Genus Fusarium. The Commonwealth Mycological Institute, Kew, Surrey, England. pp. 237.

CAB, (Commonwealth Agricultural Bureau) 1968. Plant Pathologist's Pocket Book.1st edn. The Commonwealth Mycological Institute, England. pp. 267.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 608.

Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 608.

Ellis, M.B. and Ellis, J.P. 1997. Micro Fungi on Land Plants. An identification Handbook. The Commonwealth Mycological Institute, England. pp. 868.

Gilman, J.C. 1967. A Manual of Soil Fungi. Oxford and IBH Publishing Co., New Delhi, 2nd edition (Revised). pp. x + 450.

Haynes, K., Westerneng, T. and Fell, J. 1996. Rapid detection and identification of pathogenic fungi by polymerase chain reaction amplification of large subunit ribosomal DNA. J. Med. Vet. Mycol. **33**: 319–325.

Islam, M.N. and Mukherjee, S.K. 2011. Construction of MYMIV based gene silencing vector and its use. ISBN: 978-3-8443-8820-6. LAP-LAMBERT Academic Publishing GmbH & Co KG. Dudweiler Landstr. 99, 66123 Saarbrücken, Germany.

Makimura, K., Murayama, S.Y. and Yamaguchi, H. 1994. Detection of a wide range of medically important fungi by polymerase chain reaction. J. Med. Microbiol. **40**: 358–364.

Njambere, E.N., Attanayake, R.N. and Chen, W. 2010. Applications of molecular markers and DNA sequences in identifying fungal pathogens of cool season grain legumes. Gherbawy and Voigt (eds.), Molecular Identification of Fungi. Springer-Verlag Berlin Heidelberg. pp. 79–91.

Raper, K.B. and Thom, C. 1949. Manual of the Penicillia. Williams and Wilkins, Baltimore, MD, USA.

Rossman, A.Y. and Palm-Hernandez, M.E. 2008. Systematics of plant pathogenic fungi. Why it matters. Plant Dis. **92**: 1377–1386.

Sandhu, G.S., Kline, B.C. and Stockman, L. 1995. Molecular probes for diagnosis of fungal infections. J. Clin. Microbiol. **33**: 2913–2919.

Shamsi, S., Hosen, S. and Ahmed, A. 2018. Fungi associated with leaves of *Sonneratia apetala* Buch. Ham and *Sonneratia caseolaris* (L.) Engler from Rangabali coastal zone of Bangladesh. Dhaka Univ. J. Biol. Sci. **27**(2): 155–162.

Shamsi, S., Hosen, S. and Begum, M. 2017. New record of *Gonatophragmium mori* (Sawada) Deighton on *Ficus hispida* L. from Bangladesh. Bangladesh J. Plant Taxon. **24**(1): 125–127.

Siddiqui, K.U., Islam, M.A., Ahmed, Z.U., Begum, Z.N.T., Hassan, M.A., Khondker, M., Rahman, M.M., Kabir, S.M.H., Ahmad, M., Ahmed, A.T.A., Rahman, A.K.A. and Haque, E.U. (Eds) 2007. Encyclopedia of Flora and Fauna of Bangladesh. Vol. 2. Cyanobacteria, Bacteria and Fungi. Asiatic Society of Bangladesh, Dhaka. pp. 415.

Sutton, B.C. 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. The Commonwealth Mycological Institute, England. pp. 696.

Thom, C. and Raper, K.B. 1945. A Manual of the Aspergilli. Williams and Wilkins, Baltimore, MD, USA.