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MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF FUNGI ISOLATED FROM SELECTED BRRI RICE VARIETIES

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Keywords: Fungi; Tissue planting method; ITS; PCR amplification; Sequence analysis.

Abstract

A total of 19 fungal species were isolated from the seeds of selected rice varieties (BRRI dhan 90 to BRRI dhan 99) following Tissue planting method and Blotter method. The isolated fungi were ***Aspergillus niger*****,** ***A. ochraceus*****,** *A. oryzae, A. tamarii***,** ***A. terreus*****,** *Chaetomium globosum***,** *Cladosporium oxysporum, Colletotrichum gloeosporioides, Corynespora cassiicola, Curvularia lunata, Curvularia soli, Daldinia eschscholtzii, Fusarium solani, Penicillium oxalicum,Penicillium sclerotiorum, Pestalotiopsis guepinii, Pyricularia oryzae* and ***Rhizopus stolonifer*****.** Fourteen fungi were selected for molecular identification. Out of the 19 fungal isolates, 14 were confirmed up to species level through ITS sequence based molecular analysis. Among the isolated fungi *Pnicillium sclerotiorum* and *Curvularia soli* are the new record for Bangladesh. Association of *Daldinia eschscholtzii* with rice seeds is also recorded first time from world.

Introduction

Rice (*Oryza sativa* L.) is the staple food crop for more than half of the global population including Bangladesh. It is the second largest cereal crop produced all over the world. It belongs to the family Poaceae, mostly grown in tropical and sub-tropical climate. Rice suffers from more than 60 different diseases of which fungal disease is one of them (Fakir *et al.*, 2002). For establishing effective disease control measure, quarantine measures, protecting agricultural crops from pathogenic fungi, correct identification of pathogenic fungi is very essential. For these purposes molecular identification of pathogenic fungi is important. To distinguish genetic relationships in fungi, various PCR methods such as, DNA amplification fingerprinting (Bentley *et al.* 1998 and Gerlach *et al.* 2000), DNA sequence analysis (Geiser *et al.*, 2004) etc. have been conducted previously.

Isolation of total genomic DNA from fungi suitable for polymerase chain reaction (PCR) amplification and other molecular applications was described by Amer *et al.* (2011).

The identification of *Cochliobolus carbonum* was done based on morpho-pathological characteristics and Internal Transcribed Spacer (ITS) region sequencing analysis by EL-Shafey *et al.* (2018).

Morphological characterization and molecular analysis are performed for correct identification of isolated fungi. The sequence results obtain using ITS1 and ITS4 are compared with NCBI GenBank and BOLD database using BLAST analysis. The aim of the study was to investigate the morphological and molecular identification of fungi associated with selected BRRI rice varieties.

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Material and Methods

Ten varieties of BRRI rice seeds i.e. BRRI dhan 90 to BRRI dhan 99 were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. Samples were collected during August 2021.

Isolation and morphological identification of fungi

Fungi associated with selected BRRI rice varieties were isolated with following "Tissue planting method" on PDA medium (CAB, 1968). The mycelia and spore observation were done at 40× magnification. The microphotographs of the fungi along with the measurement of spore size were taken by a high-resolution microscope facilitated with camera (Nikon optiphot-2 trinocular microscope, Japan). Identification of the isolates was determined following standard literatures (Thom and Rapper, 1945; Rapper and Thom, 1949; Benoit and Mathur, 1970; Booth, 1971; Subramanian, 1971; Ellis, 1971, 1976; Barnett and Hunter, 1972; Sutton, 1980). The specimens were preserved in the Herbarium, Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Bangladesh.

Molecular characterization of fungi

Genomic DNA extraction was done according to the methods by Amer *et al*. (2011) with minor corrections.

DNA extraction

For genomic DNA extraction, monoconidial isolates were grown on PDA medium at 28°C for 10 days. Fungal mycelium was harvested by scraping the surface of 10 days old monoconidial cultures with a sterile spatula from the Petri plates. One gm of fungal mycelium of each isolate was taken in 1.5 ml sterile Eppendorf tube. The mycelium was immediately grinded with a homogenizer machine with 400µl sterile extraction buffer (200mM Tris- HCI, 250mM NaCl, 25mM EDTA, 0.5% SDS) in each Eppendorf tube. Then 6 µL of 20 mg/ml RNase was added in each Eppendorf. Tubes were stirred with a vortex mixer so that the mixture became homogenous. The tubes were transferred to 65°C preheated water bath for 10 minutes. The samples were taken from the water bath and cooled down to room temperature. 130 µL of 3M sodium acetate, pH 5.2 was added in each tube. Tubes were vortexed for 30s at maximum speed and incubated at -20° C for 10 minutes. The samples were centrifuged at 13,000 rpm for 15 minutes. The supernatants were transferred to fresh tubes and equal volume of chloroform: isoamyl alcohol mixture (24:1) was added and mixed by gentle inversion and then tubes were centrifuged at 12000 rpm for 5 minutes. The aqueous (upper) layer was carefully transferred to new tubes and equal volume of cold isopropanol was added to each sample, mixed well and samples were incubated at 20°C for 10 minutes. Samples were then centrifuged at 6000 rpm for 20 minutes. The supernatant was discarded and the pellet was washed twice with 700 µL of 70% ethanol. The DNA pellets were subsequently air dried in an oven at 40°C for at least 10 minutes. The resultant DNA pellet was then resuspended in 100 µL of 1x TE (10 mM Tris- HCI, 1 mM EDTA) buffer (pH 8.0). The DNA was allowed to dissolve overnight at 4°C. Then it was stored at 20°C for further analyses.

PCR amplification

Samples Molecular identification of the isolates was performed using the internal transcribed spacer (ITS) region. PCR amplification was conducted using the ITS1 (5'- TCCGTAGGTGAA-CCTGCGG-3') as forward and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') as reverse primers. 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 H2O.

Reaction mixture was vortexed and centrifuged in a microcentrifuge. The PCR was initiated by an initial denaturation step at 94ºC for 5 minutes following 30 cycles of 94, 54 and 72ºC each for 30 sec, with a final extension step of 5 min at 72ºC and ended with 4ºC. PCR amplified products were stored in – 20ºC 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 30 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).

Results and Discussion

Morphological identification

A total of 19 species of fungi were isolated from ten BRRI rice seeds. They were *Aspergillus niger* Van Tieghem**,** *A. ochraceus* K. Wilh**.**, *A. oryzae* (Ahlb.) Cohn, *A. tamari* Kita G**.**, *A. terreus* Thom**,** *Chaetomim globosum* Kunze ex Fr**.**, *Cladosporium oxysporum* Berk. & Curt., *Colletotrichum gloeosporioides* Penz. & Sacc., *Corynespora cassiicola* Berk. & Curt., *Curvularia lunata* (Wakker) Boedjin, *Curvularia soli* Y. Marín & Crous**,** *Daldinia eschscholtzii* (Ehrenb.: Fr.) Rehm., *Fusarium solani* (Mart.) Sacc., *Penicillium oxalicum* Currie & Thom**,** *P enicillium sclerotiorum* J.F.H. Beyma**,** *Pestalotiopsis guepinii* (Desm.) Stey., *Pyricularia oryzae* Cavara and *Rhizopus stolonifer* (Ehrenb.) Vuill.

Key morphological features of ~~the~~ isolated fungi are given below:

1. ***Aspergillus niger*** van Tiegh., Ann. Sci. Nat.,. Bot. 8: 240, (1867) [MB#284309]

(Fig. 1. A-B)

Colonies effuse, black. Vesicle covered by closely packed more or less clavate branches. Conidia catenulate, dry, usually globose, echinulate, dark brown in color, 2-4µm in length.

Material studied: Isolated from seven varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

2. ***Aspergillus ochraceus*** K. Wilh., Beiträge zur Kenntnis der Pilzgattung Aspergillus: 66 (1877

(Fig. 1. C-D)

Conidial heads radiate, splitting into several columns with age. Conidiophore stipes brownish, commonly 3.5-5 µm in length, with roughened walls. Vesicles spherical, thin-walled, hyaline.

Material studied: Isolated from only one variety of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 10 December 2021.

3. ***Aspergillus oryzae*** (Ahlb.) Cohn, Jahresbericht der Schlesischen Gesellschaft für Vaterländische Kultur 61: 226 (1884) **(Fig. 1. E-F)**

Colonies growing rapidly, pale greenish-yellow, olive-yellow or with different shades of green, typically with dull brown shades with age. Conidiophore stipes hyaline, up to 4-5 µm in

length. Vesicles subspherical. Conidia spherical to ovoidal, smooth-walled to roughened, greenish to brownish.

Material studied: Isolated from seven varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

4. *Aspergillus tamarii* Kita, Centralbl. Bakteriol., Abt. 2: 433 (1913) **(Fig. 1. G-H)**

Conidial heads compact and spherical or loosely radiate. Conidiophore stipes usually 1-3 µm in length, hyaline, usually roughened. Conidia echinulate to tuberculate, subspherical.

Material studied: Isolated from six varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

5. *Aspergillus terreus* Thom, American Journal of Botany 5 (2): 85 (1918) **(Fig. 1. I-J)**

Colonies growing rapidly, cinnamon to orange-brown or brown, velvety smooth-walled, hyaline, Conidia globose to slightly ellipsoidal, smooth-walled, mostly 2-3 µm diam, uninucleate.

Material studied: Isolated from two varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

6. *Chaetomium globosum* Kunze, Mycologische Hefte 1:16 (1817) [MB#172545] **(Fig. 1. K-L)**

Colony is punctiform, greyish, numerous on substrate. Hyphae brown septate, profusely branched. Perithecia dark brown with long hairy wavy appendages. Ascospores lemon shaped, 11-14 × 8-11 µm.

Material studied: Isolated from only one variety BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

7. ***Cladosporium oxysporum*** Berk. & Curt.,1886, *J. Linn. Soc*., 10 (46) : 362 **(Fig. 1. M-N)**

Colonies effuse, greyish brown, thinly hairy, Conidiophore solitary or in fascicles, straight or slightly flexuous, distinctly nobose, pale to mid brown. smooth, 3-6 µm.

Material studied: Isolated from three varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 10 December 2021.

8. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., Atti dell´Istituto Veneto Scienze Sér. 6, 2: 670 (1884) **(Fig. 1. O-P)**

Conidiomata acervular, amphigenous, mostly epiphyllous, subepidermal. Setae often present on acervuli but sometimes arising alone from stomata, forming dense fascicles and bearing enteroblastic conidia apically. Appressoria with entire or sometimes slightly irregularly lobate margin, ovate, globose or ampulliform, brown to medium brown.

Material studied: Isolated from four varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

9. *Corynespora cassiicola* (Berk. & Curt.) Wei, 1950 **(Fig. 1. Q-R)**

Colonies effuse, grey or brown, thinly hairy; viewed under a binocular dissecting microscope the conidiophores appear iridescent. Conidia solitary or in chains of 2-6, very variable in shape, obclavate to cylindrical, straight or curved, subhyaline to rather pale olivaceous brown or brown, smooth.

Material studied: Isolated from only one variety of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

10. *Curvularia lunata* (Wakker) Boedijn. [Cochliobolus linatus Nelson & Haasis]. Ellis MB, Mycol. Pap. 106: 2-43, 1966. **(Fig. 1. S-T)**

Colonies effuse greenish black Conidiophores solitary, mostly unbranched, straight or slightly undulating, brown, septate up to 37-64 µm long. Conidia mostly three septate, brown, slightly curved, third cell from the base in broader and darker than others, smooth.

Material studied: Isolated from six varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

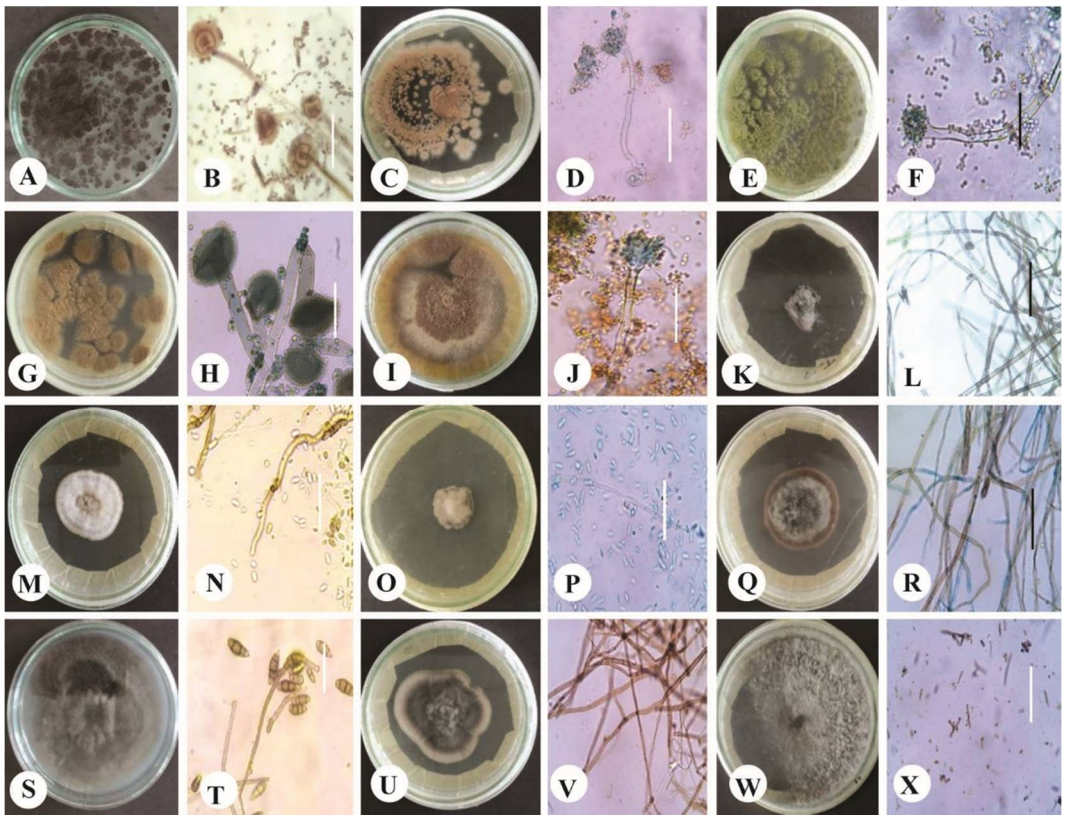

Fig. 1. Colony on PDA medium and conidiophore with conidia under microscope (Bar = 50 µm). A-B. *Aspergillus niger*, C-D. *A. ochraceus*, E-F. *A. oryzae*, G-H. *A. tamarii*, I-J. *A. terreus* and K-L. *Chaetomium globosum*. M-N. *Cladosporium oxysporum*, O-P. *Colletotrichum gloeosporioides*, Q-R. *Corynespora cassiicola*, S-T. *Curvularia lunata*, U-V. *Curvularia soli* and W-X. *Daldinia eschscholtzii*.

11. *Curvularia soli* Y. Marín & Crous, Studies in Mycology 86: 161 (2017) **(Fig. 1. U-V)**

Conidiophores arising in groups, septate, straight or flexuous, geniculate at upper part, smooth to verruculose, unbranched, Conidia verruculose, curved, rarely straight, middle cells disproportionately enlarged, reniform, rarely ellipsoidal, pale brown to brown, apical and basal

cells paler than middle cells being subhyaline to pale brown, hila protuberant, flat, darkened, thickened, 1.3–3.5 µm.

Material studied: Isolated from two varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

12. *Daldinia eschscholtzii* (Ehrenb.: Fr.) Rehm, Ann. Mycol. 2: 175. 1904**.** **(Fig. 1. W-X)**

Colonies white to smoky gray.

Material studied: Isolated from only one of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

13. *Fusarium solani* (Mart.) Sacc., Michelia 2 (7): 296 (1881) **(Fig. 2. A-B)**

Colonies sparse, floccose, greyish-white mycelium. Macroconidia developing in 4-7 days from branched and well developed conidiophores, cylindrical to falcate, often slightly wider towards the apex and with a well marked foot cell. Chlamydospores globose to oval, smooth to rough walled, 8-9 µm, developing intercalary or terminally.

Material studied: Isolated from five varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

14. *Penicillium oxalicum* Currie & Thom, Journal of Biological Chemistry 22: 289 (1915) **(Fig. 2. C-D)**

Colonies growing rapidly, reverse pale to yellow or pinkish. Conidiophores smooth, 3-3.5 µm long. Metulae appressed. Phialides in verticils of 6-10, acerose, 10-15 x 3-3.5 µm. Conidia elliptical, smooth (reticulate in SEM), very large, 5-5,5 x 3-3.5 µm.

Material studied: Isolated from five varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

15. *Penicillium sclerotiorum* J.F.H. Beyma, Zentralblatt für Bakteriologie und Parasitenkunde, Abteilung 2 96: 416 (1937) **(Fig. 2. E-F)**

Sclerotia orange-red, 500- 700 µm diam, very hard, consisting of hyaline, polygonal cells with very thick walls, surrounded by sterile. Asci and ascospores not observed. Conidiophores strictly simple, only very rarely with one lower branch-like metula. Phialides in compact, with a cylindrical base and at the apex narrowed into a short, Conidia ellipsoidal to pear-shaped, smooth-walled or nearly so, commonly a few of them globose, 2-3 µm diam, at first hyaline, later brown, finely roughened.

Material studied: Isolated from three varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

16. *Pyricularia oryzae* Cavara, Fungi Longobardiae exsiccati sive Mycetum specimina in Longobardia collecta, exsiccata et speciebus novis vel criticis, iconibus illustrata Pug. I: no. 49 (1891) **(Fig. 2. G-H)**

Cultures greyish. Conidiophores single or in fascicles, simple, rarely branched, showing sympodial growth. Conidia formed singly at the tip of the conidiophore at points arising sympodially and in succession, pyriform to obclavate, narrowed toward tip, rounded at the base, with a distinct protruding basal hilum. Chlamydospores often produced in culture, thick-walled, 5-12 µm diam.

Material studied: Isolated from only one variety of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 10 December.

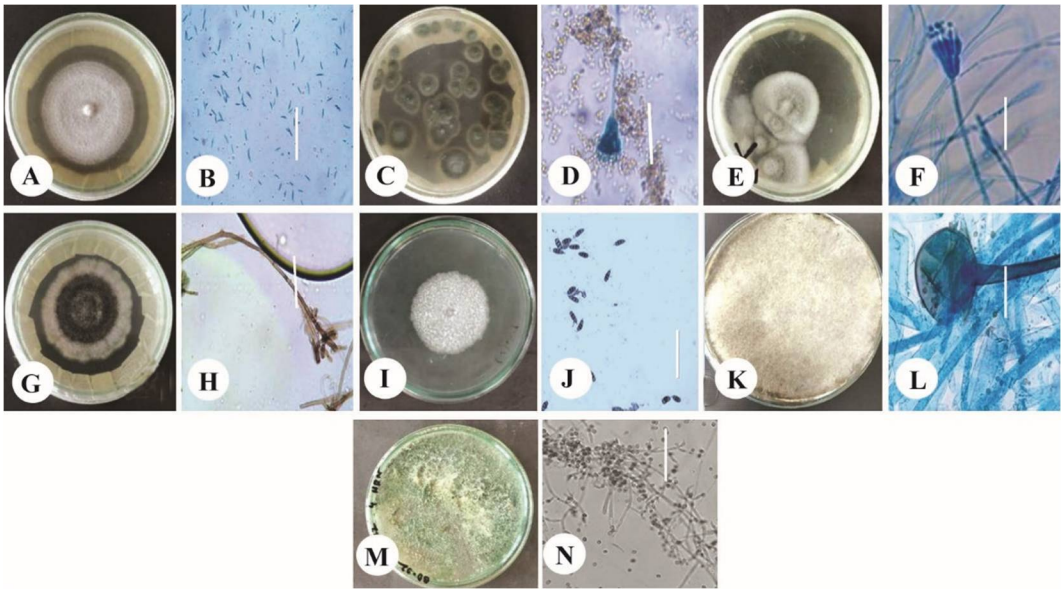

Fig. 2. Colony on PDA medium and conidiophore with conidia under microscope (Bar = 50 µm). A-B. *Fusarium solani*, C-D. *Penicillium oxalicum*, E-F. *penicillium sclerotiorum*, G-H. *Pyricularia oryzae*, I-J. *Pestalotiopsis guepinii*, K-L. *Rhizopus stolonifer*, M-N. *Trichoderma virens*.

17. ***Pestalotiopsis guepinii*** (Desm.) Stey., Bull. Jard. Bot. État Brux. **19**(3): 312**(**1949).

(Fig. 2. I-J)

Colonies white, cottony, reverse white. Hyphae septate, branched, hyaline. Acervuli black, small, shining. Conidiophores septate, branched, dark brown, cylindrical or lageniform, Conidia fusiform, straight or slightly curved, mostly 3 euseptate: basal cells hyaline, truncate, with an endogenous, cellular, appendage: apical cell conic, hyaline, with 2 or more apica, simple or branched, spathulate or spathulate appendages.

Material studied: Isolated from three varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

18. ***Rhizopus stolonifer*** (Ehrenb.) Vuill., Revue Mycologique Toulouse 24: 54 (1902)

(Fig. 2. K-L)

Mycelium coenocytic, well developed, branched and fluffy. Mycelium produces many aerial stolons that develop rhizoids at certain points. Directly above the rhizoids one or more sporangiospores are produced. The central portion of sporangium becomes highly vacuolated and it eventually surrounded by a wall that separates it's from the peripheral zone. The central portion is the columella. Sporangium produces non-motile sporangiospores.

Material studied: Isolated from ten varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 31 October 2021.

19. ***Trichoderma virens*** Pers., (1794). **(Fig 2. M-N)**

Colony effuse, light green. Conidiophores are hyaline, much branched, bearing phialides single or in groups. Conidia hyaline, powdery mass, 1-celled, ovoid, borne in small terminal clusters. It is used in the commercial production of enzyme cellulase.

Material studied: Isolated from two varieties of BRRI rice seeds (*Oryza sativa* L.) collected from BRRI, Joydebpur, Gazipur, HN Nishi, 10 December.

Molecular identification

Among the 19 fungi, some Isolates were unable to identify up to species level based on the morphological features only. Therefore, molecular characterization of the fungal isolates were conducted for proper identification using ITS sequence analysis. Out of the 19 fungi 14 were confirmed up to species level through ITS sequence based molecular analysis (Table 1).

Genomic DNA was isolated successfully from fourteen fungi. PCR was conducted using ITS1 (Forward) and ITS4 (Reverse) primers and ~550 bp DNA band was amplified. Sequence analysis of the amplified DNA through BLAST search in GenBank was conducted and found 90.43 to 99.60% (Fig. 3). 90-99% nucleotides identities with isolated fungi which was presented in Table 1.

Table 1. BLAST analysis of the amplified sequences from the isolated DNA of fungi.

Sample No.	Name of Fungi	Max score	Total score	Query coverage	E Value	Percent Identity (%)	NCBI Gene Bank Acc. No.
N3	*Aspergillus tamarii*	701	701	87%	0.0	94.61%	KX610720.1
N13	*Cladosporium oxysporum*	652	652	74%	0.0	96.50%	MF511908.1
N12	*Colletotrichum gloeosporioides*	466	466	88%	1e-126	90.43%	OK584697.2
N1	*Corynespora cassicola*	883	883	99%	0.0	97.51%	MW300948.1
N9	*Curvularia lunata*	815	815	99%	0.0	99.55%	MT647915.1
N16	*C. soli*	883	883	98%	0.0	99.59%	MT565489.1
N15	*Daldinia eschscholtzii*	484	484	98%	2e-132	99.60%	MT626601.1
N10	*Fusarium solani*	782	782	98%	0.0	98.02%	MH684735.1
N7	*A. oryzae*	268	286	99%	1e-67	99.33%	OP237512.1
N6	*Penicillium oxalicum*	534	534	96%	3e-147	98.37%	LT559084.1
N8	*P. sclerotiorum*	392	392	98%	1e-104	98.23%	MT000475.1
N14	*Pestalotiopsis guepinii*	246	246	98%	0.0	96.69%	KF171535.1
N5	*Pyricularia oryzae*	46.1	46.1	79%	0.0	97.30%	CP050920.1
N2	*Trichoderma virens*	893	893	90%	0.0	96.38%	MZ769121.1

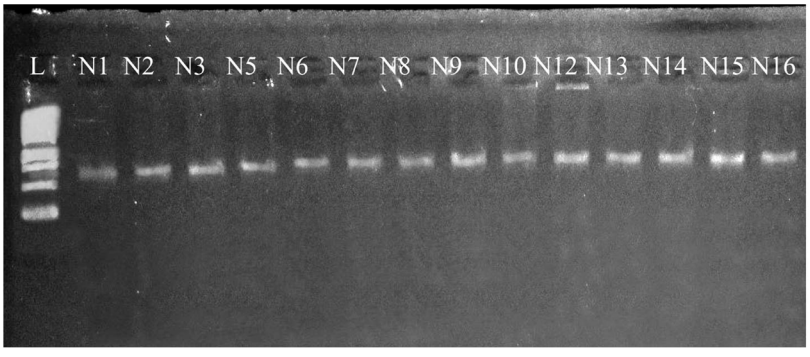

Fig. 3. Gel electrophoresis of the PCR product of 14 fungi performed by ITS1 (F) and ITS4 (R) primers and showing ~550 bp amplification.

To confirm identity, the obtained DNA sequences of the isolated fungi were matched with the available sequences in NCBI database. Results obtained from the BLAST database

Among the isolated fungi, *penicillium sclerotiorum* and *Curvularia soli* are the new record for Bangladesh as these were not documented in relevant literature (Siddiqui *et al*., 2007; Shamsi S, 2017; Nahar *et al*., 2019; Amina *et al*., 2022). Association of *Daldinia eschscholtzii* with rice seeds is also recorded first time from world.

This present investigation suggests that molecular technique is more accurate and rapid means of fungal identification. ITS-based molecular identification methods might be an important complement to conventional mycological detection by culture.

Table 2. Comparison between morphological and molecular identification of 14 fungal isolates.

Isolates No.	Morphological identification	Molecular identification
N1	Unidentified	*Corynespora cassicola*
N2	*Trichoderma* sp.	*Trichoderma virens*
N3	*Aspergillus* sp.	*Aspergillus tamari*
N5	Unidentified	*Pyricularia oryzae*
N6	*Penicillium* sp.	*Penicillium oxalicum*
N7	*Aspergillus* sp.	*Aspergillus oryzae*
N8	*Penicillium* sp.	*Penicillium sclerotiorum*
N9	*Curvularia* sp.	*Curvularia lunata*
N10	*Fusarium* sp.	*Fusarium solani*
N12	*Fusarium* sp.	*Colletotrichum gloeosporioides*
N13	Unidentified	*Cladosporium oxysporum*
N14	Unidentified	*Pestalotiopsis guepinii*
N15	Unidentified	*Daldinia eschscholtzii*
N16	*Curvularia* sp.	*Curvularia soli*

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