PREVALENCE OF SEED-BORNE FUNGI ASSOCIATED WITH SELECTED RICE VARIETIES OF BRRI

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Abstract

Selected rice varieties such as BR-78, 80, 81, 82, 83, 84, 86, 87, 88 and 89 were used in this experiment to investigate the prevalence of seed-borne fungi. A total of twelve fungi belonging to six genera was isolated from the seeds of selected BRRI (Bangladesh Rice Research Institute) rice varieties by using tissue planting method. They were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A. ochraceous* Wilhelm, *A. terreus* Thom, *Curvularia lunaata* (Wakker) Boedijn, *Curvularia* sp. 1, *Drechslera oryzae* Breda de Haan (Subramanian and Jain), *Penicillium* sp.1, *Penicillium* sp. 2 and *Rhizopus stolonifera* (Ehrenb.) Vuill., Among the isolated fungi *A. niger* was the most prevalent fungi associated with the seeds of the rice varieties with a mean per cent frequency of 16.93 and the least prevalent fungi was *A. ochraceous* with a mean per cent frequency of 0.27. Mean per cent frequency of *A. niger* (16.33) was highest which was followed by *A. flavus* (7.27), *Penicillium* sp.1 (5.93), *Penicillium* sp. 2 (3.27), *Drechslera oryzae* (2.67) and *A. terreus* (2.53). Among the identified seed-borne fungi *Curvularia lunata* and *Drechslera oryzae* were found as pathogenic and cause seed-borne diseases.

Key words: Seed-borne fungi; Prevalence; BRRI rice seeds; Released variety.

INTRODUCTION

Rice suffers from more than 60 different diseases. In Bangladesh, 43 diseases are known to occur on the rice crop. Among these diseases, 27 are seed borne of which 14 are of major importance (Ou 1985). During storage a lot of storage fungi grows in the rice seeds which causes seed health deterioration, reduction in seed germination rate, post-germination diseases and ultimately yield loss (Bakr *et al.* 2007). Some of the major diseases of rice caused by fungi are brown spot (*Drechslera oryzae* and *Bipolaris sorokiniana*), bakane (*Fusarium moniliforme*), blast (*Pyricularia oryzae*), sheath rot (*Sarocladium oryzae*), sheath blight (*Rizoctonia solani*), etc.

Currently, Bangladesh has achieved food security by cultivating high-yielding varieties. According to BRRI, there are a total of 94 hybrid rice varieties and more than 78 hybrid rice varieties are grown in the field (Bhandari *et al*. 2011). In Bangladesh several researches have been done separately on fungal association with BRRI rice varieties and their management up to BRRI dhan77 in different time span (Miah *et al.* 1985, Shamsi *et al.* 2010, Sultana *et al.* 2018, Chowdhury *et al*. 2018). After that, BRRI dhan78 to BRRI dhan 89 have been released from BRRI, but so far, no remarkable work has been done on seed borne diseases associated with the newly released varieties. Therefore, present investigation was undertaken to identify the fungi associated with selected BRRI dhan and prevalence of fungi in different storage condition.

MATERIAL AND METHODS

Collection of Samples

Seed samples of ten (500 g each) newly released varieties (BRRI dhan 78-BRRI dhan 89, except BRRI dhan 79 and BRRI dhan 85) were collected from Bangladesh Rice Research Institute, Joydebpur,

Gazipur in May 2019. The samples were then kept in brown paper bags and stored in airtight containers at room temperature (25ºC±2ºC).

Seed Quality Test

The 100 g seed sample of each variety was taken in a sterilized plastic tray. The samples were visually inspected to analyze seed quality. The samples were then separated into categories, such as: pure seeds, other crop seeds, healthy seeds, abnormal seeds and inert matter (Table 1). The per cent purity of the seeds was determined following formula:

$$\text{Per cent purity of seeds} = \frac{\text{weight of pure seeds}}{\text{total weight of seeds}} \times 100$$

Fungal Isolation

Fungal isolation was done using 'Blotter Method' (ISTA 1996) and 'Tissue Planting Method' (CAB 1968).

A. Blotter Method: A moist chamber was prepared by placing double layers of blotting paper in a 9 cm bottom diameter Petri plate. The blotter papers were soaked with sufficient amount of sterile distilled water. The plates were then sterilized in an autoclave at 121ºC temperature under 15lbs pressure for 20 minutes. 400 seed samples were taken from each variety. The seeds were surface sterilized with 10% chlorox solution for 5 minutes and then washed three times with sterile water. Then the seeds were placed in a piece of sterile filter paper containing Petri plates to remove excess water. Then the seeds were transferred in the moist chambers in a sterile condition. A total of 400 seeds was transferred in 20 moist chambers and 20 seeds were placed in each chamber. After that the moist chambers were placed in an incubator at 25ºC for 7-15 days. After incubation the associated fungi were isolated and seedling germination rate and mortality rate were calculated.

B. Tissue Planting Method: For tissue planting method, 400 seeds from each sample were taken and surface sterilized as the above stated method. The seeds were then placed in 20 pre-poured Petri plates (20 seeds per plate). Each plate contained 15 ml of Potato Dextrose Agar (PDA) medium (aqueous solution of 20% potato extract, 2% dextrose, 1.5% agar). The plates were then incubated at 25ºC for 5-7 days and after incubation associated fungi were isolated, and seedling germination rate and mortality rate were calculated. All the isolates were preserved in PDA slants at 4ºC temperature.

Identification of Fungi

Identification of the fungal isolates was determined based on morphological characteristics observed under a compound microscope following the standard literatures (Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Barnett and Hunter 2000).

Per cent frequency and seedling mortality calculation

Per cent frequency of the occurrence of the fungal isolates was calculated by adopting the formula of Spurr and Wetly (1972). Seedling mortality was determined after 10 days of incubation by the formula of Anonymous (2014).

Pathogenicity test

The pathogenicity test of the isolated fungi was determined according to the criteria of Koch's postulates and following the seed inoculation technique (Reddy and Subbayya 1989). Four hundred healthy seeds were selected from the BRRI rice variety BR-83. The seeds were then soaked in distilled water in a beaker for three hours. After that, surface sterilization was done with 10% clorox solution for 5 minutes and then the seeds were washed three times with distilled water. One hundred milliliter of spore suspensions of the isolated fungi at 10^4 concentration were prepared in a 250 ml sterilized beaker. Two hundred seeds were inoculated with spore suspension of the isolated fungi. Then the seeds were placed in sterilized 8-inch cotton plugged test tubes containing 10 ml 2% water agar medium. Healthy seeds were used as control. Observation was made for 4 weeks at 3-day intervals. Germination percentage of seeds, development of disease symptoms and mortality of seedling were recorded on healthy and inoculated seeds of BR-83. After 7 days of inoculation pathogenic fungi were re-isolated from inoculated seeds whereas the seedling from those healthy seeds remained fresh.

RESULTS AND DISCUSSION

For seed quality analysis the percentage of normal and abnormal rice seeds is presented in Table 1. Dry inspection indicated that the percentage of pure seeds ranged from 100 to 86.16. The highest percentage of pure seed was found in the variety BR-84 (100%) and lowest was recorded in BR-81 (86.16). The highest percentage of abnormal seeds was recorded in the variety BR-80 (14.01%) and lowest in BR-78 (0.56%).The highest percentage of other crop seeds was found in the variety of BR-86 (5.21%) and lowest in BR-81 (0.15%). The highest percentage of inert materials was found in the variety of BR-83 (8.00%) and lowest in BR-78 (0.49%).

Table 1. Per cent incidence of different types of rice seeds in dry seed inspection.

Name of rice varieties	Pure seeds	Healthyseeds	Dis coloured seeds	Abnormal seeds	Inert Matter
BR-78	98.49	97.93	5.74	1.02	0.49
BR-80	99.15	85.14	14.01	-	0.68
BR-81	86..16	87.26	9.62	0.15	2.98
BR-82	99.34	95.60	3.74	-	0.66
BR-83	92.00	90.00	2.00	-	8.00
BR-84	100.00	-	-	-	-
BR-86	86.93	78.33	8.6	5.21	7.86
BR-87	99.00	99.00	-	-	1.00
BR-88	93.89	89.02	4.87	4.86	1.25
BR-89	97.63	97.63	-	0.36	2.01

Absent (-)

A total of twelve fungi species belonging to six genera was isolated from the seeds of selected BRRI rice varieties following 'tissue planting method'. The isolated fungi *viz. Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceous*, *A. terreus*, *Curvularia lunata*, *Curvularia* sp.1, *Drechslera oryzae*, *Penicillium* sp.1, *Penicillium* sp. 2 and *Rhizopus stolonifer* were identified from the collected rice varieties in the tenure of July 2019-November 2019. *A. niger* was the most prevalent fungi among all of the varieties with a mean per cent frequency of 16.93 and the least prevalent fungi is *A.*

ochraceous with a mean per cent frequency of 0.27. Mean per cent frequency of *A. niger* (16.33) was highest which is followed by *A. flavus* (7.27), *Penicillium* sp.1 (5.93), *Penicillium* sp. 2 (3.27), *D. oryzae* (2.67), *A. terreus* (2.53), *Curvularia lunata* (1.87), *A. fumigatus* (1.80), *R. stolonifer* (1.60), *A. alternata* (1.40), *Curvularia* sp.1 (0.80) and *A. ochraceous* (0.27) (Table 2 and Fig.1).

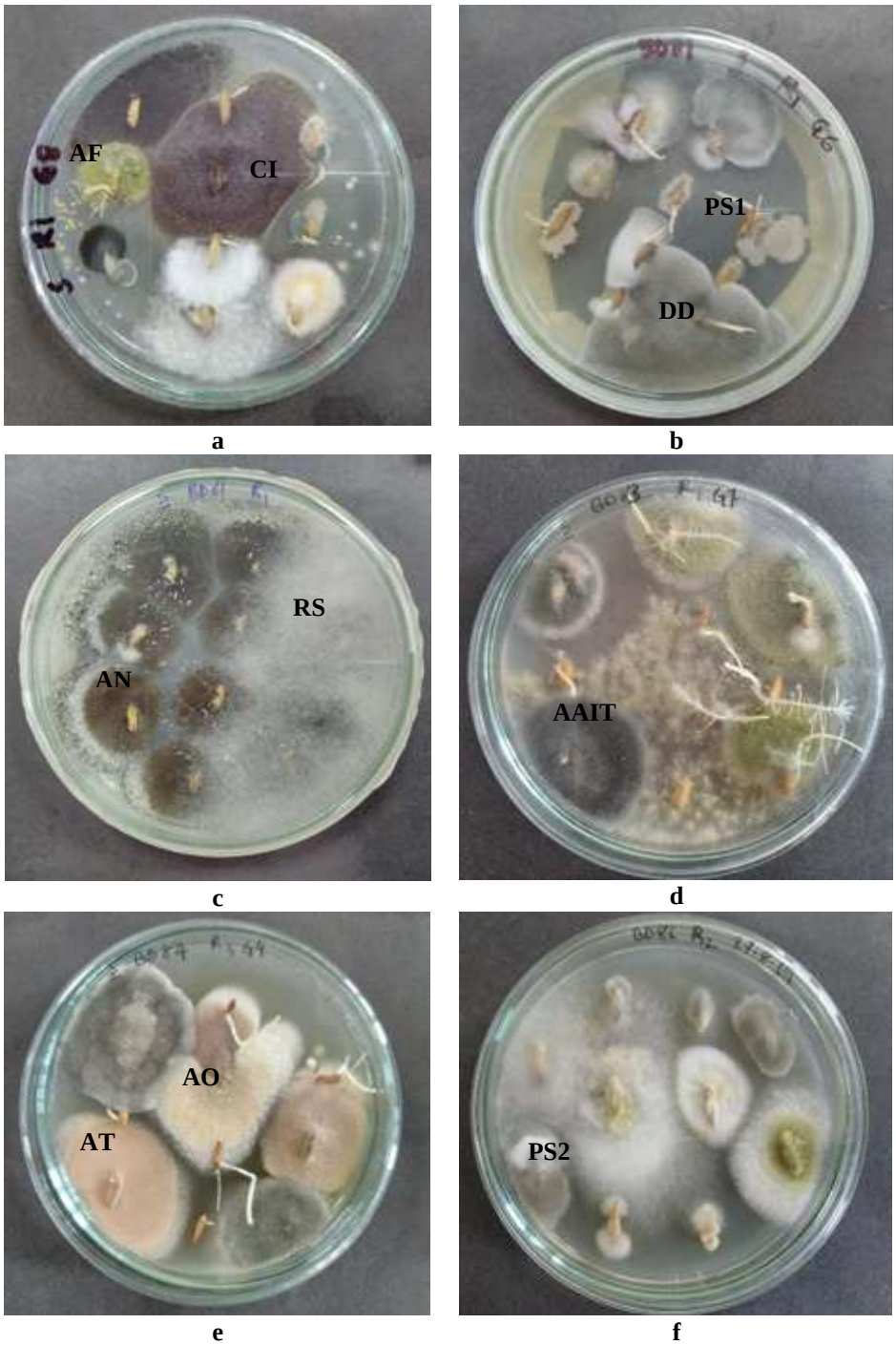

Fig. 1. Fungal colony on the seeds of selected BRRI rice varieties in Potato Dextrose Agar (PDA)medium. (**a.** AF=*A. flavus*, CL=*C. lunata*; **b**. DO=*Drechslera oryzae*, *Penicillium* sp.1; **c**. AN=*A. niger*, RS= *R. stolonifer*; **d**. AA=*Alternaria alternata*; **e**. *A. ochraceous*, *A. terreus*and **f**. PS2=*Penicillium* sp.2).

The highest frequency of *A. alternata* was found in BR-78 (6.00), *Aspergillus flavus* in BR-83 (19.33), *A. fumigatus* in BR-78 (10.00), *A. niger* in BR-88 (55.33), *A. ochraceous* in BR-84 (2.00), *A. terreus* in BR-84 (10.00), *Curvularia lunata* in BR-78 (6.67), *Curvularia* sp.1 in BR-78 (3.33), *D. oryzae* in BR-78 (9.33), *Penicillium* sp. 1 in BR-80 (12.67), *Penicillium* sp. 2 in BR-80 (14.00) and *Rhizopus stolonifer* in BR-78 (4.67) (Table 2). The total per cent frequency of fungal occurrence was the highest in BR-78 (78.67) and the lowest in BR-83 (28.00) (Table 2).

Table 2. Per cent frequency of fungi with different varieties of BRRI rice seeds in 'Tissue Planting' Method.

Name of The Fungi	Name of BRRI rice varieties										Mean
	BR-78	BR-80	BR-81	BR-82	BR-83	BR-84	BR-86	BR-87	BR-88	BR-89	
Alternaria alternata	6.00	-	-	-	-	0.67	1.33	4.67	-	1.33	1.40
Aspergillus flavus	7.33	5.33	1.33	3.33	19.33	4.67	10.67	8.67	3.33	8.67	7.27
A. fumigatus	10.00	-	3.33	-	-	1.33	3.33	-	-	-	1.80
A. niger	13.33	9.33	7.33	44.67	0.67	7.33	11.33	10.67	55.33	9.33	16.93
A. ochraceous	-	-	-	-	-	2.00	0.67	-	-	-	0.27
A. terreus	3.33	3.33	1.33	2.67	-	10.00	2.67	-	-	2.00	2.53
Curvularia lunata	6.67	-	-	-	1.33	-	-	6.00	-	4.67	1.87
Curvularia sp.1	-	1.33	2.00	-	-	-	-	3.33	-	1.33	0.80
Drechslera oryzae	9.33	2.67	1.33	-	5.33	2.00	-	2.67	-	3.33	2.67
Penicillium sp. 1	10.00	12.67	12.00	4.67	1.33	2.00	0.67	7.33	-	8.67	5.93
Penicillium sp. 2	8.00	14.00	4.67	-	-	0.67	-	-	-	5.33	3.27
Rhizopus stolonifera	4.67	0.67	0.67	2.00	0.00	1.33	1.33	2.00	2.00	1.33	1.60
Total	78.67	49.33	34.00	57.33	28.00	32.00	32.00	45.33	60.67	46.00	

Absent (-)

A total of seven fungi species, namely *A. alternata*, *Aspergillus flavus*, *A. niger*, *C. lunata*, *D. oryzae*, *Penicillium* sp. 1 and *R. stolonifer* were identified by using 'Blotter method' during the tenure of July 2019-November 2019. *Aspergillus niger* was the most prevalent fungus among theseeds of all rice varieties examined with a mean per cent frequency of 9.42 and the least prevalent fungi was *Curvula lunata* with a mean frequency of 1.75. Mean per cent frequency *A. niger* was highest which was followed by *Penicillium* sp. 1 (8.36), *D. oryzae* (5.63), *A. flavus* (4.59), *R. stolonifer* (4.09) and *C. lunata* (1.75) (Table 3).

The highest per cent frequency of *A. flavus* was in BR-89 (8.00), *A. niger* in BR-78 (12.67), *C. lunata* in BR-87 (3.60), *D. oryzae* in BR-80 (9.51), *Penicillium* sp. 1 in BR-78 (11.33) and *R. stolonifer* in BR-81 (6.58) (Table 3 and Fig. 1). The total per cent frequency of fungal occurrence was the highest in BR-78 (44.00) and the lowest in BR-88 (19.81) (Table 3).

Table 3. Per cent frequency of fungi with different varieties of BRRI rice seeds in 'Blotter method'.

Name of The Fungi	Name of BRRI rice varieties										
	BR-78	BR-80	BR-81	BR-82	BR-83	BR-84	BR-86	BR-87	BR-88	BR-89	Mean
Aspergillus flavus	4.67	6.33	4.83	3.62	4.08	5.50	4.47	3.51	0.86	8.00	4.59
Aspergillus niger	12.67	8.00	7.57	11.87	7.06	9.12	11.41	8.84	6.98	10.67	9.42
Curvularia lunata	2.67	0.67	2.21	3.79	1.33	0.84	0.00	3.60	0.67	1.69	1.75
Drechslera oryzae	7.33	9.51	8.35	5.49	4.67	6.13	2.23	5.71	2.88	3.99	5.63
Penicillium sp.1	11.33	9.13	7.72	11.03	9.71	5.80	8.53	6.23	5.12	9.02	8.36
Rhizopus stolonifer	5.33	3.75	6.58	3.42	1.69	4.18	4.88	5.64	3.31	2.14	4.09
Total	**44.00**	**37.40**	**37.27**	**39.21**	**28.53**	**31.57**	**31.52**	**33.53**	**19.81**	**35.50**	

The fungal association with rice seeds also affects germination, seedling mortality as well as seedling height. The germination percentage of seeds was highest in BR-89 (97.33%) followed by BR-84 (96.00%), BR-83 (95.34%) and lowest in BR-81 (2.00%). The percentage of seedling mortality was highest in BR-83 (50.35%) and lowest in BR-82 (1.33%) and BR-81 (1.00%). The length of root was maximumin BR-87 and minimum in BR-89, whereas shoot length was highest in BR-86 and lowest in BR-89. Germination and mortality of variety BR-81 and only mortality of variety BR-82 showed far difference in comparison to the results of the other tested samples. It seems a little bit unusual. It is to be noted here that, experiments in these cases were repeated three times and received the same results (Table 4).

Table 4. Effects of seed borne fungi on germination, seedling mortality and seedling height of rice seeds after 7 days of incubation.

Name of varieties	Germination (%)	Mortality (%)	Seedling height (cm)	
			Root	Shoot
BR-78	92.67	13.69	3.90	4.91
BR-80	91.33	36.49	3.97	4.40
BR-81	2.00	1.00	6.72	5.47
BR-82	85.33	1.33	4.29	3.20
BR-83	95.34	50.35	6.73	3.77
BR-84	96.00	19.44	5.64	4.56
BR-86	89.33	14.92	6.06	6.01
BR-87	38.67	10.78	6.91	4.93
BR-88	94.58	23.94	4.90	4.80
BR-89	97.33	36.30	3.30	3.33

In the present research work, a total of twelve fungal species was isolated from the selected ten newly released BRRI rice varieties. Among these fungi *Penicillium* sp., *Drechslera oryzae*, *A. flavus*, *A. niger* and *Rhizopus stolonifer* were predominant in most of the rice varieties. Among the isolated fungi, *Curvularia lunata* and *Drechslera oryzae* were found to be pathogenic to BRRI rice varieties. On the basis of percentage of purity, fungal association, seed germination and seedling mortality, BR-83, BR-84, BR-88 and BR-89 showed less fungal association, highest seed germination and lowest seedling mortality out of ten newly released BRRI rice varieties. These four varieties of rice may be tested at the field level before distribution to the farmers for their using purposes.

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(Manuscript received on 02 April, 2023)