Middle-East Journal of Scientific Research 10 (4): 482-488, 2011
ISSN 1990-9233
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Detection and Identification of Seed Borne Pathogens from Some Cultivated Hybrid Rice Varieties in Bangladesh

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Abstract: Seed health testing to detect seed borne pathogens is an important step in the management of crop diseases. This study was carried out to assess seeds of cultivated hybrid rice varieties (13 imported, two local hybrid rice varieties and two local varieties as check) for seed borne pathogens. Blotter method, paper towel method and agar plate method were used to identify seed borne pathogens and a total of 12 pathogens (*Xanthomonas oryzae, Rhizopus stolonifer, Aspergillus* spp. *Fusarium moniliforme, Phoma* sp. *Bipolaris oryzae, Curvularia lunata, Penicillium* sp. *Alternaria tenuissima, Nigrospora oryzae, Chaetomium globosum* and *Tilletia barclyana.*) were identified. Among these pathogens, *Xanthomonas* spp. *Rhizopus stolonifer, Aspergillus* sp. *Bipolaris oryzae* and *Fusarium moniliforme* are pre-dominent on all tested hybrid rice varieties. The lowest pathogenic incidence recording varieties showed lowest rotten seed, dead seed and highest seed germination and seedling vigour index. Hira-1 and Hira-2 showed the better performance in terms of lowest pathogenic incidence, rotten seed, dead seed seed germination and seedling vigour index.

Key words: Hybrid rice · Seed health · Seed borne pathogens · Blotter method · Paper towel method · Agar plate method

INTRODUCTION

Rice (*Oryza sativa*) is one of the world's primary food crops mostly grown in tropical and sub tropical climate. It is main staple food in Bangladesh which covers 92% of food grain production and 72% of total crop land [1]. The yield of conventional rice varieties is comparatively low and it seems impossible to change this yield with reachable resources under the prevailing situation. At this situation hybrid rice varieties may be a breakthrough which could help to achieve the goal of self-sufficiency of food. Therefore, development and introduction of hybrid varieties is the topmost priority for the government [2]. In Bangladesh, more than 78 hybrid rice varieties are grown in the field and about 75% of seed demand is meet from import mostly from China [3]. Major constraints in hybrid rice adoption were identified; these were high cost of seed, requirement of more crop care and management time, high pest and disease attack, low profits and lack of suitability for home consumption [4]. Seed borne diseases are very important from the following points of view; (i) introduction of new pathogens (ii) quantitative and qualitative crop losses and (iii) permanent contamination of soil [5].

Most of the major diseases of rice are seed borne [6]. 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. Fungi are the principal organisms associated with seed in storage. Of the seed borne diseases of rice, 22 are caused by fungi [7]. Bacteria are also commonly carried internally and externally by the seeds. The extremely seed borne pathogens of rice are Brown spot (*Bipolaris oryzae*), Bakanae (*Fusarium moniliforme*), Blast (*Pyricularia oryzae*), Sheath blight (*Rizoctonia solani*), Sheath rot (*Sarocladium oryzae*), Stem rot (*Sclerotium oryzae)* are associated with seed infection of rice and causes yield reduction, quality deterioration and germination failure [8-10]. Rice seed play an important role to carry pathogen in quarantine aspect. Farmers generally use different hybrid rice varieties and face the difficulties of many diseases. In the last few years

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the cultivation of imported hybrid rice in Bangladesh increased rapidly. Recently bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae,*), bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) disease appeared seriously in the boro (cultivation period: December-February) rice. As the pathogens of BLB and BLS are seed borne, there is a chance to transmit new race of the pathogen in the country by imported hybrid rice seed. Increasing seed demand and subsequent increase of international import, endemic plant pathogens continue to be a challenge in safeguarding plant health in Bangladesh. Therefore, early and accurate diagnoses and pathogen surveillance will allow time for development and application of mitigation strategies. So, assessment of the seed health standard of imported hybrid rice is very important for farmer and food security. Considering the above facts the present experiment were undertaken with hybrid rice varieties collected from the seed importer and local market of the country with the following objectives: i) to identify different seed borne pathogens and their incidence from imported hybrid rice varieties in Bangladesh ii) to know the comparative seed health status of hybrid rice varieties in Bangladesh.

MATERIALS AND METHODS

Altogether 15 seed samples hybrid rice varieties and two HYVs were collected from different seed importer, Bangladesh Rice Research Institute (BRRI) and local market of Bangladesh. Detailed information of the used hybrid varieties and check varieties are given in Table 1.

Detection of Seed Borne Pathogens: To identify the pathogens on different hybrid rice varieties, the following three methods were used in this experiment:

Blotter Method: The collected seed samples of rice were analyzed for the presence of major seed borne fungal pathogens by blotter method following the International rules for Seed Testing [11]. Two hundred seeds were tested for each variety maintaining four replications. Twenty-five seeds were placed on three layers of moist blotting paper (Whatman No.1) in each glass petridish. The petridishes were incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed was observed under strereomicroscope in order to record the presence of fungal colony and bacterial ooze 7 days after incubation based on growth habit. In doubtful cases temporary slides were prepared from the fungal colony and observed under compound microscope. Appropriate keys [12-15] were consulted for identification of the fungi and bacteria. The results were presented as percent incidence for individual pathogen. Germination of the seeds was also recorded. Each individual incubated seed was observed under stereomicroscope at 16x and 25x magnification in order to record the incidence of seed borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by Mathur and Kongsdal, 2003 [16]. For proper identification of fungi temporary slides were prepared from the fungal colony and observed under compound microscope at 100x and 400x and identified with the help

Table 1: Detailed information of the used hybrid varieties and check Varieties of boro rice in the experiment

Sl. No.	Name of varieties	Line/Cross combination	Origin	Source of collection
1.	Hira-1	99-5	China (Imported)	Supreem Seed Co. Ltd.
2.	Hira-2	HS-273	China (Imported)	Supreem Seed Co. Ltd.
3.	ACI 1	F1 Seed	China (Imported)	ACI Limited
4.	Surma 1	F1 Seed	China (Imported)	Syngenta Bangladesh Ltd.
5.	Taj-1	GRA2/06	China (Imported)	National Seed Co. Ltd.
6.	Modumoti-2	WBR-2	China (Imported)	United Seed Co. Ltd.
7.	Krishan-2	S-2B/07	China (Imported)	Mukterpur Bhandar
8.	Sonar bangla-6	HTM-6	China (Imported)	Mallika Seed Co. Ltd.
9.	Richer-101	R-101	China (Imported)	Lal Teer Seed Co. Ltd.
10.	Moyna	HTM 303	China (Imported)	Lal Teer Seed Co. Ltd.
11.	Tia	HTM 707	China (Imported)	Lal Teer Seed Co. Ltd.
12.	Tinpata	T-40	China (Imported)	Tinpata Quality Seed Bangladesh Ltd.
13.	Aloron	HB-8	China (Imported)	BRAC Seed Enterprise
14.	BRRI hybrid dhan-1	IR58025A x BR827R	Bangladesh (Local)	Bangladesh Rice Res. Inst.
15.	BRRI hybrid dhan-2	BRRI A x BR168R	Bangladesh (Local)	Bangladesh Rice Res. Inst.
16.	BR 28	check variety	Bangladesh (Local)	Bangladesh Rice Res. Inst.
17.	BR 29	check variety	Bangladesh (Local)	Bangladesh Rice Res. Inst.

of Keys suggested by Malone and Muskette, 1964; Booth, 1971; Ellis, 1971; Chidambaram and Mathur, 1975 and Neergaard and Saad, 1962 [12, 13, 17, 14, 18]. The fungi from the incubated seeds were also transferred to PDA when needed. The culture was incubated at 25±10°C for 3-7 days. Temporary semi permanent slides were prepared from the fungal colony and observed under compound microscope. The fungi were identified with the help of different books, manuals and publications [13, 19]. The results were presented as percent incidence for individual pathogen.

Rolled Paper Towel Method: The method developed by Warham (1990) was followed. Germinability of the seeds were determined in the laboratory at room temperature (30±2°C). 200 seeds were randomly taken from each variety and 40 seeds were placed between a pair of moist paper towels [20]. There were four replications for each variety. The towels were rolled and the ends were closed by threads and covered by polyethylene paper to prevent drying. After 10 days of incubation period observations pertaining to (a) % germination, (b) Non germinated seed (hard seed and rotten seed), (c) Post-emergence death, (d) Shoot length (e) Root length (f) Vigor Index and (g) Incidence of different organism. For determination of organisms some portion of the fungi growth on the infected seeds were taken with the needle and observed under compound microscope. For determination of seedlings vigour 10 seedlings (normal /abnormal) were randomly selected from each paper and their individual shoot and root length was measured. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from the starting point of the root to the largest available lateral root apex. Vigour of the seedling was determined by the following formula [21].

Vigour Index = (mean of root length+ mean of shoot length) × percentage of seed germination.

Agar Plate Method: In the agar plate method, two hundred seeds were tested for each maintaining four replications. Surface disinfected seeds (0.1% mercuric chloride) were plated on the PDA medium and the plated seeds were usually incubated for 5-7 days at 22-25°C under 12h altering cycles of light and darkness. At the end of the incubation period, fungi growing out from the seeds on the agar medium were examined and identified. Identification was done based on colony characters and morphology of sporulation structures under a compound microscope. In the agar plate method more than one type of fungal colonies were produced. In this case, identification was done on the most frequently occurring colony present in all the petridishes and then the second most frequent, the third most frequent and so on. Thereafter, the identification of the different colonies were done visually and then under a stereomicroscope and followed by an examination of the fruiting structures under a compound microscope. Once the identification was done, the colonies were assigned names and their acronyms written on the reverse [16].

Design of the Experiment: The laboratory experiment was conducted following Completely Randomized Design (CRD) with four replications. The recorded data on various parameters under the present study were statistically analyzed using MSTAT-C statistical package.

RESULTS AND DISCUSSION

In blotter method, a total of 12 seed borne pathogens were identified. These were *Xanthomonas oryzae, Rhizopus stolonifer, Aspergillus* spp. *Fusarium moniliforme, Phoma* sp. *Bipolaris oryzae, Curvularia lunata, Penicillium* sp. *Alternaria tenuissima, Nigrospora oryzae, Chaetomium globosum* and *Tilletia barclyana* (Table 2). The highest incidence of these pathogens were recorded on Sonar Bangla-6, BRRI hybrid dhan-2, Hira-2, ACI-1, Aloron, Modhumoti-2, and Hira-1. Some new pathogens were recorded from one variety and others were multiple varieties. *Phoma* sp. *Nigrospora* sp. *Chaetomium globosum* and *Tilletia barclayana* were not dominent on all varieties whereas *Xanthomonas* spp. *Rhizopus stolonifer, Aspergillus* sp. *Bipolaris oryzae* and *Fusarium moniliforme* were predominant on most of the varieties. *Phoma* sp. *Nigrospora* sp.and *Tilletia barclayana* were identified only from hybrid varieties and these pathogens were absent in check local varieties. Among the different hybrid rice varieties, Hira-1 and ACI-1 showed reduced or no incidence in case of major identified seed borne pathogens. Although the findings about presence of seed borne pathogens on hybrid rice seed is limited but some researchers reported some pathogens that were found on rice seed. Bhutta and Ahmed (1994) reported that maximum seed infection due to *Xanthomonas oryzae* pv. *oryzae* was 11% and 12% in variety IRRI-6 at Lahore and Hydrabad, respectively [22]. Sharma *et al*. (1987) detected 10 fungal species of fungi from the rice seeds where *Fusarium moniliforme (Gibberella fujikuroi), Curvularia*

lunata (Cochliobolus lunata), Aspergillus flavus and *Rhizopus* spp. were the most common [23]. Of all the pathogens *Xanthomonas* spp. *Rhizopus stolonifer, Aspergillus* spp. *Bipolaris oryzae, Fusarium moniliforme* were predominant.These pathogens were designated as predominant, because each of them constituted at least 5.0% of the total seed borne pathogens infection. Mian and Fakir (1989) reported that the most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus* spp. and *Trichoconis padwickii* [24].

In case of rolled paper towel method, the highest seed germination (96.38%) was observed on Hira-1 and the lowest seed germination (8.25%) was observed on BRRI hybrid dhan-2. The minimum percentage of non germinated hard seed and rotten seed was recorded on ACI-1 (1.63%) and Hira-1 (0.75%), respectively (Table 3). The maximum percentage of post emergence death recorded on Aloron (4.38%) and no post emergence mortality was recorded on BRRI Hybrid Dhan-2 (0.00%) followed by Surma-1, Taj-1 and Krishan-2. Hira-1 showed the highest vigour index whereas lowest vigour index was recorded on BRRI hybrid dhan-2. These findings indicate that percent seed germination was decreased due to hard seed and rotten seed. Rotten seed and post emergence mortality of seedling were directly associated with seed borne pathogenic infection.

In agar plate method, 10 seed borne pathogens were identified associated with hybrid rice seeds. These were *Xanthomonas* spp. *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Bipolaris oryzae, Curvularia lunata, Penicillium* sp. *Alternaria tnuissima* and *Nigrospora oryzae* (Table 4). The highest incidence of *Xanthomonas* spp. was noticed on Tinpata where as *Bipolaris oryzae* on Aloron, *Fusarium moniliforme* on ACI-1, *Rhizopus stolonifer* on Tia, *Alternaria tenuissima* on Hira-1, *Curvularia lunata* on Aloron, *Penicilium* sp and *Aspergillus flavus* on BRRI hybrid dhan-1, *Aspergillus niger* on Taj-1 were observed. *Nigrospora* sp. was recorded only on Hira-1. Of all the pathogens *Xanthomonas* spp. *Bipolaris oryzae, Aspergillus* sp. *Fusarium moniliforme* and *Rhizopus stolonifer* were predominant. The fungi and bacteria identified in the present studies comprise the genera *Bipolaris, Rhizoctonia, Fusarium, Aspergillus, Penicillium, Alternaria, Nigrospora, Phoma, Chaetomium* and *Xanthomonas* have also been reported in rice seeds by different scientists at home and abroad [25, 26, 10]. *Bipolaris oryzae, Trichoconis padwickii, Curvularia lunata, Nigrospora oryzae, Alternaria tenuis, Aspergillus* spp. and *Penicillium* spp. were identified by Rahman *et al*. (2000) on BR 11 [27]. Gopalakrishnan *et al*. (2010) conducted an experiment in India to identify the seed borne pathogen associated with rice seed and they recorded 8 genera of fungi *viz*. *Alternaria, Aspergillus,*

Table 2: Percent seed germination and incidence of different seed borne pathogens on imported hybrids and high yielding verities of rice seed by blotter method

Hybrids and		% Pathogen incidence											
High yielding varieties	% Seed germination	*Xanthomons* spp.	*Rhizopus stolonifer*	*Aspergillus* spp.	*Fusarium moniliforme*	*Phoma* sp.	*Bipolaris oryzae*	*Curvularia lunata*	*Penicillium* sp.	*Alternaria tenuissima*	*Nigrospora* sp.	*Chaetomium globosum*	*Tilletia barclayana*
Hira-1	90.25 b-d	0.00 e	0.38 e	0.00 c	1.00 de	0.00 b	3.25 b-d	0.00 c	0.00 b	0.63 a	0.25a	0.00 b	0.00 b
Hira-2	99.50 a	0.00 e	0.00 e	12.00 a	3.00 b	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
ACI-1	94.00 bc	1.75 c-e	1.50 e	0.25 c	9.50 a	0.00 b	0.00 e	2.00 b	0.00 b	0.25ab	0.00 b	0.00 b	0.00 b
Surma-1	90.25 b-d	4.25 c-e	1.25 e	1.50 bc	0.00 e	0.00 b	3.75 b	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Taj-1	82.75 f	4.75 cd	9.25 b	1.63bc	2.50 bc	0.50 a	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Modhumoti-2	95.50 ab	2.50 c-e	2.00 e	2.50 bc	1.50 cd	0.00 b	3.50 bc	0.50 c	1.75 a	0.25ab	0.00 b	1.25 a	0.00 b
Krishan-2	86.00d-f	6.00 c	3.63 c-e	1.75 bc	1.25 c-e	0.00 b	0.00 e	0.50 c	0.50 b	0.00 b	0.00 b	0.00 b	0.00 b
Sonar Bangla-6	74.50 g	18.13 a	0.50 e	0.25 c	0.00 e	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Richer-101	82.00 f	14.75 ab	3.50 c-e	0.00 c	0.00 e	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	2.75 a
Moyna	83.00 f	14.00 ab	7.00 bc	3.25 b	0.00 e	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Tia	84.25 ef	11.63 b	6.75 bc	0.00 c	0.00 e	0.00 b	0.00 e	0.50 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Tinpata	87.75 df	17.50 a	0.25 e	0.00 c	0.00 e	0.00 b	0.00 e	0.50 c	0.25 b	0.25ab	0.00 b	0.00 b	0.00 b
Aloron	86.75d-f	0.25 e	2.75 de	0.00 c	0.00 e	0.00 b	18.00a	4.25 a	0.25 b	0.50ab	0.00 b	0.00 b	0.00 b
BRRI hybrid dhan-1	63.25 h	0.75 de	19.75 a	2.00 bc	1.00 de	0.00 b	1.25 c	0.00 c	1.00ab	0.00 b	0.00 b	0.00 b	0.00 b
BRRI hybrid dhan-2	54.63 i	10.63 b	5.86 b-d	0.50 c	0.00 e	0.00 b	0.00 e	0.50 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
BRRI dhan-28	89.00 c-e	5.50 c	0.50 e	1.50 bc	1.25 c-e	0.00 b	0.25 e	0.25 c	1.00ab	0.00 b	0.00 b	0.25 b	0.00 b
BRRI-dhan-29	89.50 c-e	1.00 de	1.25 e	0.75 c	0.00 e	0.00 b	1.50 cde	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
LSD 0.05	5.83	5.37	4.793	2.914	1.477	0.238	2.149	0.531	1.054	0.536	0.114	0.983	1.716
CV%	6.28	4.16	5.46	4.88	3.99	2.26	2.43	3.89	1.45	2.79	1.83	1.56	1.79

Table 3: Seed germination, post emergence mortality and seedling vigor of hybrids and high yielding verities of rice recorded by rolled paper towel method

Hybrids & High yielding varities	% Germination	%Non germinated seed		%Post-emergence mortality	Root length (cm)	Shoot length (cm)	Vigor index
		Hard	Rotten				
Hira - 1	96.38 a	3.00 j	0.75 g	2.25 ef	15.53 bc	8.73 e	2329.28 a
Hira - 2	93.75 ab	2.00 j	4.00 def	2.00 efg	14.68 c	8.46 e	2168.32 b
ACI- 1	91.75 b	1.63 j	6.25 b-d	3.88 ab	15.31 bc	8.39 e	2165.60 b
Surma - 1	63.25 h	33.25 e	3.25 e-g	0.75 h	10.68 g	6.02 h	1056.96 h
Taj - 1	20.88 k	63.13 b	5.50 c-e	0.75 h	9.30 h	5.13 i	310.75 j
Modhumoti - 2	91.63 b	5.63 j	2.88 e-g	3.63 bc	12.24 ef	7.06 g	1734.31 e
Krishan - 2	68.88 g	25.63 f	5.50 c-e	0.88 h	11.59 fg	7.28 fg	1296.92 g
Sonar Bangla - 6	37.25 j	51.25 c	12.00 a	1.88 efg	15.85 ab	9.91 c	977.46 h
Richer - 101	72.63 ef	18.75 h	9.13 ab	1.38 gh	16.77 a	11.38 a	2044.11 c
Moyna	73.13 ef	66.00 b	5.50 c-e	2.25 ef	14.90 bc	7.70 f	726.85 i
Tia	74.25 e	20.25 gh	8.00 bc	1.75 fg	14.98 bc	9.31 d	1798.21 de
Tinpata	70.13 fg	18.13 h	11.00 a	1.63 fg	15.63 bc	10.70 b	1843.73 d
Aloron	78.88 d	17.63 h	3.25 e-g	4.38 a	11.16 g	5.09 i	1280.77 g
BRRI hybrid dhan - 1	83.50 c	10.75 i	5.50 c-e	3.00 cd	13.11 de	8.58 e	1810.70 de
BRRI hybrid dhan - 2	8.250 l	86.50 a	5.25 c-e	0.00 i	6.94 i	4.75 i	96.36 k
BRRI dhan - 28	58.88 i	40.50 d	1.75 fg	3.13 cd	10.91 g	6.46 h	1048.49 h
BRRI dhan - 29	74.88 e	23.88 fg	1.25 fg	2.50 de	13.55 d	7.34 fg	1564.07 f
$LSD_{0.05}$	3.31	4.901	2.892	0.7235	1.028	0.5579	106.7
CV(%)	3.42	2.01	3.11	3.76	5.51	5.04	5.26

Values with different letters within a column differ significantly at 5% level of significance as per DMRT.

Table 4: Incidence of different seed borne pathogens of imported hybrids and high yielding varieties of rice seed by agar plate method

Hybrids and high yielding	% Pathogen incidence									
	Xanthomonas spp.	*Bipolaris oryzae*	*Fusarium moniliforme*	*Rhizopus stolonifer*	*Alternaria tenuissima*	*Nigrospora oryzae*	*Curvularia lunata*	*Penicilium* sp.	*Aspergillus flavus*	*Aspergillus niger*
Hira - 1	1.63 f	9.63 b	2.25 cd	1.63 bc	1.63 a	1.38 a	0.00 f	0.00 d	0.00 d	0.00 e
Hira - 2	6.63 d	2.13 ef	3.88 b	0.00 d	0.00 c	0.00 b	0.00 f	0.00 d	6.50 a	0.00 e
ACI - 1	5.38 de	0.00 g	7.63 a	1.75 a-c	0.00 c	0.00 b	2.88 b	0.00 d	1.50 b-d	0.00 e
Surma - 1	4.50 de	5.00 c	0.00 e	1.00 cd	0.00 c	0.00 b	0.00 f	0.00 d	2.13 bc	1.13 b
Taj - 1	3.43 ef	0.00 g	3.63 bc	1.50 bc	0.00 c	0.00 b	0.00 f	0.00 d	3.25 b	1.38 a
Modhumoti - 2	3.25 ef	3.00 de	3.38 bc	0.00 d	1.50 ab	0.00 b	2.25 bc	1.63 ab	1.88 b-d	0.88 c
Krishan - 2	6.75 d	0.00 g	2.75 b-d	2.25 a-c	1.25 b	0.00 b	1.88 bc	1.50 ab	1.13 cd	0.63 d
Sonar Bangla - 6	12.75 a-c	0.00 g	0.00 e	1.50 bc	0.00 c	0.00 b	0.25 ef	0.00 d	2.75 bc	0.00 e
Richer - 101	11.50 bc	0.00 g	0.00 e	2.38 ab	0.00 c	0.00 b	0.00 f	1.00 c	0.00 d	0.00 e
Moyna	6.25 d	0.00 g	0.00 e	2.50 ab	0.00 c	0.00 b	1.25 c-e	0.00 d	2.63 bc	0.00 e
Tia	14.00 ab	0.00 g	0.00 e	3.00 a	0.00 c	0.00 b	0.75 d-f	0.00 d	0.00 d	0.00 e
Tinpata	15.00 a	0.00 g	0.00 e	1.00 cd	1.38 ab	0.00 b	1.25 c-e	1.25 bc	0.00 d	0.00 e
Aloron	3.38 ef	15.13 a	0.00 e	1.50 bc	0.00 c	0.00 b	6.38 a	0.00 d	0.00 d	0.00 e
BRRI hybrid dhan - 1	4.25 d-f	3.13 de	3.50 bc	2.44 ab	0.00 c	0.00 b	0.00 f	1.88 a	3.38 b	0.00 e
BRRI hybrid dhan - 2	10.13 c	0.00 g	0.00 e	1.88 a-c	0.00 c	0.00 b	1.63 cd	0.00 d	2.75 bc	0.00 e
BRRI dhan - 28	5.88 de	1.38 fg	1.50 d	1.50 bc	0.00 c	0.00 b	1.63 cd	0.88 c	2.00 bc	0.00 e
BRRI dhan - 29	4.38 d-f	4.13 cd	0.00 e	1.25 b-d	0.00 c	0.00 b	0.00 f	0.00 d	2.63 bc	0.00 e
$LSD_{0.05}$	2.786	1.394	1.403	1.361	0.3364	0.08992	1.024	0.4863	1.954	0.2203
CV(%)	4.99	3.32	5.87	3.13	2.10	4.97	6.85	1.63	1.87	6.12

Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium and *Trichoderma* comprising twelve species Among them, the most predominant one was *Bipolaris oryzae* which was associated with 58.89 per cent seed samples, followed by *Alternaria padwickii* (52.96%) [28]. Local varieties of rice (*Oryza sativa* L.) viz. KS-282, Basmati-385, Basmati-370, Basmati Kernal and Basmati-198 were studied in Pakistan using blotter paper method to investigate the occurrence of seed-borne mycoflora. There was 27%, 19%, 17%, 16% and 14% mycoflora found associated with the seeds of Basmati kernel, Basmati-385, Basmati- 370, Basmati-198 and KS-282, respectively. Four fungal species namely *Fusarium moniliforme, Alternaria* sp. *Helminthosporium* sp. and *Curvularia* sp. were isolated from different test rice varieties [29].

CONCLUSION

The result of the present study reveals that seed borne pathogens are present on most of the cultivated hybrid rice varieties in Bangladesh including imported hybrid rice seed. Although in certain instances they occurred in trace levels but under suitable environmental condition they may create the disease in epidemic level. Pathogen free seed is the vital input in agriculture. Most of the hybrid rice seeds are imported in Bangladesh. Although the imported hybrid varieties are treated with seed treating chemicals for maintaining quarantine regulations. But from the present study it was revealed that seed borne pathogens were associated with those seeds. Some patogens were recorded only from hybrid varieties that were not present on check varieties and it was also noticed that a particular pathogen was observed in a particular variety. So the seed health status of imported hybrid rice seed needs to be improved to prevent the introduction of new pathogen.

ACKNOWLEDGEMENT

The authors are greatly acknowledged to the authority of Ministry of Science &Information and Communication Technology, People's Republic of Bangladesh and Sher-e-Bangla Agricultural University for providing financial support and research facilities. The authors also acknowledge to Lal teer seed and Supreme seed companies for providing seed materials to do this experiment.

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