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Study of seed-borne fungal pathogens of Kataribhog aromatic rice and comparison of field intensity with laboratory counts

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Abstract

Eight landraces of Kataribhog aromatic rice viz. Philippine, Radhunipagal, Bolder, Zota, Tajmohal, Zira, Behulabator and Badhua were grown to investigate the prevalence of seed-borne fungal pathogens during 2010 and 2011. Disease severities in field level were recorded once on leaf blast and brown spot at 80 days after transplanting and neck blast, node blast and grain spot at 30 days after heading. The blast severity was recorded in the mother crop during 2010 ranged from 19.22 to 26.30% in leaf, 23.27 to 64.89% in neck and 15.19 to 25.45% in node whereas in the subsequent crops of 2011 recurring from 2010 were 19.21 to 34.60% in leaf, 21.54 to 60.48% in neck and 15.98 to 31.77% in node. The yield losses due to neck blast were 13.26 to 36.99% and 12.28% to 34.48% in 2010 and 2011, respectively. The brown spot severity was found 26.60 to 46.76% in 2010 and 28.28 to 48.45% in 2011 whereas the grain spot severity was 19.88 to 64.94% in 2010 and 23.83 to 70.82% in 2011. In laboratory study, the *Bipolaris oryzae* infected seed ranged 8.64 to 18.37% by blotter test. The growth of *Pyricularia grisea*, *Alternaria padwickii* and *Curvularia* spp. were absent in blotter test but presence of conidia ranged from 6.38 to 10.77, 3.36 to 7.52 and 8.62 to 16.55, respectively per 50 seeds in washing test. In dry inspection test, apparently healthy seeds, discolored and spotted seeds were found 2.50 to 70.25 %, 0.75 to 10.75% and 1.50 to 43.25 %, respectively. The germination of seeds in soil substrate ranged 87.00 to 93.00%, abnormal seedlings 18.00 to 48.00%, infected seedlings 16.00 to 38.00% and dead seeds 7.00 to 13.00%. The yield were 2.01 to 3.28 t/ha during 2010 whereas 1.81 to 3.21 t/ha during 2011. The average yield gap among the landraces was 77.34%. Finally, the yield gap value indicated that there was much risk of seed-borne fungal diseases for all landraces of Kataribhog rice.

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Introduction

The people of Dinajpur district are cultivating Kataribhog aromatic rice before the arrival of Arjo in this region. The enthusiastic farmers are interested to cultivate different landraces of Kataribhog rice as its farming has become popular due to huge demand and lucrative market prices. In Bangladesh, 43 diseases are known to occur on rice crop. Among these 27 are seed-borne of which 14 are of major importance (Fakir, 2000). In total, rice is attacked by 47 seed-borne fungal diseases (Richardson, 1990). The pathogens of these diseases are disastrous as they reduce seed vigor and weaken the plant at its initial growth stages. Fungi including *Pyricularia grisea*, *Drechslera oryzae*, *Sclerotium*, *Fusarium solani*, *Alternaria alternata*, *A. padwickii*, *A. longissima*, *Aspergillus niger*, *Curvularia oryzae*, *C. lunata*, *Fusarium moniliforme*, *F. semitectum*, *F. oxysporum*, and species of *Phoma*, *Cercospora*, *Chaetomium*, *Penicillium*, *Myrothecium* and *Colletotrichum* have been landraced from seeds of different varieties of rice (Khan *et al.*, 2000; Habib *et al.*, 2012). However, history has taught us that occurrence and resurgence of rice diseases can cause misery, death and economic losses. Sporadic occurrence of rice blast (*P. grisea*) on susceptible varieties reaches epidemic proportions in southeastern United States (Guerber, and TeBeest, 2006).

The disastrous epiphytotic of rice blast cause losses up to 90% of rice yield in California (Greer *et al.*, 1997). It is also a major disease of aromatic rice even under low land irrigated system and is most serious problem in Iran, Pakistan and India. This destructive disease may cause substantial yield loss that can be as high as 50% when the disease occurs in epidemic proportions (Anonymous, 2007). Different types of blast caused 40% yield loss in Dinajpur region (Rashid *et al.*, 2011). The fungus *Bipolaris* was one of the principal causes of Bengal famine of 1942-43. Severe infection of this disease reduced both yield (20-40%) and seed quality (Vidhyasekaran and Ramadoss, 1973). In USA, stack burn disease (*A. padwickii*) caused losses of seedlings and reduction of

the leaf area, eventually leading to serious direct damage to grain before and during storage. Black kernel of rice caused by *Curvularia* spp. has been reported in the boro rice seed sample in Dinajpur region of Bangladesh (Rashid, 2001). Fungi associated with discolored rice seed resulted in poor germination and vigor and cause diseases to emerged seedling or growing plants (Sachin and Agarwal, 1994). Seed is the basic input of agriculture. Using good quality and cleaned seed of rice, yield could be increased by 7-20% (Diaz *et al.*, 1998). For routine seed health test, detection of pathogenic fungi is of prime importance. As the nature of infection in seed, their detection methods may also vary.

Seed health of rice is evaluated by different method for detection of fungi frequently. But growth of *P. grisea*, *A. padwickii* and *Curvularia* spp. on rice seed by blotter method was found in our present lab facilities. In Bangladesh, considerable work has been done on seed health of rice by different methods. However, information about the seed health of Kataribhog rice is not available and the comparison between field intensity and laboratory counts has not yet been reported in Dinajpur district and not even throughout the country. Considering the above facts, the present piece of research work has been aimed to evaluate the field intensity of blast, brown spot and grain spot diseases and to compare between field intensity and laboratory counts.

Materials and methods

Disease severity study at field

The field experiment was conducted during the period of August 2010 to November 2010. The seedlings of eight Kataribhog landraces viz. Philippine, Radhunipagal, Bolder, Zota, Tajmohal, Zira, Behulabasar and Badhua were used and collected from the farmers used as treatments. The research plot was laid out in Randomized Complete Block Design (RCBD) with three replications. Thirty (30) days old seedlings were transplanted on 9th August, 2010 in the experimental plot. Plant spacing was maintained 20cm. × 15 cm. using 3 seedlings/ hill where total hills were 200 per plot. The same

experiment was carried out in the next cropping season. Data were recorded from the ten out of 200 hills per plot on the severity of leaf blast, neck blast, node blast and brown spot once when maximum disease appeared. The percent spotted grain was recorded at maturation stage at 30 days after heading (DAH). Data related to disease was recorded by following the Standard Evaluation System for Rice (IRRI, 1996) for sorting out resistant genotypes. The disease severity was evaluated by using the following formulae:

Disease severity (DS) =

$$\frac{\text{Area of plant tissue affected}}{\text{Total assessed area}} \times 100$$

and Panicle Blast Severity (PBS) =

$$\frac{(10 \times N_1) + (20 \times N_3) + (40 \times N_5) + (70 \times N_7) + (100 \times N_9)}{\text{Total assessed area}}$$

Where N_1 - N_9 are the number of panicles with score 1-9.

The estimation of the relationship between panicle blast and yield loss was calculated by using Critical Point Model for rice blast. Losses had been estimated by the equation $Y = 0.57x$, where Y is the percentage loss and x is the percentage of blast of panicles at 30 days after heading (Mehrotra and Agarwall, 2003). The crop was reaped plot wise on 28 November, 2010 at fully ripening stage and threshed carefully to avoid mixing with other landraces. The harvested rice was sun dried several times to reach the moisture limit of 12.5%. The yield per unit area (g/m^2) were taken from each of the plot. The yield per plot was converted to the yield per hectare. Data were subjected to statistical analysis to find out the levels of significance of the experimental results. The mean of all the treatments were calculated and analyzed. The significance of differences among pairs of treatment means were evaluated by Duncan's New Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

Prevalence of seed-borne fungal pathogens:

The seeds of previous experiment were used in this

investigation. The sample of each landraces were taken in the month of April, 2011 and enclosed in polythene bag with proper labeling, brought to Plant Pathology laboratory and kept in the refrigerator at $5 \pm 10^\circ \text{C}$ until used for subsequent studies. Two sets of study were conducted concurrently on the quality of seed and seedling evaluation. In one, seeds belonging to the samples were first categorized in apparently healthy seeds, discolored and spotted seeds in different grades ($<20\%$, $20 \leq 50\%$, $>50\%$) and unfilled grains. The amount of seeds in different categories had expressed in percentage. Separation of different categories of seeds was done with unaided eyes. However, a stereomicroscope was used wherever necessary. Inert matter, variety mixture, deformed seed and other plant part were also observed but not counted in this experiment. In the second set of study, seeds of the samples were sown in sand. One hundred seeds were sown in each tray. For each sample altogether 400 seeds in four trays were tested for germination. Observations were made on percentage of germination, seedling abnormalities, infected seedling and dead seeds. Data were recorded after 7 days of sowing. Abnormal and infected seedlings were evaluated following the Handbook for Seedling Evaluation (ISTA, 2003). The standard blotter method was used to detect the occurrence of seed-borne fungi associated with the seeds in seed samples by following International Rules for Seed Testing (ISTA, 1993; Aveling and Blanco, 2009). Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by Malone and Muskette (1964), Neergaard (1979) and Ellis (1971). Examination of a suspension obtained from washing of seeds has done for recording the presence of inoculum of *P. grisea*, *B. oryzae*, *A. padwickii* and *Curvularia spp.* on the surface of seeds. In washing test, a working sample of 50 seeds was prepared from eight seed lots of Kataribhog landraces using the halving method. The 50 seeds were divided into two portions of 25 seeds. The seeds were transferred to a 50 ml Erlenmeyer flask where 10 ml of water and one drop of liquid detergent were added to it. The seeds were shaken vigorously for 5

minutes on an electrically operated shaker in order to separate the spores adhering to the seed surface. The water was poured off from the flask into a 25 ml beaker through a piece of cheese cloth. Equal amount of water was transferred from the beaker into centrifuge tubes. Centrifugation was done at 4000 rpm for 2 minutes. The water was poured off carefully and the tubes were kept inverted. The edge was wiped with a paper towel to absorb the remaining drops. One ml of water was added to the sediments in each of the centrifuge tubes. The sediment was mixed by sucking in and out of the small tip of the pipette using a long-stem Pasteur micropipette. The solution was not sucked up to the large part of the pipette as it will stay there and be lost to the test. The sediment obtained after centrifugation was examined on four slides in 8 drops of water under the compound microscope for the presence of conidia. The slide was scaled with fine pointed permanent marker on the both ends by 20 equal squares. Conidia were counted from each squares and total number of conidia were recorded. The recorded data on various parameters under the present study were statistically analyzed.

Results and discussion

The prevalence of seed-borne fungal pathogens *P. grisea* and *B. oryzae* and their intensity in field level of fine aromatic Kataribhog rice was investigated during 2010 and 2011 due to heavy severity in this locality. Disease severity in field level were recorded at 80 days after transplanting for leaf diseases and 30 days after heading for neck blast, node blast and grain spot during both the seasons of 2010 and 2011. The disease severity of leaf blast, neck blast and node blast were found significant among the different Kataribhog landraces (Table 1). The blast severity of mother crops during 2010 ranged from 19.22 to 26.30% in leaf, 23.27 to 64.89% in neck and 15.19-25.45% in node. In the subsequent crops of 2011 recurring from 2010, the disease severity of blast ranged from 19.21 to 34.60% in leaf, 21.54 to 60.48% in neck and 15.98-31.77% in node. The yield losses due to neck blast were noticed 13.26-36.99% and 12.28% to 34.48% in the year of 2010 and 2011, respectively. The highest yield loss was recorded

36.99% in Zira katari in 2010 and the lowest 12.28% in Philippine katari in 2011. *P. grisea*, the cause of blast disease attacks the leaf, neck and node and can severely damage boro rice and Kataribhog rice and reduced considerable yield that occurs commonly in Dinajpur region (Rashid *et al.*, 2011; Rashid, 2012). Among the landraces, parallel relationships existed except Zira katari where the highest neck blast infection was found. To our knowledge, these are the first quantitative studies among the different blast severities and the node blast of Zira katari might be caused by different virulent race of *P. grisea*. There was infection of *P. grisea* in seed samples of eight Kataribhog landraces, harvested 2010 but found free of infection by blotter method. Obviously, in obtaining zero percent infection in these heavily infected seed samples by blotter method, the test had simply not been properly carried out nor had proper laboratory facilities in the Department of Plant Pathology, Hajee Danesh Science and Technology University, Dinajpur. The growth of *P. grisea* were absent in the blotter test but the seeds were found to be presence of conidia ranged from 6.38-10.77 per 50 seeds in the washing test. The washing test is, however may be considerable under the following reasons:

Detection of conidia in washing test, may be dead or alive, indicates that the Kataribhog rice has been infected by *P. grisea*.

Absent of conidia in blotter test, does not mean that the Kataribhog rice has not been infected, because heavy field infection may lead to invasion of mycelium into the seed without development of conidia.

Detection of conidia in washing test was the evident of the possibility of transmission to the subsequent crop.

The conidia found in washing test were positively viable on the ground that the subsequent crop was heavily infected.

Discrepancies in recording *P. grisea* in the blotter test

that have been observed in the washing test, being decidedly preferable to the blotter method for any seed testing scheme. However, the washing test being a less time consuming procedure, can be used for easy

health screening of seed sample providing viability of the conidia is made and final check the blotter method may be eventually followed (Mathur *et al.*, 1972).

Table 1. Percent severity of leaf blast, neck blast, node blast, brown spot and grain spot of Kataribhog landraces at different growth stages during 2010 and 2011.

Kataribhog landraces	% Leaf blast (80 DAT)		% Neck blast (30 DAH)		% Node blast (30 DAH)		% Brown leaf spot (80 DAT)		% Spotted grain (30 DAH)	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Philippine	21.41	19.21	24.42	21.54	15.19	15.98	28.59	30.70	20.15	23.83
Radhunipagal	25.94	26.85	30.15	31.09	21.36	22.87	30.67	33.64	23.32	25.93
Bolder	19.22	20.08	23.27	23.60	18.97	31.77	39.74	41.52	64.94	70.82
Zira	25.34	34.60	64.89	60.48	16.53	17.84	27.69	32.53	27.36	32.34
Jota	20.42	19.46	25.74	21.76	15.94	16.10	26.60	28.28	19.88	24.95
Behulabasar	24.99	28.68	30.11	31.84	20.48	21.30	44.55	46.18	24.91	26.29
Tajmahal	26.30	28.73	30.29	30.96	25.45	26.36	45.75	48.45	24.11	25.77
Bodhua	24.39	26.48	30.97	31.75	22.60	23.32	46.76	47.44	25.56	28.90
LSD (5% level)	4.10	3.84	2.54	4.27	2.88	3.06	4.51	5.74	6.60	2.54

'DAT' and 'DAH' denote the days after transplanting and days after heading, respectively.

Table 2. Percent infected seeds by *Bipolaris oryzae* of different Kataribhog landraces by blotter method at laboratory.

Kataribhog landraces	% <i>B. oryzae</i>	% <i>P. grisea</i>	% <i>A. padwickii</i>	% <i>C. spp.</i>
Philippine	9.96	0.0	0.0	0.0
Radhunipagal	9.21	0.0	0.0	0.0
Bolder	18.37	0.0	0.0	0.0
Zira	16.22	0.0	0.0	0.0
Jota	8.64	0.0	0.0	0.0
Behulabasar	14.86	0.0	0.0	0.0
Tajmahal	14.01	0.0	0.0	0.0
Bodhua	18.17	0.0	0.0	0.0
LSD (5% level)	2.716	0.0	0.0	0.0

Regarding brown spot severity in the field level among the Kataribhog landraces ranged from 26.60 to 46.76% in 2010 and 28.28 to 48.45% in 2011 (Table 1). The prevalence of *B. oryzae* infected seeds ranged from 8.64 to 18.37% by blotter test. So, the *B. oryzae* was found high percent in seed of all Kataribhog landraces when seeds were incubated for 8 days in normal laboratory conditions. The association of *B. oryzae* as a seed-borne fungus with rice had been observed by a good number of researchers (Mia *et al.*, 1994; Sharma *et al.*, 1997;

Mathur *et al.*, 2004; Naeem *et al.*, 2001 and Ahmad *et al.*, 1989). The results obtained in the comparison of the field intensity and seed infection by blotter method reflect correlation, furthermore, special interest also show the presence of conidia of *Bipolaris oryzae* in the washing test following the principles of blast pathogen (Table 2; Fig. 1). Practically all the seed samples recorded higher number of conidia ranged 69.77-107.23 per 50 seeds. This may be due to the reason that in most samples present conidia on the seed surface were not detectable, and hence they

were unable to produce any growth on the seed surface in the blotter method in the laboratory conditions.

Table 3. Physical quality of seed and health status of Kataribhog landraces by dry inspection method.

Kataribhog landraces	Percent seed under different grades							Unfilled grain
	Apparently healthy seeds	discolored seed			Spotted seed			
		<20% discolored	20-50% discolored	>50% discolored	<20% spotted	20-50% spotted	>50% spotted	
Philippine	70.25	2.00	10.75	3.25	4.00	2.25	1.50	6.00
Radhunipagal	63.80	5.05	10.40	5.15	3.20	2.35	1.55	8.50
Bolder	2.50	0.75	1.25	2.80	43.25	27.25	18.25	4.00
Zira	61.25	3.50	4.25	5.50	7.05	2.20	3.00	13.25
Jota	68.25	4.20	10.25	3.50	3.45	4.10	2.75	3.50
Behulabasar	56.50	8.25	8.50	7.50	4.00	4.25	4.00	7.00
Tajmahal	58.75	6.75	6.00	7.75	2.25	4.25	4.75	9.50
Bodhua	59.00	7.25	8.25	5.00	5.75	4.50	4.00	6.25
LSD (5% level)	14.63	2.09	3.94	2.52	5.06	3.34	2.61	3.60

Table 4. Physiological quality and seedling evaluation of Kataribhog landraces in sand media.

Kataribhog landraces	% Germination	% Abnormal seedlings	% Infected seedlings	% Dead seeds
Philippine	91.75	20.00	20.00	8.25
Radhunipagal	90.00	25.00	27.00	10.00
Bolder	88.00	48.00	38.00	12.00
Zira	87.00	23.00	21.00	13.00
Jota	93.00	18.00	16.00	7.000
Behulabasar	89.00	28.00	22.75	11.00
Tajmahal	88.00	27.00	30.00	12.00
Bodhua	87.50	25.00	28.25	12.50
LSD at 5% level	6.22	4.62	4.87	4.49

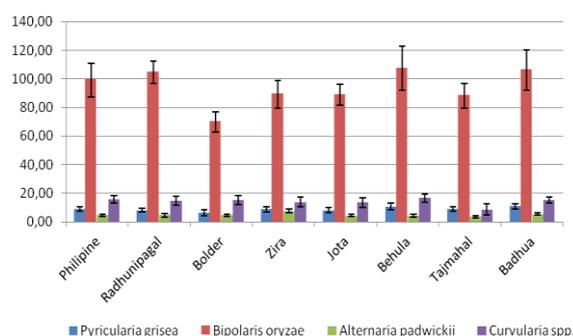
The growth of *A. padwickii* and *Curvularia* spp. were also absent in the blotter test but the seeds were found to be presence of conidia ranged from 3.36-7.52 and 8.62-16.55, respectively per 50 seeds in the washing test. This may be due to the fact that in the seed samples the conidia present on the seed surface were not viable or were not able to grow in the laboratory conditions, and hence they were unable to produce any growth on the seed surface in the blotter test. The viability tests of conidia from the washing test were not done due to the same reasons as of *P. grisea*. However this inconsistency in recording these two fungi in the blotter test that have been observed in the washing test, their conidial intensity on individual seeds were reflected in the amount of damage caused to these seeds in terms of loss of seeds and seedlings in the soil. According to Ridgman (1990) damage caused by *A. padwickii* has generally been neglected since the leaf phase is not very

important. However, when seeds are attacked, the damage can be considerable through seed decay and seedling blight. The associations of *Curvularia* spp are common on rice seed, and damage the seeds and seedling in the soil (Rashid, 2001).

Two sets of study were conducted concurrently on the physical quality of seed and seedling status. In one, seeds belonging to the samples of eight selected Kataribhog landraces were first categorized into healthy seeds, discolored seeds, spotted seeds and unfilled grains on dry inspection (Table 3). The apparently healthy seeds were found 2.50 to 70.25 % and unfilled seeds 3.50-13.25%. The results obtained on discolored and spotted seeds were categorized in the following threes:

Table 5. Yield per unit area, yield per hectore and yield loss (%) of Kataribhog landraces in 2010 and 2011.

Kataribhog landraces	Yield per unit area (g/m ²)		Yield (t/ha)		% Yield increase over Behulabasar katari		%Yield loss due to neck blast (%Y=0.57X)	
	2010	2011	2010	2011	2010	2011	2010	2011
Philippine Katari	315.6	302.30	3.28	3.12	62.18	72.38	13.92	12.28
Radhuni Pagal Katari	202.0	189.90	3.00	2.90	49.25	60.22	17.19	17.72
Bolder Katari	298.6	270.90	2.41	2.25	19.90	24.31	13.26	13.45
Zira Katari	170.2	163.50	2.35	2.32	16.91	28.18	36.99	34.48
Jota Katari	282.1	244.10	3.46	3.21	72.14	77.34	14.67	12.40
Behulabasar Katari	143.5	133.50	2.01	1.81	-	-	17.16	18.15
Tajmahal Katari	303.6	288.60	2.24	1.98	11.44	9.39	17.27	17.65
Bodhua Katari	255.50	255.50	2.12	1.89	5.47	4.42	17.65	18.10
LSD at 5% level	98.31	30.09	0.56	0.44	-	-	1.453	2.430

**Fig. 1.** Average number of conidia of *Pyricularia grisea*, *Bipolaris oryzae*, *Alternaria padwickii* and *Curvularia spp.* per 50 seeds by 'washing test'.

Categories I: < 20% severity found 0.75-8.25% discolored seeds and 2.25 to 43.25 % spotted seeds.

Categories II: 20 - ≤ 50% severities found 1.25-10.75% discolored seeds and 2.20 to 27.25 % spotted seeds.

Categories III: > 50% severities found 2.80-7.75% discolored seeds and 1.50% to 18.20 % spotted seeds.

The seeds of different Kataribhog landraces were relatively good quality in terms of discolored seed and spotted seed. This results partially supported by several researchers such as Sharma *et al.* (1997) found 4.35 to 8.82% discolored seed, Fakir *et al.* (2000) found 14.43-24.44% discolored seed and 33.72-37.71% spotted seeds in BRRI released varieties in Rajshahi region.

In the second set of study, seeds of the samples were sown directly in the sand, one hundred seeds per tray. Observations were made on percentage of

germination, seed rot and seedling abnormalities (Table 4). The germination ranged was 87.00-93.00%, abnormal seedlings were 18.00-48.00%, infected seedlings were 16.00-38.00% and the dead seeds were 7.00-13.00%. These results are partially agreement with Huda (1992) who found 71.00 to 78.00% germination of farmers' stored rice seed. Considering the percentage of germination, frequencies of abnormal, infected seedlings and dead seeds which the fungi are capable of inducing in the seed beds of rice and presumably in the fields as well related correlation between laboratory observations and field observation could be established.

The yield of different Kataribhog landraces was found to be significant in 2010 and 2011 (Table 5). The yield of per unit area ranged from 143.5- 315.60 g/m² and per hectore 2.01-3.28 t/ha during 2010 where the yield per unit area 133.50-302.30 g/m² and yield per hectore 1.81-3.21 t/ha during 2011. The average percent yield gap among the landraces was highest 77.34% over low yield landrace for both the subsequent experiments. This yield gap value indicated that there was much risk for severity of the fungal disease during the growth season and a subsequent transfer of fungal pathogens to the following crops.

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