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Effect of Seed Cleaning and Washing on the Incidence of Bacterial Leaf Blight of Rice

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Abstract: The study was conducted to determine the effect of seed cleaning and washing on the incidence of bacterial leaf blight (BLB) of rice cv. BR11. Six different treatments were used. They were farmer's seeds clean seeds, diseased seeds, farmer's seeds washed with tap water, farmer's seeds washed with 20% brine solution and clean seeds washed with tap water. Three seed health testing methods namely paper rolled towel, cassette slide holder and liquid assay method were used for detection of BLB infection in seed. Seed health both before sowing and freshly harvested seeds from six different treatments were determined. Seedling vigour of clean seeds and brine solution washed seed were also higher than farmer's seed and diseased seed. *Xanthomonas oryzae* pv. *oryzae* was identified through physiological, biological, serological, hypersensitive reaction and pathogencity tests in seeds of all treatments. Incidence of BLB in field plots were recorded at tillering stage and flag leaf stage. Maximum and minimum BLB disease was recorded in diseased seeds and brine solution washed seeds respectively for both situation.

Key words: Seed cleaning, washing, bacterial leaf blight, rice

Introduction

Rice (*Oryza sativa* L.) is the prime food crop of Bangladesh. About 90 % or more or the people of Myanmar, Srilanka, Vietnam, Kampuchia and Bangladesh depend on rice for their major food intake (Anonymous, 1991). Rice is also grown in tropical and sub tropical countries of the world. In Bangladesh 75 % of the total cropped area, about 9.926 million hectares land covers with rice (Anonymous, 1996). It is the source of cash income for many farmers. The average per hectare production of rice in Bangladesh lower compared to the other rice growing countries of the world. The national yield of rice is 3.75 t ha^{-1} (Anonymous, 1998).

There are many constraints responsible for low yield of rice in Bangladesh. Among the constraints disease is considerable as the most important one. All of the diseased bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* has been recognized as one of most damaging diseases of rice in Bangladesh (Anonymous, 1992). *X. oryzae* pv. *oryzae* is a seed borne pathogen which survive on seed from 170-180 days and act as a source of inoculums from season to season (Murtym and Devadath, 1984).

In this tropics the loss due to this diseases may be as high as 60-70 % (Ou, 1973). The disease is systemic and may cause on a average 20-30 yield loss (Ou, 1985) depending on the severity of infection. Srivastava and Kapoor (1982) obtained 6-37 % yield loss against 1-9 infection grade in India. In Bangladesh, 10-30 % yield loss has been reported by Shajahan (1992).

Seed cleaning and washing is a easiest and cheapest method which reduce the bacterial population in seed. It is reported that *P. glumae* was markedly reduce if seeds were washed and shaken with distilled (Ashizawa *et al.*, 1997). On the other hand cleaning and washing of farmers rice seeds reduce the seedling diseases up to 53.87 % over the unlearned farmers seed (Hossain and Doullah, 1998). Cultural practices are the best choice for controlling BLB. Seed cleaning and washing is one kind of cultural practice that can apply our poor farmer without any technical knowledge. Disease severity of BLB caused by *X. oryzae* pv. *oryzae* were suppressed in the field when seeds were sown after washed with 50 ppm solution of Zhongshengmycin (Zhang *et al.*, 1996). The effective control of BLB is very limited so researchers have been given emphasis on cultural practices to reduce its severity. The objectives of this study were to:

- record the bacterial leaf blight (BLB) under different treated before sowing and freshly harvested seeds by cassette holder method, paper roll towel method and liquid assay.
- identify the *Xanthomonas oryzae* pv. *oryzae* and its characterization associated with rice seeds and disease symptoms in the plants
- observe the effect of different treatment on the incidence of BLB of rice.

Materials and Methods

Three seed samples of rice cv. BR11 were collected from three different areas under Mymensingh District of Bangladesh during July 1999 to March 2000.

Laboratory experiment: One hundred grams working samples were prepared from each sample contained 10 kg farmer's saved BR11 rice cultivar according to ISTA rules. The samples were enclosed in polyethylene bags with proper labeling and were dept in the refrigerator at 5 $\pm$ 1°C for further studies. Working seed samples were separated on the basis of following categories:

Farmer's saved seed, apparently healthy seed (bright coloured/without spoting) and diseased/discoloured seed. Fourty grams seeds were taken from working sample and separated according to the following groups for purity test: pure seed, other's seeds, inert matter. Six different treatments viz. farmer's seed, clean seed, diseased/spotted seed, farmer's seed washed with normal water, farmer's seed washed with brine solution (20% NaCl) and clean seed washed with water were used in the experiment.

Germination test was conducted in sand and soil. Twenty four glass petridish were filled coarse sand. Four hundred seeds of each treatment were sown in 4 replicated dish using 100 seeds per petridish. The petridishes were then kept in the glass house. Watering was done regularly. Recording on germination was taken after 7-12 days of sowing. Soil was sterilized in autoclave with 121°C temperature and 15 lb pressure. Sterilized soil was filled in the petridish. Seed sowing procedure and mode of water application were similar to those followed in sand method. The vigour of seedlings were determined by the following formula (Baki and Anderson, 1972).

Field experiment: This was conducted following the randomized complete block design (RCBD) with four replication for each treatment. The fertilizer were applied at the rate of 180 kg ha^{-1}. One hundred kg TSP ha^{-1}, 70 kg MP ha^{-1}, gypsum 60 kg ha^{-1} and zinc sulphate 10 kg ha^{-1}. All fertilizer except urea was incorporated with soil during final land preparation. Urea was applied in equal three installment at 15, 30 and 60 days after transplanting. Three parameters viz. a) total no. of leaves/hill, b) % no. of healthy

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leaves/hill and c) % no. of diseased leaves/hill were considered for data collection. Disease index in each treatment was made following standard evaluation system (Anonymous, 1986). Following methods were used for detection and identification of the pathogen (*X. oryzae* pv. *oryzae*) from collected rice seed samples, seedling symptom test:

- Rolled paper towel method
- Slide cassette holder method, washing test and liquid assay were done following the method of Mortensen (1997a, b, c).

Results and Discussion

Percent healthy, discolored and empty seeds were observed 53.50, 30.50 and 15.00, respectively. Number of healthy seeds was higher (53.50 %) then other seeds (Table 1). This finding was consistent with Shahijahan *et al.* (1988). He cited modern rice varieties had more then 1% spotted grain then the local one and also reported that 23 fungal species (17 genera), 1 actinomycetes and 2 bacteria were associated with spotted rice grains.

In soil, the highest germination of before sowing seeds was counted in treatment T6 (81.75%) and the lowest was (56.50%) in treatment T3 (Table 2). The effect of treatment T2 and T5 were statistically similar to T6. In sand, statistically similar results to soil test were found for before sowing seeds. Highest germination of freshly harvested seeds (89.00 %) was observed in treatment T6 followed by T2 and lowest were counted in T3 (65.50 %) in soil. In sand highest percent of germination were 91.75 % in treatment T6 and lowest were 67.00% in T3. These values were statistically different. This result suggested that percent germination of freshly harvested seeds were higher than the before sowing seeds for all treatments.

Among the treatment highest seedling vigour was 2064.80 in T6 and the lowest was in T3 representing 974.83 for before sowing treated seeds. For freshly harvested seeds, highest seedling vigour (2614.00) were observed in treatment T5, lowest was 1330.00 in treatment T3. From both situation T5, T6 and T2 showed good performance than T1 (farmer's seeds) and T3 (diseased seeds). Root length and shoot length was also higher (Table 3). It is suggested that seed cleaning and washing have good impact on the vigour of rice seedling.

Table 1: Dry inspection of collected farmer's seeds of rice var. BR11

Categories	% of categories
Apparently healthy seed	53.50a
Discolored and spotted seed	30.50b
Empty seed	15.00c
Level of significance	00.05

400 seeds were tested for each samples

In case of before sowing seeds, highest number of blight symptoms was observed in T3 and lowest was in T6 (25.25 & 20.25 and 6.50 & 6.25 %) in both methods. In case of freshly harvested seeds, highest was recorded in T3 (16.75 %) and lowest in T5 (3.00%), respectively that were statistically significant and different (Table 4).

Incase of tillering stage highest disease index was 41.26 and lowest was 12.16 in treatment T3 and T5, respectively (Table 5). Treatment T2, T4 and T6 also showed good result than T3 and T1. Highest BLB disease index at flag leaf stage was 65.70 % and lowest was 21.04 % in diseased seeds (T3) and farmer's seeds washed with brine solution (T5), respectively. Zhang *et al.* (1996) found that rice seeds were soaked in 50 ppm solution of Zhongshengmycin which reduced the BLB index in the field. This is a support of the present findings that seed cleaning and washing with normal water as well as brine water reduced the BLB incidence under natural condition.

Several number of different coloured bacterial colony were observed in NA (Nutrient agar) and KB (King's medium B) media from both before sowing and freshly harvested seeds of all treatments by liquid assay. From these, six bacterial strains were selected for identification after purification (Table 6). On the other hand, thirteen strains from blight symptoms showing leaf were obtained from slide cassette holder and rolled paper towel method and their physiological, biochemical, serological, hypersensitivity and pathogenicity test were performed. All the strains showed gram negative reaction and negative test for soft rooting in potato slices. Many workers used similar test and reported the same results (Agarwal *et al.*, 1989; Ashura *et al.*, 2001; Gottyn *et al.*, 1996; Xie *et al.*, 1999) for identification of bacterial pathogens from rice seeds.

Table 2: Germination of rice seeds (before sowing and freshly harvested) of different treatments in sand and soil before sowing

	% germination			
	Soil		Sand	
Treatments	Before sowing	Freshly harvested	Before sowing	Freshly harvested
T_1	63.75c	74.75d	66.25c	76.25d
T_2	81.25a	87.25ab	82.50a	90.25ab
T_3	56.50d	65.50e	59.50d	67.00e
T_4	69.50b	79.50c	71.50b	80.75c
T_5	77.75a	85.50b	80.25a	88.50b
T_6	81.75a	89.00a	83.00a	91.75a

Table 3: Seedling vigour of rice seeds (before sowing and freshly harvested) of different treatments evaluated by rolled paper towel method before sowing in sand and soil

	% germination		Root length (cm)		Shoot length (cm)		Vigour index (VI)	
Treatments	Before sowing	Freshly harvested	Before sowing	Freshly harvested	Before sowing	Freshly harvested	Before sowing	Freshly harvested
T_1	66.00b	78.00b	13.03c	15.02cd	5.87c	6.31cd	1248.45d	1663.00bc
T_2	83.75a	86.75a	15.67d	18.00ab	7.95a	7.94ab	1979.60a	22.5800a
T_3	59.75e	69.50c	11.60d	13.63d	4.71d	5.50d	974.83e	1330.00c
T_4	71.25c	80.25b	13.30c	15.98bc	6.37c	6.80bcd	1403.03c	1830.00b
T_5	78.75b	91.00a	14.68b	20.06a	7.15b	8.61a	1689.63b	2614.00a
T_6	85.25a	88.50a	16.07a	18.03a	8.15a	7.69abc	2064.80a	22.95.00a

Means with different letters differ significantly at $P<0.05$, T_1= Farmer's seed, T_2= Clean seed, T_3= Diseased seed, T_4= Farmer's seed washed with water, T_5= Farmer's seed washed with brine solution (20 % NaCl), T_6= Clean seed washed with water

Table 4: Rice seeds (before sowing and freshly harvested) of different treatments evaluated by cassette holder method and rollod paper towel method

	% seedling with bacterial symptom			
	Cassette holder		Rollod paper towel	
Treatments	Before sowing	Freshly harvested	Before sowing	Freshly harvested
T_1	19.50ab	16.25ab	16.00ab	11.50ab
T_2	7.25c	5.00c	6.50c	3.50c
T_3	25.25a	17.75a	20.25a	16.75a
T_4	16.50b	13.50b	11.00c	8.75bc
T_5	9.75c	4.00c	7.50c	3.50c
T_6	6.50c	6.00c	6.25c	3.00c

Means with different letters differ significantly at $P<0.01$

Table 5: Effect of different treatments on the incidence of BLB at maximum tillering and flag leaf stage of rice under field conditions

	Total no. of leaves/hill		% of healthy leaves/hill		% of diseased leaves/hill		% of disease index (PDI)	
Treatments	MTS	FLS	MTS	FLS	MTS	FLS	MTS	FLS
T_1	57.55c	44.05d	70.00d	53.67d	30.00b	46.33b	28.41b	42.51b
T_2	70.20ab	59.64ab	87.38b	75.70b	12.63d	24.30b	15.66d	27.88d
T_3	48.70d	39.88e	60.03e	44.00c	39.97a	50.00a	41.26a	65.70a
T_4	59.33c	48.58c	77.71c	60.25c	22.29c	29.55c	23.69c	35.60c
T_5	67.58b	57.05b	91.34a	82.00a	8.66e	18.00c	12.16e	21.04e
T_6	72.63a	62.72a	89.64ab	80.65a	10.36de	19.35e	14.15de	26.39d

Means with different letters differ significantly at $P<0.05$, T_1 = Farmer's seed, T_2 = Clean seed, T_3 = Diseased seed, T_4 = Farmer's seed washed with water, T_5 = Farmer's seed washed with brine solution (20% NaCL), T_6 = Clean seed washed with water, MTS = Maximum tillering stage, FLS = Flag leaf stage

Table 6: Bacterial strain isolated from different symptoms of different treatments of rice and their characterization

Strain No.	Source of bacterial strain	Strain on Na media	Strain on KB media	KOH solubility test	Grain straying test	Soft rotting test	Oxidase test	O/F test	Slide agglut ination test	Under NUV light	Chilli HR test	Tabacoo HR test	Site of inoculation: Rice leaf	Site of inoculation: Stem puncture of rice seedling	Identify of the pathogens
1	Blight symptom	Yellow	Yellow	+	-	-	-	+/-	+	-	+	+	+	-	*X. o. o.*
2	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
3	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
4	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
5	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
6	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
7	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
8	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
9	,,	White	White	+	-	-	-	+/-	+	-	+	+	+	+	*P. fuscovaginae*
10	,,	,,	,,	+	-	-	-	+/-	+	-	+	+	+	+	*P. fuscovaginae*
11	,,	F.green	F.green	+	-	-	-	+/-	ND	+	+	+	+	-	*P. fuscovaginae*
12	,,	,,	,,	+	-	-	-	+/-	ND	+	+	+	+	-	*P. fuscovaginae*
13	,,	,,	,,	+	-	-	-	+/-	ND	+	+	+	+	-	*P. fuscovaginae*
14	Seed (liquid assay)	Yellow	Yellow	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
15	,,	,,	,,	+	-	-	-	+/-	ND	-	+	+	+	-	*X. o. o.*
16	,,	White	White	+	-	-	-	+/-	+	-	+	+	+	+	*P. fuscovaginae*
17	,,	F.green	F.green	+	-	-	-	+/-	ND	+	+	+	+	-	*P. fuscovaginae*
18	,,	White	White	+	-	-	-	+/-	+	-	+	+	+	+	*X. o. o.*
19	,,	,,	,,	+	-	-	-	+/-	+	-	+	+	+	+	*X. o. o.*

X.o.o. = *Xanthomonas oryzae* pv. *oryzae*, A.a.a. = *Acidovorax avenae* pv. *avenae*, + = Positive reaction, - =Negative reaction, ND = Not done, F. green = Fluorescent green, KB = King's medium B, NA = Nutrient agar

Seed cleaning and washing is a cultural practices that can reduces the bacterial infection in seeds both before sowing and in freshly harvested seeds. Moreover, it suppress the BLB disease a considerable amount in the field. So it can be suggested our farmer's to use.

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