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STUDIES ON SEED-BORNE FUNGI, BACTERIA AND NEMATODE OF RICE IN THE PUNJAB

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One hundred and seventy paddy seed samples were collected from Punjab paddy growing area, and tested for fungal, bacterial and nematode seed-borne pathogens by using standard methods. Seven seed-borne fungi were recorded. *Trichoconis padwickii* was recorded in 57 seed samples with maximum percent seed infection of 84.5. The infection of *Fusarium moniliforme, F semitectum, Drechslera oryzae* and *Curvularia lunata* was 21.0% on variety B-6129, 14.5% on IRRI-6 and 21.5% on KS-282 and B-385 respectively. Other seed-borne fungi such as *F. graminearum* and *Nigrospora oryzae* were recorded at infection of 1.0%.

Forty four seed samples were assayed for identification of bacterial pathogens using seedling symptom method. The seed infection of *Xanthomonas oryzae* and *Pseudomonas avenae* was 11.0% in variety IRRI-6 at Lahore and 8.5% in variety B-385 from Sahiwal area. All the seed samples were also assayed for rice with tip nematode *(Aphelenchoides basseyi)*. Out of 170 seed samples tested, 13 were found infected with white tip nematode. It was observed that percent seed infection with fungi, bacteria and nematode varied from cultivar to cultivar and locality to locality.

Key words: Seed-borne pathogen, Blotter paper method, Abnormality, Rice seed samples (Paddy).

Introduction

Seed-borne pathogens can reduce the germination resulting in poor stands of crop leading to reduction in yield. Mathur *et al.* [1] reported 33% grain yield loss in rice grain in a paddy crop raised from seed infected with *Trichoconis padwickii* Ganguly. Mukerjee [2] isolated *Xanthomonas campestris* pv. *oryzae* (Uyeda and Ishiyama) Dowson from parenchyma, embryo and endosperm of rice seed. 20 to 30% crop losses have been reported by this disease in the Philippine and Indonesia [3]. Seed-borne nature of bacterial stripe of rice caused by *Pseudomonas avenae* Mann, have been reported [4,5].

It is estimated that rice nematodes reduce yields upto 10% [6]. Several nematodes have been reported on rice crop in Pakistan [7] but little information is available on the nematodes occurring in rice seed as pathogen.

Keeping in view the importance of seed-borne diseases in rice production areas and available little information on such aspects, the present study was undertaken with the objectives to isolate, identify and to determine the occurrence of each pathogen on the paddy seed of commercially grown cultivars in different ecological zones of the Punjab Province.

Materials and Methods

Collection of seed samples. One hundred and seventy paddy seed samples of eight cultivars (Table 1) were collected during 1987-88 from rice growing areas of the Punjab and Rice Research Institute, Kala Shah Kaku. Seed samples (1000 gms) were drawn according to the procedure as laid down in ISTA rules [8]. Out of 170 samples, 58 were tested for seed-borne fungi, 44 for bacteria and 170 for nematodes. In this study, seeds were not surface disinfected.

Isolation of fungi. Fungi from the paddy seed were detected from four hundred seed of each sample by the Standard Blotter Methods [8]. Twenty five seed were plated in each petri dish (diameter 9 cm) containing three pieces of blotter paper moistened with distilled water. The plates were incubated at 20° (± 2°) for eight days under alternating cycle of twelve hrs. day/night by using flourescent tubes. At the end of incubation period, the seeds were examined for fungal growth under stereoscopic binocular microscope.

Germination test. Eleven rice seed samples showing a high incidence of seed-borne pathogen were tested by the Between Paper Method [8]. Four hundred seeds of each sample were sown on the four wet filter paper (Anker brand size, 24 x 48 cm), each with 100 seed. Papers were rolled and put in polyethylene bags, and incubated at 20° (± 2°) for twelve days. At the end of incubation period, seed germination was recorded.

Seedling symptom test. The method used to detect *X. campestris* pv. *oryzae* and *P. avenae* were based on technique described by Singh *et al.* [9] and Shakya *et al.* [10]. Two hundred rice seeds were plated in petri dishes on three layers of filter paper moistened with 230 ppm of

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nitrogen solution in four replication with 50 seed per plate. The petri dishes were incubated at 30° (± 2°) for 17 days. After three days, the lids were removed to allow seedling to grow further. The petridishes were covered with polyethylene sheet to maintain high humidity. Nitrogen solution was added twice a day during first week and then dishes were flooded with sterile water. The leaves exhibited water soaked stripe were examined under stereoscopic microscope for confirming the bacterial streaming.

Pathogenicity test. The test was done by injecting a suspension of each bacterial culture (10^6 cells/ml) separately in the stem of 3-4 weeks old rice seedling of a susceptible variety B-385 raised in pots. Seedlings injected with sterile water served as control. The pots were incubated at 27°-30° for 4-5 days for observation of bacterial symptoms [10].

Extraction of nematodes. A 10 gm composite rice seed subsample from each sample was placed in the blender containing 100 ml water and agitated for 45 seconds. The resultant rice grain and rice hull suspension was loaded in the Baermann apparatus and examined for rice nematode larvae after 24 hr.

Results and Discussions

Seed infection percentage ranges of the fungi recorded in paddy are listed in Table 1. All the fungi detected in this study are reported to be seed-borne in nature [11].

Occurrence of *Trichoconis padwickii* was found in high frequency in all the samples except one (B-198) from Kala Shah Kaku. The recovery of this fungus ranged from 1.0 to 84.5% with maximum (84.5%) on variety, B-385 collected from Lahore area. The varieties, KS-282 (Sahiwal) and B-385 (Hafizabad and Sheikhupura) showed the recovery of this fungi as 55 and 20-30% respectively. *Fusarium moniliforme* was the common fungus pathogen observed in 35 samples out of 58 tested and its infection ranged from 0.5 to 21.0%. Maximum (21.0%) recovery of this genus was recorded on variety B-6129. In case of varieties KS-282 (Kala Shah Kaku) and B-385 (Sialkot) its recovery from seed was 10.5 and 9.0% respectively. *Fusarium semitectum* Berk and Rav. was also a common fungus recorded in 46 seed samples. Maximum recovery of this fungus was from variety IRRI-6 (14.5%) and B-385 (12.5%) at Sahiwal and 10.5% on B-385 in Lahore area. *F. graminearum* Schwabe was recovered in only 1 sample of variety B-198, collected from Kala Shah Kaku.

Drechslera oryzae (Van Breda de Haan) subramanian and Jain, an important seed-borne pathogen was found in 42 seed samples. Maximum recovery was from varieties B-198 and IRRI-6 (8.5%) at Lahore and KS-282 (21.5%)

TABLE 1. SEED-BORNE FUNGI DETECTED IN PADDY SEED SAMPLES COLLECTED FROM THE PUNJAB DURING 1987-88.

Locality	Cultivars	No. of sample tested	seed-borne Fungi	Range seed percentage showing fungal colonies
Lahore	B-198	1	*Curvularia lunata*	1.5
			Drechslera oryzae	8.5
			Fusarium moniliforme	3.5
			F. semitectum	1.0
			Nigrospora oryzae	1.0
			Trichoconis padwickii	1.0
	B-385	18	*C. lunata*	0.0 - 6.0
			D. oryzae	0.5 - 6.0
			F. moniliforme	0.0 - 1.5
			F. semitectum	0.0 -10.5
			T. padwickii	15.0 -84.5
	B-370	2	*C. lunata*	0.5 - 3.0
			D. oryzae	1.0 - 2.0
			F. semitectum	3.0 - 5.0
			T. padwickii	3.0 - 9.5
	IRRI-6	2	*C. lunata*	0.5 - 1.0
			D. oryzae	1.5 - 8.5
			F. moniliforme	1.0
			F. semitectum	1.0 - 3.0
			T. padwickii	51.5 -62.5
Sahiwal	B-370	2	*C. lunata*	0.5
			D. oryzae	0.5 - 2.5
			F. semitectum	4.0 - 6.5
			T. padwickii	36.5 -48.5
	B-385	15	*C. lunata*	0.0 - 6.5
			D. oryzae	0.0 - 5.5
			F. semitectum	1.5 -12.5
			T. padwickii	7.0 -45.5
	IRRI -6	4	*C. lunata*	0.0 - 8.5
			D. oryzae	0.5 - 3.0
			F. semitectum	3.0 -14.5
			T. padwickii	3.0 -34.0
	KS-282	4	*D. oryzae*	10.5 -21.5
			F. moniliforme	0.5
			N. oryzae	2.0
			T. padwickii	21.5 -55.0
Sialkot	B-385	2	*C. lunata*	0.0 -21.5
			F. moliniforme	0.0 - 9.0
			T. padwickii	3.0 - 7.0
Gujrat	B-385	1	*F. moniliforme*	0.5
			T. padwickii	13.0
Sheikhu-pura	B-385	1	*F. moliniforme*	6.0
			T. padwickii	30.0
Hafiz-abad	B-385	1	*F. moliniforme*	6.0
			T. padwickii	20.0
Rice Res. Inst. Kala Shah Kaku	B-198	1	*F. moniliforme*	6.5
			F. graminearum	1.0
			F. semitectum	5.0
	B-6129	1	*D. oryzae*	5.0

(Continued)

(Table 1, Continued)

		F. moniliforme	21.0
		F. semitectum	1.5
		T. padwickii	4.5
IRRI-6	1	*T. padwickii*	10.5
		F. moniliforme	8.0
Jhona-349	1	*C. lunata*	0.5
		T. padwickii	13.0
		F. miniliforme	5.0
KS-282	1	*F. moniliforme*	10.5
		T. padwickii	1.5
Total	58		

from Sahiwal. *Curvularia lunata* (Wakker) Boedijn and *Nigrospora oryzae* (Berk and Broome). Petch., were also detected in a few samples with highest infection (21.5%) on variety B-385 and 2.0% on KS-282 (Sialkot and Sahiwal).

Fungi previously, reported on peddy seed by Bajwa *et al.* [12] were identified at the generic level while in the present study, the fungi are identified at the species level. A number of saprophytes such as *Alternaria alternata, A. Longisma, Aspergillus* spp., *C. geniculata, D. hawiensis, D. tetrramera, Epicoccum* spp., *Myrothecium roridum* and *Rhizopus* spp., were also observed but their incidence was not recorded.

Ninety eight percent of the seed samples were found infected with *T. padwickii,* the cause of stack burn/leaf spot. The infected seed by this fungus became rotted before germination leading to poor stand of nursery seedlings. The infection of roots and coleoptile cause the death of young seedlings [1]. In this study sever infection of coleoptile was observed . *D oryzae*, the cause of leaf blight/leaf spot was found in 72% of the samples tested. The mild seed infection of this pathogen (few conidia in group but no mycelium) can lead to seed rot and seedling mortality upto 100% [13] being a multiple cycle disease.

The effects of seed-borne fungi and bacteria on seed germination was studied following Between Paper Method. The results are given in Table 2. The results were based on infection percentages. Seedlings exhibiting discolouration and rottening of tissues were considered as abnormal. The maximum abnormality of seedlings was recorded on B-6129 (38.0%) from Kala Shah Kaku while it was minimum (3.0%) on IRRI-6 from Sahiwal. The difference in seedling abnormality and ungerminated seed in these cultivars obviously due to infection level of seed-borne pathogen.

Rotted and discoloured seedlings were plated on blotter paper and incubated for eight days at 20° to confirm the cause of rottening and discolouration in seedlings. The isolation of *T. padwikii, F. moniliforme, F. semitectum, D. oryzae and C. lunata* from the diseased seedlings suggest the involvement of the fungi in the causation of abnormal seedling and prohibiting the seed to germinate.

The samples assayed for detection of *X. oryzae* showed maximum infection (11.0%) from Lahore on variety IRRI-6. *P. avenae* found in 37 seed samples out of 44 tested, showed maximum infection of 8.5% on variety B- 385 from Sahiwal (Table 3). Minimum infection (0.5-1.5%) was found on varieties B- 198 and B-370 collected from Lahore, Sahiwal and Kala Shah Kaku. Variable

TABLE 2. EFFECT OF SEED-BORNE FUNGI ON GERMINATION OF RICE SEEDS.

Locality	Variety	Normal seedling % age.	Abnormal seedling %age.	Ungerminated seeds % age.
Lahore	B-370	93.0	6.0	1.0
	B-385	92.0	4.0	4.0
	IRRI-6	86.0	6.0	8.0
Sahiwal	B-370	84.0	10.0	6.0
	B-385	88.0	7.0	5.0
	IRRI-6	96.0	3.0	1.0
Kala Shah Kaku	B-198	78.0	12.0	10.0
	B-6129	57.0	38.0	5.0
	IRRI-6	77.0	21.0	2.0
	Jhona-349	81.0	19.0	0.0
	KS-282	88.0	8.0	4.0

TABLE 3. DETECTION OF SEED-BORNE BACTERIUM FROM PADDY SEEDLING SYMPTOM TEST IN THE PUNJAB DURING 1987-88.

Locality	Cultivar	No. of samples tested	Infection percentage range	
			X. oryzae	*P. avenae*
Lahore	B-198	1	1.0	1.5
	B-370	2	0.0	0.5
	B-385	10	0.0 - 6.5	1.5 - 5.0
	IRRI-6	2	0.0 -11.0	1.0 - 6.0
Sahiwal	B-198	2	0.0 - 1-0	0.5 - 1.5
	B-370	2	0.0 - 1.0	0.0 - 2.0
	B-385	8	2.0 - 5.0	1.0 - 8.5
	IRRI-6	4	0.5 - 1.5	0.5 - 2.5
	KS-282	1	0.5	2.5
Rice Res. Inst. Kala Shah Kaku	B-198	1	1.0	1.0
	B-370	1	0.5	0.0
	B-388	1	5.5	1.5
	IRRI-6	1	6.5	3.0
	Jhona-349	1	6.5	3.5
	KS-282	1	7.5	2.5
Sialkot	B-385	2	1.0 - 2.0	5.0 - 6.0
Gujrat	B-385	1	2.0	4.0
Hafizabad	B-385	1	1.0	3.0
Sheikhupura	B-385	1	2.0	4.0
	Total	44		

infection on different cultivars might be due to climatic conditions and the degree of resistance exhibited by the individual cultivars [14]. This finding confirm the infection of paddy seed by *P. avenae* as reported by Shakya *et al.* [5] in the Punjab where as the dectection of *X. oryzae* is a new report for Pakistan. Although the disease, bacterial blight has been reported in the field based on visual foliage symptoms [14,15].

The suspension of each bacterial pathogen injected in to a separate set of rice seedlings which produced typical symtoms i.e. leaf stripe for *P. avenae* and blighted spots in case of *X. oryzae*. Such symptoms have been reported by Shakya *et al.* [5,16].The seedlings inoculated with sterile water (control) produced neither stripe nor blight symptoms.

Of the 170 seed samples tested for the occurrence of nematodes, 13 samples indicated the presence of white tip nematode *A. bassevi* (Table 4). The variation in reaction to nematode incidence among cultivars might be correlated with the inherited level of resistance of each cultivar. Strong correlation have been reported between attraction of white tip disease nematode and susceptibility of rice cultivars in Korea [17].

TABLE 4. PRESENCE OF RICE WHITE TIP NEMATODE EXTRACTED FROM VARIUOS CULTIVARS OF PADDY SEED SAMPLE COLLECTED FROM THE PUNJAB.

Locality	Cultivar	No. of seed samples	
		Assayed	Infected
Lahore	B-385	15	0
	IRRI-6	2	0
Sahiwal	B-385	13	0
	B-370	02	0
	IRRI-6	4	0
Rice Res. Inst.	B-198	20	1
Kala Shah Kaku	B-370	30	4
	B-385	40	5
	B-6129	15	0
	IRRI-6	9	3
	KS-282	15	0
Sialkot	B-385	2	0
Gujrat	B-385	1	0
Hafizabad	B-385	1	0
Sheikhupura	B-385	1	0
	Total	170	13

Conclusion

The present investigations have indicated the prevalence and frequency of seed-borne pathogens associated with paddy seed in the Punjab. Similar studies in detail, are suggested to know the extent of seed infection level for better understanding of epidemiological studies.

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