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DETECTION AND LOCATION OF SEED-BORNE FUNGI IN SORGHUM SEED

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Abstract

Using ISTA techniques, 27 species of fungi were isolated from 6 varieties of sorghum seed. Deep freezing method was found superior to the standard blotter and agar plate methods for the detection of *Fusarium* spp., *Drechslera spicifera* and *Curvularia lunata*. Seed components showed infection of *Alternaria alternata, Curvularia lunata, Drechslera spicifera, D. hawaiiensis, Fusarium moniliforme* and *F. semitectum* but *F. moniliforme* was frequently recorded in the embryo.

Introduction

Sorghum, an important summer crop is cultivated on an area of about 296,000 ha in Pakistan with an estimated production of 450,000 metric tons (Anon., 1983). Of the fungi infecting sorghum, atleast 37 fungi are reported to be seed-borne (Bain, 1950; Noble & Richardson, 1968). Reports of the fungi isolated from sorghum seeds include species of *Alternaria, Cladosporium, Curvularia, Drechslera* and *Fusarium* (Ghafoor & Khan 1974). The present work was carried out to detect the seed-borne fungi of sorghum and their location in the seed.

Materials and Methods

Fifteen seed samples of 6 different varieties of sorghum were collected from different places in Sind during 1986-87. Using ISTA techniques (Anon., 1976), 400 seeds of each variety were tested by the standard blotter, deep freezing and agar plate methods. Seeds were also tested by the component plating method for the detection and location of seed-borne fungi. Details of each method are given below:

Standard Blotter Method: Untreated seeds and seeds after 10 minutes treatment with 1% NaOCl were placed on three layers of moistened blotters, 25 seeds per dish. The dishes were incubated for 7 days at 24°C ± 1°C under 12 h of alternating cycles of A.D.L. (artificial day light supplied by cool white flourscent tube) and darkness.

Deep Freezing Blotter Method: Untreated seeds were incubated for 1 day each at 20°C and –20°C followed by 5 days incubation at 24°C ± 1°C under 12 h of alternating cycle of ADL and darkness.

Table 1. Fungi recorded in 15 samples of sorghum and tested by standard blotter method.

Seed-borne fungi	No. of samples infected	Percentage Incidence	
		Range	Average
Alternaria alternata	9	0-5-9.0	6.5
*Aspergillus candidus**	5	0.5-3.5	1.0
A. flavus	6	0.5-5.0	2.5
A. fumigatus	2	0.2-0.5	0.4
A. niger	7	0.5-7.0	6.8
*A. sulphureus**	2	0.5-1.0	0.8
*A. terreus**	3	0.5-2.0	1.0
Cladosporium sphaerospermum	4	1.5-4.5	2.5
Hormoconis sp.*	2	0.5-1.0	0.8
*Curvularia clavata**	5	1.0-5.5	3.5
C. lunata	11	1.5-20.5	12.5
C. pallescens	3	0.5-1.0	0.6
*C. robusta**	2	0.5-1.0	0.7
*C. tuberculata**	2	1.0-1.5	1.2
Drechslera hawaiiensis	3	0.2-0.5	0.4
*D. halodes**	3	0.2-1.0	0.5
D. rostrata	3	0.2-1.0	0.5
D. sorghicola	3	0.5-1.0	0.6
*D. spicifera**	8	0.5-10.5	6.8
Fusarium chlamydosporum	6	0.5-9.5	6.5
F. moniliforme	15	2.0-48.5	42.5
F. semitectum	9	0.5-22.5	19.5
*Memnoniella echinata**	2	0.5-1.5	1.0
*Myrothecium roridum**	2	0.5-1.0	0.7
Phoma glumarum	1	0.5	0.5
*Sporotrichum pruinosporum**	6	2.0-8.5	6.5
*Stachybotys atra**	3	0.5-1.5	1.0

*The new records have been marked by asterisk.

Agar Plate Method: Chlorine pretreated seeds were plated on potato dextrose agar (PDA), ten seeds per dish and incubated at 24°C under 12 h of alternating cycles of ADL and darkness. Fungi were identified after references to Barnett (1972), Booth (1971), Ellis (1971) and Raper & Fennell (1965).

Component Plating Method: For the location of deep infection, the seeds were washed in distilled water in a test tube and then soaked for 10-12 h in distilled water. The

seeds were dissected aseptically under a stereobinocular and each component like stylet remnant, pericarp, endosperm, embryo and tip cap, was surface disinfected in 1% sodium hypchlorite solution and plated directly on water soaked blotter in Petri dishes. Different parts of the seed were plated in a dish, and after 7 days of incubation under 12 h of alternating cycles of ADL and darkness at 24°C ± 1°C, the seed parts were examined for fungal infection.

Results and Discussion

A total of 27 species of fungi isolated by standard blotter method from 15 seed samples of 6 sorghum varieties are given in Table 1. Of these, 13 fungal species are being reported for the first time from Pakistan. However, the remaining fungal species have been previously reported from sorghum seeds (Ghafoor & Khan, 1974; Mirza & Qureshi, 1978, Ahmad, 1956). Among the fungi isolated from sorghum seeds *Fusarium moniliforme* was the most predominant because it was found in all the seed samples with an infection range of 2-48.5% (Table 1). *Fusarium semitectum* and *Curvularia lunata* were detected in 60-70% of seed samples with an average infection percentage of 19.5 and 12.5, respectively. Other seed-borne fungi isolated from sorghum seeds were: *Alternaria alternata, Drechslera spicifera, Aspergillus niger, Fusarium chlamydosporum* and *Sporotrichum pruinosporum* showing 6.5-6.8% infection (Table 1). Presence of these fungi especially *F. moniliforme* affected seed germination. These observations are similar with the finding of Feliciano *et al.*, (1981) who reported that seed-borne fungi affect the germinability of seed.

Out of the three methods employed, deep freezing method yielded maximum counts of *Fusarium* spp., *Drechslera* sp., and *Curvularia* sp., from seeds of the 6 sorghum varieties tested (Table 2). These observations corroborate with the findings of Mathur *et al.*, (1975). Disinfection of the seed with 1% sodium hypochlorite showed an increase in the incidence of *Curvularia lunata, Drechslera spicifera, Fusarium moniliforme* and *F. semitectum* due to elimination of ubiquitous type of fungi such as *Aspergillus* spp., *Rhizopus* spp., and *Cladosporium* spp.

Results of the components plating of 3 varieties of sorghum showed that *Alternaria alternata, Curvularia lunata, Drechslera hawaiiensis, D. spicifera* and *Fusarium semitectum* were recovered more frequently from the pericarp with an average incidence of 14.5, 11, 10, 4.5 and 15% whereas endospermic infection of these fungi were 2.5, 5.5, 2.0, 2.5 and 10% respectively (Table 3). *Fusarium moniliforme* was the only fungus which was recovered from the embryo in all the 3 highly infected sorghum varieties. The fungus was more abundantly present in the endosperm than in the pericarp. It is inferred from these results that the infection of *F. moniliforme* could be detected more conveniently in the endosperm by the component plating method than pericarp and embryo of the seed..

Table 2. Percent incidence of seed-borne organisms associated with six varieties of sorghum seeds.

Sorghum cultivar	*Curvularia lunata*				*Drechslera spicifea*				*Fusarium moniliforme*				*Fusarium semitectum*			
	Blotter Method				Blotter Method				Blotter Method				Blotter Method			
	Untreated seeds	Pretreated seeds	Deep Freezing Method	Agar Plate Method	Untreated seeds	Pretreated seeds	Deep Freezing Method	Agar Plate Method	Untreated seeds	Pretreated seeds	Deep Freezing Method	Agar Plate Method	Untreated seeds	Pretreated seeds	Deep Freezing Method	Agar Plate Method
Red Janpur	1.5	7.0	15.0	18.5	1.5	0.5	4.5	1.5	30.5	38.6	42.8	28.0	10.0	12.8	20.5	14.0
Bhagdar	11.5	15.8	20.0	12.0	3.5	2.5	5.6	2.5	30.5	32.8	39.2	15.8	8.5	10.5	12.5	16.0
Sarokartuho	17.0	11.5	16.0	12.5	5.5	7.2	8.5	8.0	28.0	36.8	40.5	20.5	3.0	6.5	8.0	6.5
Depar	8.5	7.0	20.5	10.0	0.0	0.0	0.0	0.0	6.5	8.5	22.2	3.5	11.0	11.5	22.0	12.5
Kaghan	0.0	0.0	0.0	2.0	0.0	1.0	2.0	6.0	17.0	19.5	30.0	12.0	1.5	6.0	14.6	11.5
Sweet Jowar	3.0	0.0	9.0	9.5	5.0	4.0	7.5	3.8	2.0	3.5	8.0	4.5	0.0	0.0	2.0	0.0

Table 3. Percentage recovery of fungi from different seed parts of three varieties of sorghum.

Varieties	Seed-borne Fungi	Style Remnant	Tipcap	Pericarp	Endos-perm	Embryo
Red Janpur	*Alternaria alternata*	15	17	9	0	0
	Drechslera spicifera	6	7	5	4	0
	Fusarium moniliforme	30	20	30	32	25
	F. semitectum	5	4	15	10	4
Bhagdar	*Curvularia lunata*	4	4	12	6	0
	Drechslera spicifera	2	3	4	2	0
	Fusarium moniliforme	26	24	29	30	15
Sarokartuho	*Alternaria alternata*	20	19	20	5	0
	Curvularia lunata	8	12	10	5	0
	Drechslera hawaiiensis	0	0	10	2	0
	Fusarium moniliforme	0	0	0	25	10

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