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Isolation of seed-borne fungi of sorghum (*Sorghum vulgare* pers.)

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Abstract
Sorghum (*Sorghum vulgare* Pers.) is the fifth most important cereal crop in the world after wheat, rice, maize and barley. The grain had been used for consumption of both humans and livestock and also different genes of the plant serve many other important uses. The crop has been suffer from various type of diseases, majority of them are known to be caused by fungi, which are mostly seed born. In present study Seed-borne fungi of sorghum in Marathwada region were surveyed. A total of 24 seed samples of eight different varieties from various locations, collected were tested, using the blotter and agar plate methods. Twenty eight fungal species of eighteen genera appeared in the seeds of eight different variety of Sorghum. In untreated seeds of the entire varieties maximum incidence was of *Curvularia lunata.* Treated seeds showed complete absence of *Cladosporium herbarum, Trichothecium roseum* and *Absidia ramose.* Agar plate method was found to be favorable for the maximum counts of saprophytic fungi and also favorable for detection of some specific fungi. Presence of many pathogenic fungi in considerable number of seed samples indicates the need of field surveys for these and other pathogens.

Keywords: Isolation, Seed-borne Fungi, Sorghum, Marathwada

INTRODUCTION

Sorghum (*Sorghum vulgare* Pers.) is the fifth most important cereal crop in the world after wheat, rice, maize and barley. It is found in the arid and semi arid parts of the world, due to its feature of being extremely drought tolerant. The nutritional value of sorghum is same as of that of corn and that is why it is gaining importance as livestock feed. Sorghum is also used for ethanol production, producing grain alcohol, starch production, production of adhesives and paper other than being used as food and feed.

Sorghum is popularly known as "Jowar" in India. The crop in the country stands at the third place in context of importance after wheat and rice. The grain had been used for consumption of both humans and livestock and also different genes of the plant serve many other important uses. The crop was introduced in India in the first millennium and since then it has been actively cultivated in the subcontinent. The production of sorghum in India reaches up to 9 million metric tons mark each year but last few years have shown a marginal but gradual decline in the production and productivity of the crop. The area under cultivation of the crop too has a steep decline in the last 15 years i.e. 50% and 25% in the khariff and rabi season respectively (CRNindia, 2010).

In sorghum (*Sorghum bicolor*), covered smut (*Sphacelotheca sorghi*), head smut (*Sphacelotheca reiliana*) and long smut (*Tolyposporium ehrenbergii*) have been reported to be the most destructive pathogens, causing heavy losses in third world countries (Frowd, 1980). *Peronosclerospora sorghi,* the downey mildew pathogen in sorghum and maize, and *Sclerospora graminocola* in pearl millet transform the floral primordial into vegetative leafy structures causing 30 to 70% losses in seed production in the semi-arid tropics (Williams,1984). A yield loss of 58 to 70% of hybrid sorghum and millet with 60 to 76% ergot severity has been reported in most sorghum and millet growing countries (Thakur and Chahal, 1987). Besides, these losses in potential yield, mold fungi which grow on the seed substratum produce mycotoxins which are hazardous to man and animals (Halt, 1994).

Commercially, discolored sorghum seeds caused by fungi are of poor quality (Castor and Frederikser, 1980; Gopinath and Shetty, 1987), reducing their acceptability and thus, the market value of the produce. Grain mold causes crop loss by reducing seed size and weight, the food value and keeping quality of grains (Gopinath, 1984: Bandyopadhyay, 1986). Many of the diseases that cause reduced yields in sorghum have seed-borne phases. Seed borne inoculums therefore, has severe implications for yield, seed production and distribution systems, trade, human nutrition and germplasm. The management of these pathogens during the seed-borne phase is considered to be the cheapest disease control strategy (Shenge, 2007).

However, details on the role of seed born fungi and their metabolites in the deterioration of seed quality and viability are meager. The effective management can only be implemented effectively if the pathogens are correctly identified. It is in view of this that the current study aimed at detecting seed borne pathogens on sorghum seeds.

In present work isolation of mycoflora, using recent techniques, from the seeds at their various developmental stages, collected from different field of marathwada region. Fungi were isolated from the naturally discolored, rotten, immature and shriveled seeds, collected from the standing crops. Isolation of seed mycoflora

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using standard blotter method as well as agar plate method is studied.

MATERIALS AND METHODS

Experimental location

The laboratory experiment was conducted in the Plant Pathology Laboratory of the Department of Botany, Dr. B.A.M. University, Aurangabad (M.S.) India.

Sources of experimental materials

Twenty four samples of eight different varieties of sorghum seeds were collected (Neergaard, 1973) from sorghum growing areas of Marathwada Region, of Maharashtra, India. The Eight Varieties of Sorghum are as: CSH-1, CSH-4, CSH-5, CSH-6, CSH-7, CSH-9, MSH-37 and Yellow Jowar. A composite sample of each variety was prepared by mixing the individual variety samples and was preserved in cloth bags at laboratory temperature were used for the isolation and detection of seed-borne fungi.

Physical inspection of the seeds

The samples of sorghum seeds were physically inspected with the unaided eye on the basis of which they were separated into pure seeds, seeds of other crops and inert matter. One kilogram (kg) of each sample was poured into a plastic tray. Inert matter included soil, sand, stones, plant debris, fungal fruiting bodies etc.

Plating and Examination of incubated seeds

The standard blotter method was used to detect a wide range of fungi which are able to arise easily from seeds in presence of humidity. Four hundred untreated pure seeds from each sample were plated on moisten blotters in plastic Petri dishes (90 mm diameter) at the rate of 10 seeds per dish and incubated for 7 days at 20-25°C under alternating cycles of 12 h Light and 12 h darkness. Individual seeds were examined for the presence or absence of fungi under a stereomicroscope and identification was confirmed by examining mycelium and/or conidia under a compound microscope. The various types of fungi were identified using identification keys and cross-checked for each seed plated to identify the type of fungus growing on each seed. The fungal species present on each seed were recorded and the percentage incidence of each fungus per sample was computed.

For agar plate method, the untreated seeds and seeds after surface sterilization with 0.1% $HgCl_2$ (Mercury chloride) for two minutes were placed on potato dextrose agar (PDA), pH 5.5. Ten seeds were placed in each Petri dish and the dishes were incubated at 24± 1°C under 24 hours of alternating cycle of light and darkness for 7 days.

RESULTS

Sorghum seed mycoflora (Blotter test Msthod)

A total of twenty fungal species belonging to sixteen genera found on eight different cultivars tested. In untreated seeds maximum incidence was of *Curvularia lunata* followed by *Rhizopus nigricans*, *Fusarium moniliforme, Drechslera longirostrata, Alternaria tenuis, Phytopthora* spp., *Aspergillus flavus, Alternaria alternata* and *D. tetramera* while *Chaetomium globosum, Colletotrichum graminicola* and *Absidia ramose* were reported poorly (Table 1).

Seed treated with surface sterilizer (0.1% $HgCl_2$) showed complete absence of certain fungi (*Absidia ramose, Cladosporium herbarum, Chaetomium globosum, Trichothecium roseum*) or low incidence of *Aspergillus niger, A. flavus, Alternaria tenuis, A. alternata, Curvularia geniculata, Drechslera longirostrata,* etc. On the other hand counts of *Curvularia geniculata, Drechslera tetramera, Macrophomina phaseoli* and *Colletotrichum graminicola* were found to be increased (Table 1).

Table1. Percent seed mycoflora of Sorghum (Blotter test method)

Sl. No.	Name of Fungus	Maximum Percent incidence		Number of Samples (Variety*)with which fungus was associated (Out of 8)
		Untreated	Pretreated	
1	*Absidia ramosa*	2	00	02
2	*Alternaria tenuis*	30	20	06
3	*Alternaria alternata*	20	12	06
4	*Aspergillus flavus*	20	04	08
5	*Aspergillus niger*	10	03	04
6	*Chaetomium globosum*	04	00	02
7	*Cladosporium herbarum*	06	00	04
8	*Colletotrichum graminicola*	02	05	02
9	*Curvularia geniculata*	10	30	06
10	*Curvularia lunata*	60	40	08
11	*Drechslera longirostrata*	40	18	06
12	*Drechslera tetramera*	20	30	05
13	*Fusarium moniliforme*	40	20	06
14	*Macrophomina phaseoli*	10	30	03
15	*Nigrospora sphaerica*	10	02	02
16	*Phytopthora* spp.	30	20	06
17	*Pythium* spp.	10	10	02
18	*Rhizopus nigricans*	40	10	08
19	*Syncephalstrum* spp.	10	05	04
20	*Trichothecium roseum*	10	00	03

*Varieties tested: CSH-1, CSH-4, CSH-5, CSH-6, CSH-7, CSH-9, MSH-37 and Yellow Jowa

Sorghum seed mycoflora (Agar Plate Method)

The data summarized in Table 2 shows that the saprophytic mycroflora increased on agar medium, similarly some new fungi were detected which were absent in blotter test. These fungi are *Aspergillus terreus, Cephalosporium acremonium, Curvularia pallescens, Drechslera sorghina, Fusarium oxysporium, Gonatobotrys ramose, Harmodendron* spp., *Myrothecium roridum, Nigrospora sphaerica, Penicillium chrysogenum, P. oxalicum, Torula herbarum* and *Trichoderma* spp. On other hand *Chaetomium globosum, Syncephalstrum* spp. and *Trichothecium roseum* were not detected in agar plate method. The dominant fungi in agar test were *Aspergillus flavus, Curvularia lunata, Cephalosporium acremonium, Rhizopus nigricans, Cladosporium herbarum* and *Drechslera longirostrata.*

Table 2. Percent seed mycoflora of Sorghum (Agar Plate Method)

Sr. No.	Name of Fungus	Maximum Percent incidence		Number of Samples (Variety*)with which fungus was associated (Out of 8)
		Untreated	Pretreated	
1	*Absidia ramosa*	06	00	04
2	*Alternaria alternata*	04	06	08
3	*Alternaria tenuis*	10	08	08
4	*Aspergillus flavus*	40	10	08
5	*Aspergillus niger*	26	04	06
6	*Aspergillus terreus*	10	02	02
7	*Cephalosporium acremonium*	30	00	04
8	*Cladosporium herbarum*	20	00	03
9	*Colletotrichum graminicola*	02	06	01
10	*Curvularia geniculata*	10	08	08
11	*Curvularia lunata*	40	30	08
12	*Curvularia pallescens*	10	14	04
13	*Drechslera longirostrata*	20	15	08
14	*Drechslera tetramera*	15	15	06
15	*Drechslera sorghina*	04	02	06
16	*Fusarium moniliforme*	15	20	08
17	*Fusarium oxysporium*	10	15	06
18	*Gonatobotrys ramosa*	10	06	04
19	*Harmodendron* spp.	15	08	05
20	*Myrothecium roridum*	03	00	04
21	*Nigrospora sphaerica*	01	02	02
22	*Penicillium chrysogenum*	02	00	06
23	*Penicillium oxalicum*	03	00	03
24	*Phytopthora* spp.	06	08	06
25	*Pythium* spp.	04	07	04
26	*Rhizopus nigricans*	40	06	04
27	*Torula herbarum*	10	00	08
28	*Trichoderma* spp.	08	00	03
29	Nonsporulted mycelium	06	01	02

*Varieties tested: CSH-1, CSH-4, CSH-5, CSH-6, CSH-7, CSH-9, MSH-37 and Yellow Jowar

DISCUSSION

Eight cultivars of sorghum (CSH-1, CSH-4, CSH-5, CSH-6, CSH-7, CSH-9, MSH-37 and Yellow Jowar) when screened for seed born pathogen by blotter test method yielding 20 fungi. It is clear from results (Table 1) that the seeds of none of the varieties were found to be free from mycoflora. This shows that irrespective of the environmental conditions and varieiesthe fungi developed the contact withseed either very superficial, semideep or completely inside the seed. From the results, it is also clear that *Absidia ramose, Cladosporium herbarum, Chaetomium globosum* and *Trichothecium roseum* were found to be associated purely externally as they were not reported in the surface sterilized seeds. At the same time due to the absence of these superficial fungi, superficial count of *Curvularia geniculata, Drechslera tetramera, Macrophomina phaseoli* and *Colletotrichum graminicola* were found to be increased. This clearly suggest that the superficial mycoflora might be having certain inhibitory characters against the above mentioned fungi, which appeared in low counts. The dominant fungi were *Rhizopus nigricans, Phytopthora* spp., *Fusarium moniliforme, Curvularia lunata, Alternaria tenuis, Alternaria alternate,* and *Aspergillus flavus.* The number of fungal species on Sorghum seeds reported by different workers are found to be variable, as Godbole (1982) reported 21 species on seed samples collected from Marathwada, predominance being of *Curvularia lunata* (62.50%)*, Fusarium moniliforme* (48%), *Drechslera tetramera* (38.60%), *D. rostrata* (32%), *Alternaria tenuis* (31.40%), and *Phoma* spp. (30.50%). Khetrapal and Ramnath (1982) recorded 48 fungal species on seed imported from Nigeria.

The percent incidence of saprophytic mycoflora in agar plate method (Table 2) increased regularly, and also appeared some new fungi. This suggests that the above mycoflora might have appeared due to nutrients in the medium. Appearance of some new fungi, only on agar and which did not found in blotter method indicates that these fungi needs some external supply of nutrients. On the contrary, absence of *Chaetomium globosum, Syncephalstrum* spp., *Trichothecium roseum* in agar plate might be due to antagonistic effect of *Aspergillus terreus, Cephalosporium acremonium, Rhizopus nigricans, Cladosporium herbarum, Drechslera tetramera, Curvularia lunata* which were dominant in agar plate. Similar type of observations have been made by Aulakh *et al* (1976) that in agar method *Aspergillus niger, Penicillium* spp., *Rhizopus arrhizus* suppressed the growth of other fungi of maize seeds. In most of cases agar plate was found to be superior than blotter for the isolation of seed mycoflora (Agrawal,1972; Godbole, 1982; Bhikane

and Mukadam, 1981).

CONCLUSIONS

In all, 28 fungal species of 18 genera appeared in the seeds of eight different variety of Sorghum. In untreated seeds of the entire varieties maximum incidence was of *Curvularia lunata.* Treated seeds showed complete absence of *Cladosporium herbarum, Trichothecium roseum* and *Absidia ramose.* agar plate method was found to be favorable for the maximum counts of saprophytic fungi and also favorable for detection of some specific fungi.

The presence of so many pathogenic fungi at high levels in various geographical areas indicates a clear need for field surveys for these and other pathogens. There also is a clear need to increase public awareness on aspects related to seed health and to develop suitable management practices for improving the quality of the seeds. Testing seed health of major crops should be introduced in the national seed quality control system.

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