

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.707.478>**Histopathological Studies of Seed Infected with Seed Borne Fungi**

A. R. Gulhane, Shilpa V. Khambalkar* and G. K. Giri

Sorghum Research Unit, Dr. PDKV, Akola, Maharashtra, India

*Corresponding author

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Fifty seed of sorghum, wheat and pearl millet from infected seed lot having characteristics symptom of seed borne fungi. Seed of such symptoms got infected of *Curvularia lunata*, *Bipolaris sorghicola* and *B. sorokiniana* infected seed of sorghum wheat and pearl millet. Histopathology of apparently in infected seeds confined to mycelium fragments of *Curvularia lunata* in seed coat and endosperm, embryonic region of sorghum, wheat and pearl millet seed. Sorghum and pearl millet seed indicates the presence of brown coloured thick mycelial fragment of *Bipolaris sorghicola* in pericarp and embryo region. The mycelial fragment of *B. sorokiniana* was observed in pericarp region of wheat seed. Thus, transmission of *C. lunata*, *Bipolaris sorghicola* and *B. sorokiniana* was confirmed from infected seeds of sorghum, wheat and pearl millet.

Introduction

Rice, wheat, sorghum and pearl millet is an important crop in India. Seed borne diseases cause economic losses to our crop. The infected seed may fail to germinate, transmit diseases from seed to seedling and from seedling to growing plant.

Brown to black discoloration of wheat were analysed to identified the *Drechslera* spp. associated with black point disease. The fungi the embryos endosperm and seed coats of the Kernels (Ozer, 2005). The *C. lunata* is one of the major grain mould of sorghum, the fungus causes black discoloration of seed, degradation of endosperm and infect the embryo. Histologically sorghum seed consist and pericarp seed coat, endosperm and embryo (Deshmukh and Raut, 1993; Girish et

al., 2004; Singh and Agrawal, 1989). *B. oryzae* and *C. oryzae* infected all parts of seed coat embryo, black dots and brown spot of fungus present on seed coat. Infection of *C. oryzae* and average percent of incidence was 10% from seed coat (Pandy *et al.*, 2000; Ibrahim and Abo-Ei-Dahab, 2014; Somsiri sangchote and Van, 2006).

Histology of brown discolor seed to confirm the presence of mycelium of *C. penniseti* in different part of seed mycelium was confirmed to pericarp and Endosperm and embryo of pearl millet seed (Singh and Singh, 2004; Singh *et al.*, 2008).

The present studies were conducted to determine the location of seed borne fungi and their seed transmission in different seed of sorghum, wheat, pearl millet and rice.

Materials and Methods

Fifty seed of sorghum, wheat, paddy and pearl millet from infected seed lot having characteristics symptoms of seed borne fungi were boiled in distilled water for 30 min at 60°C.

Later, seeds were soaked in 70% ethanol for 48 hours. The seed become soft and was ready to give a cut.

Seed of sorghum, wheat, paddy and pearl millet were cut transversely into two pieces at the center of seed to ensure better dehydration, infiltration and embedding.

The fixed seeds were dehydrated through acetone I, II, III, IV series for 40 minutes per treatment and benzene I, II for 20 min/treatment and infiltrated using paraffin wax at 52°C for 2 hours. The solid paraffin wax was liquidified and poured in the blocks, cut seeds were placed at the centre of per block, so that the seeds were embedded in the solid wax and the blocks were cooled overnight. Next day, blocks were cut into squares.

The blocks were used for serial microtome sections of 7-15 µm thickness by using hand rotary microtome. The ribbon of sections were uplifted, kept floating in water bath having 40°C temperature to deparaffinise the wax and the sections were lifted on the slides. The slides were heated on spirit lamp to make ready for staining and mounted in DPX.

Results and Discussion

Histopathology of infected seed and colonization of major seed borne fungi viz., *Curvularia*, *Bipolaris* and *Drechslera* in sorghum, wheat, paddy and pearl millet seeds were carried out with the help of hand rotary microtome.

Location of seed borne fungi in seed component

Curvularia lunata

Microphotograph (Plate 1a, b) of sorghum seed section showed distribution of mycelial fragments of *Curvularia lunata* in seed coat and floury endosperm. Microtomy section of wheat seed revealed the presence of brown colored thick mycelial fragments of *Curvularia lunata* in endosperm and embryonic region (Plate 1c). It was also similarly seen that (Plate 1d) the brown / black colored thick mycelial fragments of *Curvularia lunata* present in embryo region of pearl millet seed. Mycelium of *Curvularia lunata* was also noticed in the pericarp region (Plate 1e) of paddy seed.

Thus, it can be concluded that the *Curvularia lunata* were widely distributed in various components of the seed including seed coat, endosperm, pericarp and embryonic region.

Bipolaris sorghicola

The microtomy sections of sorghum and pearl millet seed indicates the presence of brown colored thick mycelial fragments of *Bipolaris sorghicola* in pericarp and embryo region of sorghum and pearl millet seed, respectively (Plate 2a, b).

Bipolaris sorokiniana

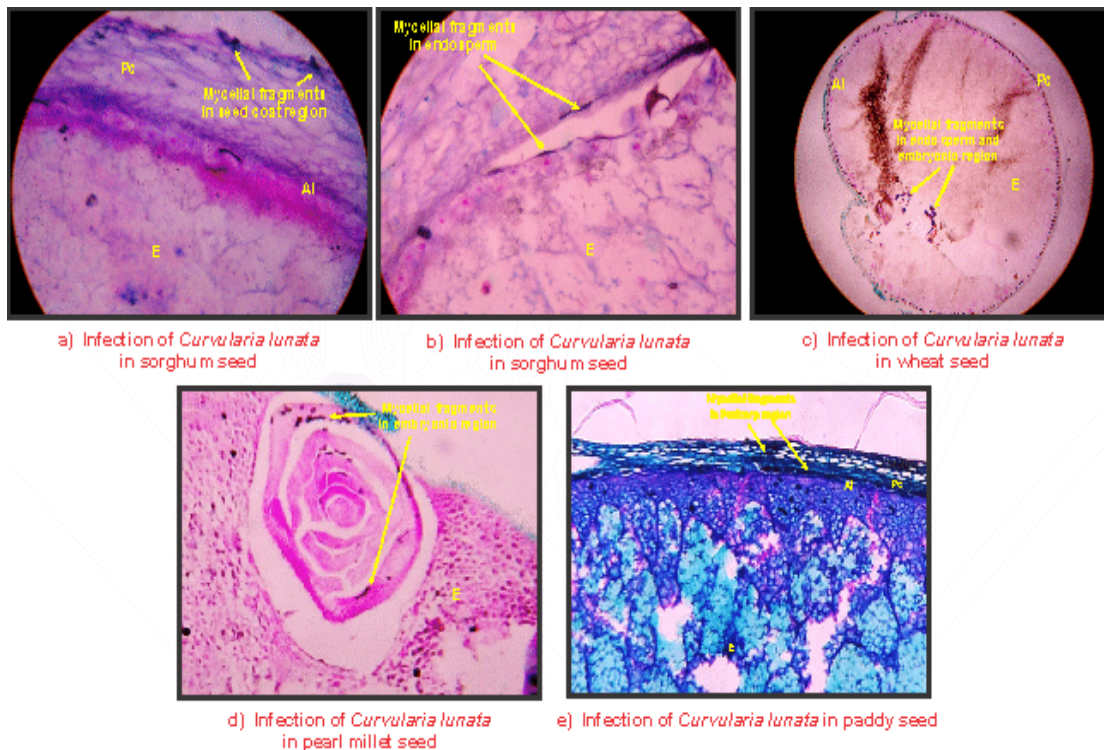
The brown colored thick mycelial fragments of *Bipolaris sorokiniana* was observed in pericarp region (Plate 3) of wheat seed.

Results of histopathology of *Curvularia* and *Bipolaris* infected seed showed the distribution of fungi in the pericarp, seed coat, endosperm and embryo. The septate, brown, thick and knotty mycelium invaded the various seed parts.

Staining procedure of slides

Sr. No.	Staining procedure steps	Time required (min)
1	Xylene –I (dip)	3
2	Xylene –II (dip)	3
3	Absolute alcohol (100%) I	3
4	Absolute alcohol (100%) II	3
5	Rectified spirit I	3
6	Rectified spirit II	3
8	Washing under tap water	5
9	Cotton blue stain	5
10	Washing under tap water	10
11	Blotting and drying	10
12	Carbol fuschin	3-5
13	Rectified spirit	1 dip
14	Rectified spirit	1 dip
15	Absolute alcohol	2 dip
16	Absolute alcohol	3 dip
17	Xylene – I	3
18	Xylene – II	3
19	Mounting in DPX	

Plate.1 Histopathology of *Curvularia* sp. in pearl millet, sorghum, wheat and paddy seed



Pc - Pericarp, Al - Alurone layer, E - Endosperm

Plate.2 Histopathology of *Bipolaris* spp. in pearl millet, sorghum and wheat seed

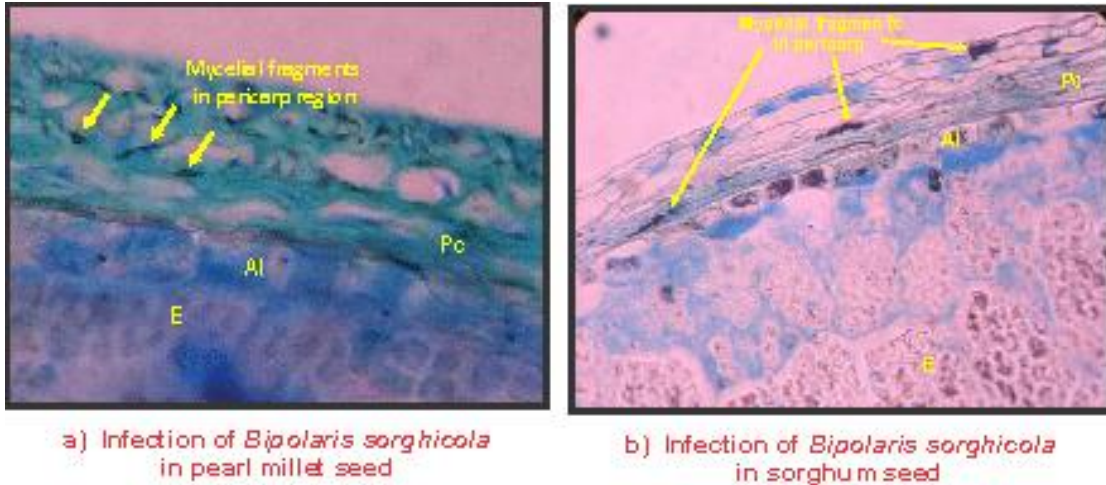
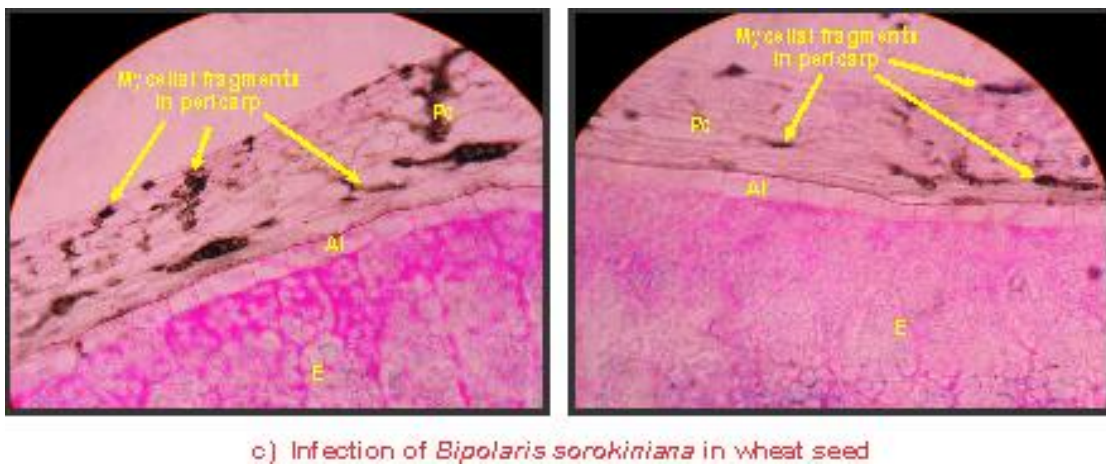


Plate.3 Histopathology of *Bipolaris* spp. in pearl millet, sorghum and wheat seed



The fungi were found colonize in seed coat, pericarp, and endosperm and embryo region. Thus, histopathological studies revealed that, *Curvularia lunata*, *Bipolaris sorokiniana* and *Bipolaris sorghicola* infect the seed and colonize in various seed tissues and damage the embryo and affect seed viability. These findings are similar to those of Caster (1977) and Caster and Frederiksen (1980) who recorded the infection of *Curvularia lunata* in ovary wall, pericarp, endosperm and embryonic tissue of sorghum seed. The presence of *Curvularia lunata* and *Curvularia oryzae* in all part of seed including embryo, seed coat and endosperm of sorghum and rice

seed have also been reported by Deshmukh and Raut (1993) and Pandey *et al.*, (2000), respectively. Thus, confirms the results of present investigation.

In the present investigation, the colonization of *Curvularia lunata* was observed in the embryonic region of wheat seed. Observation on same line have been reported by Khulbe *et al.*, (2011) in the form of light brown and dark brown to black discoloration due to *Bipolaris sorokiniana* and *Curvularia lunata* respectively in the embryonic region of wheat seed, which supports the findings of present investigations.

Histopathology of *Curvularia* and *Bipolaris* infected seed showed the distribution of fungi in the pericarp, seed coat, endosperm and embryo. The septate, brown, thick and knotty mycelium invaded the various seed parts. The fungi were found colonize in seed coat, pericarp, and endosperm and embryo region. Thus, histopathological studies revealed that, *Curvularia lunata*, *Bipolaris sorokiniana* and *Bipolaris sorghicola* infect the seed, colonize in various seed tissues and damage the embryo and affect seed viability.

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