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Detection and pathogenicity of fungal pathogens causing black point disease of wheat

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Abstract
The present investigation was carried out on the **"**Studies on Seed Borne Mycoflora of Wheat with Special Reference to Black Point Disease" with a view to assess seed borne pathogens associated with black point disease of wheat and their pathogenicity. Three seed borne pathogens associated with black point disease of wheat *viz.*, *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* were detected on wheat seeds. The internally two fungi identified were *Alternaria alternata* and *Drechslera sorokiniana.* All the isolated pathogens were found pathogenic to wheat resulting into reduction in seed germination and seedling vigour index. The seed borne pathogen *viz., Alternaria alternata,* was found more dominant and damaging.

Keywords: *Alternaria alternata, Drechslera sorokiniana, Curvularia lunata*

Introduction
Wheat (*Triticum aestivum* L.), a principal member of the family *Poaceae*, is among the most significant cereal crops cultivated globally. As a fundamental dietary staple, wheat plays a vital role in ensuring food security by providing a substantial portion of the daily caloric and nutritional intake for human populations across the world. Optimal growth conditions for wheat include cool and dry climates, particularly during the germination and early vegetative phases. The ideal temperature range for germination lies between 20°C and 25°C. India is currently ranked as the second-largest producer of wheat globally, a position that underscores its strategic importance in the nation's food economy. According to the Department of Agriculture and Farmers Welfare, wheat production in India reached a record-breaking 113.29 million tonnes during the 2023-24 crop year. Numerous infections harm the wheat crop, with the majority being seed-borne. Karnal bunt (*Tilletia indica*), loose smut (*Ustilago nuda tritici*), head blight or scab (*Fusarium* spp.) and tundu or ear cockle (*Clavibacter tritici* and *Anguina tritici*) are the major seed borne diseases of wheat. One of the main factors lowering wheat production is seed borne mycoflora. Internally and externally, mycoflora related to seeds are the cause of seed abortion, grain mortality, decreased germination ability, seed necrosis, and ultimately, harmful to severe illnesses at various phases of plant development (Niaz and Dawar, 2009) [7]. Black point, also referred to as kernel smudge, is a notable seed borne disease of wheat that has been reported in multiple wheat growing regions globally, including across diverse agro-ecological zones in India. The disease is primarily characterized by dark brown to black discoloration, typically localized at the embryonic end of the wheat kernel. In mild to moderate infections, the discolouration is confined to this region; however, under severe conditions, the entire grain may exhibit extensive blackening, shrivelling, and deformation. El-Gremi *et al.*, (2016) [3] reported that *Alternaria alternate* (Fr.) Keissl, *Cochliobolus sativus*, *Drechsler* spp. and *Fusarium graminearum* had been repeatedly isolated from kernel black pointed wheat and found to be pathogenic either alone or in combination. Ghosh *et al*., (2018) [4] reported that black point is a disease of cereal seeds, exhibiting a brown to black tip at the embryo end of the grain. The disease is caused by *B. sorokiniana*. In addition, the association of *Alternaria alternata, Fusarium* spp. *and Penicillium* spp. with wheat seeds developing black point symptoms. Early pathogen detection is an important stage in wheat diagnosis and control strategies. Major impact of seed borne diseases in wheat is not only the yield reduction but also deteriorate marketable quality of grains.

Early detection of pathogens is a crucial step in diagnosis and management programmes in wheat. The present investigation is carried out to detect various seedborne pathogens associated with black point disease of wheat.

Material and Methods

The seeds of wheat varieties/genotypes will be collected from ARS, Niphad. The ISTA's standard blotter test as described by Neergaard (1979) [6] was used for detection of pathogens associated with black point disease of wheat. ISTA's standard agar plate method was used to detect internal pathogens associated with black point disease of the wheat seed. Kotch's postulate was used for proving the pathogenicity.

The Koch's postulate of the mycoflora isolated from black point affected wheat seeds were proved in Seed Pathology Laboratory at MPKV, Rahuri. The method followed to study the pathogenicity of seedborne mycoflora is as below.

1. The seeds were surface sterilised for 5 minutes with 1.0 per cent NaOCl solution, then washed three times with sterilised water to eliminate any corrosive sublimate.
2. To inoculate the seeds with the individual pathogen, seeds were dipped in concentrated suspension of spores (10^6cfu/ml) for 12 hours. Then these seeds were dried in shade for 12 hours (Agarwal and Sinclair, 1993) [1].
3. For each isolate, seeds were placed to sterilised plastic petri plates containing three discs of blotter paper.
4. Seeds which were not smeared with fungal isolates served as control.
5. In the incubation room, these petri plates were incubated for seven days at 20±2^{0}C.
6. On the seventh day after incubation, observations on symptoms produced and the per cent occurrence of seedborne infections were recorded.
7. Care was taken to keep the blotter moist by adding sterilized water as per requirement.

Re-isolation of Pathogens

By transferring fungal growth on sterilized petri plates containing potato dextrose agar, different seedborne fungi were reisolated from pathogenic growth on rotten seeds. The re-isolation was done to ensure that the original isolates were true. Pathogenic fungi were selected and used in further research work.

Results and Discussion

Three pathogens *viz., Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* were found associated with black point disease of wheat. Internal pathogens associated with black point disease of wheat seeds showed that two fungi *viz., A. alternata* and *Drechslera sorokiniana* were found internally associated with black point disease of wheat seeds. Abdullah and Atroshi (2016) [2] reported that several fungi were found to be associated with the black-point disease of wheat. *Alternaria, Cochliobolus, Fusarium, Cladosporium, Curvulavia, Penicillium, Aspergillus* and *Stemphylium* were predominant. Li *et al*., (2019) [5] reported that seed infection by *B. sorokiniana* can result in black point disease, which may result in root rot and seedling blight. All of the pathogens detected were found to be pathogenic to wheat seeds. Table 2 show list of pathogenic organisms, along with their symptoms and percent incidence on artificially inoculated wheat seeds. Among all the seed borne mycoflora of wheat seeds, *Alternaria alternata* showed highest incidence (66.00 %) followed by *Drechslera sorokiniana* (61.00 %) and *Curvularia lunata* (53.00 %).
Ghosh *et al*. (2018) [4] reported that black point is a disease of cereal seeds, exhibiting a brown to black tip at the embryo end of the grain. The disease is caused by *B. sorokiniana.* In addition, the association of *Alternaria alternata, Fusarium* spp. *and Penicillium* spp. with wheat seeds developing black point symptoms.

Conclusions

1. There are three pathogens associated with black point disease of wheat *viz., Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata*.
2. All pathogen were pathogenic and resulting into reduction in seed germination and SVI. Among them *Alternaria alternata* was found most damaging.

Table 1: Pathogens associated with black point disease of wheat seed

Sr. No.	Pathogen associated with black point of wheat seeds
1.	*Alternaria alternata*
2.	*Drechslera sorokiniana*
3.	*Curvularia lunata*

Table 2: Pathogenicity test of pathogens associated with black point disease of wheat

Sr. No.	Pathogen associated with black point of wheat seeds	Incidence of organism (%)	Symptoms produced by pathogen on wheat seed days of inoculation)
1.	*Alternaria alternata*	66.00	Infected seeds become water soaked, discolored and shrivelled with dirty black velvety appearance.
2.	*Drechslera sorokiniana*	61.00	Brown to black discoloration usually restricted embryonic end of the graine.
3.	*Curvularia lunata*	53.00	Grey to black colored fungal growth on seeds, rotting of seeds.

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