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Detection of seed mycoflora associated with wheat (*Triticum aestivum* L.) seeds through dry seed inspection and agar plate method

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops of the world and plays a vital role in global food and nutritional security. Seed-borne fungal infection is a major biotic constraint that reduces seed quality, germination and seedling vigour in wheat. The present investigation was undertaken to detect the seed mycoflora associated with five wheat cultivars, namely GW 496, GW 499, GW 322, GADW 3 and Lok 1 through dry seed inspection and the agar plate method at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand during 2025-26. Dry seed inspection revealed the presence of healthy, black pointed, shrivelled/sunken and discoloured seeds in all the cultivars, with healthy seed percentage ranging from 46.94 to 81.69 per cent. Cultivar GW 496 and Lok 1 recorded the highest proportion of healthy seeds and seed purity whereas GW 499 showed the highest black point incidence (38.00%) and the lowest seed purity (45.36%). Detection of associated mycoflora by the agar plate method on potato dextrose agar (PDA) medium revealed the presence of six fungal species, namely *Bipolaris sorokiniana*, *Fusarium oxysporum*, *Curvularia lunata*, *Rhizopus stolonifer*, *Aspergillus flavus* and *Aspergillus niger*. Among the isolated fungi, *A. niger* recorded the highest mean percentage frequency (46.50%) and relative abundance (43.98%) followed by *A. flavus* and *B. sorokiniana* while *R. stolonifer* recorded the lowest occurrence. The results indicate that seed health and the extent of fungal association vary considerably among wheat cultivars and that *A. niger* is the predominant seed-borne fungus of wheat under the agro-climatic conditions of Anand, Gujarat.

Keywords: Wheat, Seed mycoflora, Agar plate method, Black point, Percentage frequency, Relative abundance

1. Introduction

Wheat (*Triticum aestivum* L.) belongs to the family Poaceae and is popularly known as the “King of Cereals.” It is the second most important cereal crop of India after rice and contributes substantially to food and nutritional security. Nearly 55 per cent of the world population depends on wheat for about 20 per cent of its calorie intake and the edible portion of wheat grain (100 g) contains about 11.50 per cent protein, 59.40 per cent carbohydrate, 9.10 per cent fat, 10.60 per cent crude fibre and 1.80 per cent ash. India is the world's second-largest producer of wheat and Gujarat is one of the important wheat-growing states of western India, with wheat cultivated over about 1.28 million hectares.

Wheat production is constrained by several biotic and abiotic factors, of which seed-borne fungal infection is among the most important. Wheat is reported to be affected by around 120 diseases, of which 42 are seed-borne and 35 are caused by fungi. A seed-borne pathogen growing externally, internally, or associated with the seed as a contaminant can cause seed abortion, rot, necrosis or a reduction in germination capacity, ultimately leading to seedling damage and disease development at later crop growth stages. The most predominant fungal genera reported on cereal seeds are *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium*, several of which are also known to be associated with deterioration of wheat seed quality during storage.

Since the health status of farmer-saved and certified wheat seed is not always precisely known and successful wheat cultivation depends largely on the use of healthy, quality seed, timely and accurate detection of seed-borne mycoflora is a prerequisite for effective seed health management. Keeping this in view, the present investigation was undertaken with the specific objective of detecting the seed mycoflora associated with wheat seeds of different cultivars through dry seed inspection and the agar plate method.

2. Materials and methods

The present investigation was carried out at the Seed Pathology Laboratory, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat (22° 35' N, 72° 55' E; 45 m above mean sea level; Central Gujarat Agro-climatic Zone-III) during 2025-26.

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2.1 Collection of seeds

Approximately 0.5 kg seed of each of five wheat cultivars viz., GW 496, GW 499, GW 322, GADW 3 and Lok 1 was procured from the Regional Research Station, AAU, Anand. Seeds were stored in brown paper bags covered with polythene at 5-7 °C until further use.

2.2 Assessment of seed abnormality

The method of Gilchrist (1985) [1] was followed. From each cultivar 400 seeds were drawn at random (four replicates of 100 seeds) and examined with a hand lens or stereo binocular microscope. Seeds were classified into healthy, black-point infected, shrivelled/sunken and discoloured categories. Black-point severity was further graded on a 0-5 scale.

Grade	Description
0	Seed free from any discolouration (apparently healthy)
1	Only the tip of the embryo is brown to blackish
2	Discolouration covering the whole embryo
3	Embryo with ¼ of the seed discoloured
4	Embryo with ½ of the seed discoloured
5	Embryo with more than ½ of the seed discoloured and shrivelled

Different categories of wheat seed abnormalities

Discoloured

Shriveled/Sunken

Black point severity grades (0-5 scale)

Healthy (G₀)



Seeds and their impurities were pooled separately and their percentage was calculated by using this formula.

$$\text{Purity of seed (\%)} = \frac{\text{Weight of pure seeds (g)}}{\text{Total Weight of seeds (g)}} \times 100$$

Black point seeds were sorted from the collected seed samples manually by visual observation.

$$\text{Black point incidence (\%)} = \frac{\text{Total no. of black pointed seeds}}{\text{Total number of seeds examined}} \times 100$$

2.3 Assessment of seed mycoflora by agar plate method

Mycoflora associated with the seed samples were detected by the agar plate method (ISTA, 1976) [2] using potato dextrose agar (PDA) as the basal medium. Ten seeds were placed at equal distance on 20 ml solidified PDA per plate (four replicates of 50 seeds per treatment;

200 seeds per cultivar in total) and incubated at 25 ± 2 °C under alternating cycles of light and darkness for seven days. Fungal colonies developing on the seed surface were examined under a stereoscopic binocular microscope and identified. The percentage frequency (PF) and relative abundance (RA) of each fungus were calculated as follows (Naqvi *et al.*, 2013; Adhikari *et al.*, 2016) [4, 3]:

$$\text{PF} = \frac{\text{Number of seeds on which fungus appears}}{\text{Total number of seeds}} \times 100$$

$$\text{RA} = \frac{\text{Number of particular fungi on seeds}}{\text{Total number of all fungi on seeds}} \times 100$$

Sterilized glassware and media were used throughout and all cultural work was carried out under aseptic conditions in a laminar air flow cabinet. The experiment was laid out in a factorial Completely Randomized Design (CRD).

3. Results and discussion

3.1 Seed abnormalities on dry inspection

Dry inspection of the five wheat cultivars revealed significant variation in the extent of healthy, black-pointed, shrivelled/sunken and discoloured seeds (Table 1). The percentage of healthy seeds ranged

from 46.94 to 81.69 per cent. The highest healthy seed percentage was recorded in Lok 1 (81.69%), statistically at par with GW 496 (80.88%) while GW 499 recorded the lowest (46.94%). Black point incidence varied from 5.31 to 38.00 per cent and was significantly highest in GW 499 (38.00%), whereas GW 496 (5.31%), GW 322 (5.69%) and GADW 3 (5.75%) remained statistically at par with each other at the lower end. Shrivelled/sunken seed was maximum in GADW 3 (30.31%) and minimum in Lok 1 (2.31%) while seed discolouration was highest in GW 322 (9.88%) and lowest in GADW 3 (2.63%).

Table 1. Seed abnormalities in different wheat seed samples

Sr. No.	Cultivar	Healthy (%)	Seed abnormalities (%)		
			Black pointed (%)	Shrivelled/ sunken (%)	Discoloured (%)
1	GW 496	80.88	5.31	11.00	2.81
2	GW 499	46.94	38.00	7.19	7.75
3	GW 322	62.19	5.69	22.25	9.88
4	GADW 3	61.31	5.75	30.31	2.63
5	Lok 1	81.69	10.56	2.31	5.44
	S.Em. ±	0.36	0.22	0.16	0.16
	CD at 5%	1.08	0.66	0.48	0.47
	CV %	1.08	3.36	2.18	5.49

The data presented in Table 2. indicated significant differences among wheat cultivars with respect to black point severity grades. GW 496 and Lok 1 showed lower severity with maximum grade 0 seeds

whereas GW 499 exhibited higher susceptibility with greater distribution of severe black point grades. GW 322 and GADW 3 showed comparatively lower to moderate severity levels.

Table 2. Distribution of seeds under black point severity grades (0-5 scale)

Cultivars	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
GW 496	324	10	4	2	1	3
GW 499	188	52	66	11	16	7
GW 322	247	17	5	1	-	-
GADW 3	246	8	6	4	2	3
Lok 1	325	31	9	3	-	-

(Note: From randomly selected 400 seeds, only black point-affected seeds were graded on a 0-5 scale based on discoloration and shriveling while, other shriveled or discoloured seeds were not considered for grading.)

The corresponding seed purity data (Table 3) showed the highest purity in GW 496 (83.16%), statistically at par with Lok 1 (83.18%) and the lowest in GW 499 (45.36%) confirming that GW 499 carried the greatest proportion of abnormal and affected seed among the cultivars tested.

These results are in agreement with Agarwal *et al.* (1997) [5] and Yadav *et al.* (2020) [6] who reported that black point, shrivelling and discoloration are closely associated with deterioration in wheat seed quality and that increasing severity of black point is accompanied by a marked reduction in overall seed purity.

3.2 Assessment of seed mycoflora by agar plate method

Six fungal species namely *Bipolaris sorokiniana*, *Fusarium oxysporum*, *Curvularia lunata*, *Rhizopus stolonifer*, *Aspergillus flavus* and *Aspergillus niger* were detected in association with the wheat seed samples on PDA. The percentage frequency and relative abundance of these fungi varied significantly among cultivars (Tables 4 and 5).

Among the isolated fungi, *A. niger* recorded the highest mean percentage frequency (46.50%) followed by *A. flavus* (17.00%) and *B.*

sorokiniana (16.50%) while the lowest frequency was recorded for *R. stolonifer* (4.00%). Cultivar-wise, *A. niger* showed maximum frequency in GADW 3 (80.00%) followed by GW 322 (47.50%) and Lok 1 (40.00%) whereas *B. sorokiniana* was most frequent on GW 499 (22.50%).

Table 3. Seed purity of different wheat cultivars

Sr. No.	Cultivar	Seed purity (%)
1	GW 496	83.16
2	GW 499	45.36
3	GW 322	70.32
4	GADW 3	62.15
5	Lok 1	83.18
	S.Em. ±	0.36
	CD at 5%	1.08
	CV %	1.04

Table 4. Percentage frequency of seed mycoflora associated with different wheat cultivars

Mycoflora	Percentage frequency					
	GW 496	GW 499	GW 322	GADW 3	Lok 1	Mean
<i>B. sorokiniana</i>	20.00	22.50	20.00	2.50	17.50	16.50
<i>F. oxysporum</i>	17.50	7.50	10.00	0.00	17.50	10.50
<i>C. lunata</i>	10.00	15.00	0.00	0.00	2.50	5.50
<i>R. stolonifer</i>	0.00	2.50	10.00	7.50	0.00	4.00
<i>A. flavus</i>	20.00	20.00	12.50	10.00	22.50	17.00
<i>A. niger</i>	32.50	32.50	47.50	80.00	40.00	46.50
					S. Em. ±	0.51
					CD at 5 %	1.44
					CV %	5.36

The relative abundance data followed a similar trend with *A. niger* being the dominant seed-associated fungus (mean 43.98%) followed by

A. flavus (18.93%) and *B. sorokiniana* (16.69%) while *R. stolonifer* recorded the lowest relative abundance (3.86%).

The higher prevalence of *Aspergillus* spp., particularly *A. niger* may be attributed to their ability to survive under storage conditions, rapid colonization of the seed surface and adaptation to relatively low moisture environments as compared to field fungi such as *Fusarium* and *Curvularia*. Variation in mycoflora among cultivars is likely associated with differences in seed coat characteristics, biochemical composition and genetic background of the cultivars. These findings are in close agreement with Adhikari *et al.* (2016) [3] and Agarwal *et al.* (1997) [5] who similarly reported *Aspergillus*, *Fusarium*, *Curvularia*

and *Rhizopus* species as the predominant seed-borne fungi of wheat, with storage fungi such as *Aspergillus* spp. tending to dominate over field fungi. Comparable results were also reported by Pathak and Zaidi (2013, 2014), Khan *et al.* (2023) [7] and Pagar *et al.* (2025) [8] who detected *A. niger*, *A. flavus*, *Fusarium* spp. and *C. lunata* as major seed-borne fungi of wheat using blotter and agar plate methods.

GADW 3

Lok 1

Table 5. Relative abundance of seed mycoflora associated with different wheat cultivars

Mycoflora	Relative abundance (%)					
	GW 496	GW 499	GW 322	GADW 3	Lok 1	Mean
<i>B. sorokiniana</i>	20.00	18.65	21.97	4.55	18.30	16.69
<i>F. oxysporum</i>	17.50	6.28	7.86	0.00	15.76	9.48
<i>C. lunata</i>	10.00	23.01	0.00	0.00	2.27	7.06
<i>R. stolonifer</i>	0.00	2.08	8.15	9.09	0.00	3.86
<i>A. flavus</i>	20.00	22.82	15.60	11.36	24.89	18.93
<i>A. niger</i>	32.50	27.17	46.43	75.00	38.79	43.98
					S. Em. ±	0.48
					CD at 5 %	1.34
					CV %	5.65

GW 322
GW 499
GW 496



Seed mycoflora load on seeds of wheat cultivars as detected by PDA plate method

Note: A = *B. sorokiniana*, B = *F. oxysporum*, C = *C. lunata*, D = *R. stolonifer*, E = *A. flavus* and F = *A. niger*



Pure culture of seed mycoflora isolated from wheat cultivars

4. Conclusion

Dry seed inspection and the agar plate method together revealed considerable variation among five wheat cultivars in respect of seed abnormalities and associated seed mycoflora. GW 496 and Lok 1 pos-

sessed comparatively healthy and pure seed, whereas GW 499 was the most susceptible cultivar with the highest black point incidence and the lowest seed purity. Six fungal species were detected in association with wheat seed which *A. niger* was the predominant fungus followed by *A. flavus* and *B. sorokiniana*. These findings underline the need for regular seed health testing and selection of cultivars with lower inherent susceptibility and provide a baseline for further studies

on the pathogenic potential and management of these seed-borne fungi.

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