ACTA MYCOLOGICA
Vol. XIV (1, 2) : 143-149
1978

Studies on certain aspects of seed-borne fungi

VI. Fungi associated with different cultivars of wheat (Triticum aestivum L.)

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Pandey K. K.: *Studies on certain aspects of seed-borne fungi VI. Fungi associated with different cultivars of wheat (Triticum aestivum L.).* Acta Mycol. XIV (1, 2): 143-149, 1978.

Fungi associated with eight cultivars of wheat have been investigated. Twenty seven species were isolated from external and internal surface of all the wheat (*Triticum aestivum* L.) cultivars respectively. Out of five dominant and subdominant fungi only *Aspergillus terreus* and *Alternaria tenuis* were able to colonize internally. The culture filtrates of test fungi reduced the germination of all wheat varieties up to different degrees.

INTRODUCTION

The microflora of wheat have been investigated by various workers (James et al. 1946; Christensen, Gordon 1948; Christensen 1951; Hyde, Galleymore 1951; Flannigan 1970). The fungi associated with seed cause 0.5 to 10 per cent destruction of the world's grain production (Harman et al. 1972). Association of certain fungi reduces the viability (Harman, Nash 1972) and produces toxins in many seeds and renders them inedible for human beings.

The interrelationship between seed and seed fungi is very complex. In India information related to fungi associated with wheat needs study in detail. This forms the basis of present investigation. The aim of the present paper was to study mycoflora of different cultivars of wheat collected from one locality.

MATERIALS and METHODS

The seeds of eight cultivars of wheat (*Triticum aestivum* L.) viz. 'Raj-821', 'MACS-9', 'Ridley', 'HD-2012', 'Lal Bahadur', 'Khapli', 'Kalyansona'

and 'Sonalika' were collected separately from the suburbs of Gorakhpur University under aseptic conditions. Mycoflora associated on and inside seeds was separately assessed by 'Agar plate method' and 'Blotter Technique'. The findings of the two methods are presented jointly. For internal mycoflora seeds were surface-sterilized with mercuric chloride (0.1%) solution and then rinsed in sterilized distilled water. Thereafter, seeds were plated on agar plates as well as in moist chamber.

The effect of culture filtrate of *Rhizopus nigricans, Aspergillus sydowi, A. terreus, A. niger, Alternaria tenuis* and *Curvularia lunata* on seed germination was studied. The culture filtrates were prepared as described by Mishra and Kanaujia (1973). Percentage germination of each cultivar was determined by the method described by Kanaujia (1974).

RESULTS

External mycoflora of seeds. Twenty seven fungal species were isolated from all the 8 cultivars of wheat, out of which 16, 15, 21, 15, 13, 18, 11 and 15 species were recorded from 'Raj-821', 'MACS-9', 'Ridley', 'HD-2012', 'Lal Bahadur', 'Khapli', 'Kalyansona' and 'Sonalika' respectively. *Aspergillus niger* and *Alternaria tenuis* were found in only. All the wheat cultivars were inhabited by on the other hand, *Rhizopus nigricans* ('Kalyansona'), *Chaetomium globosum* ('HD-2012'), *Aspergillus nidulans* ('Lal Bahadur'), *A. terreus* ('HD-2012'), *Curvularia lunata* ('Khapli') were not isolated only from the cultivars mentioned under brackets. *Aspergillus sydowi, A. flavus* and *Cladosporium epiphyllum* were isolated from the six cultivars. The remaining species were associated with only few cultivars (Table 1).

Rhizopus nigricans, Aspergillus nidulans, A. niger, Curvularia lunata and *Alternaria tenuis* (dominant); *Chaetomium globosum, Aspergillus sydowi, A. flavus, A. terreus* and *Cladosporium epiphyllum* (subdominant) are the forms mentioned under bracket. Other species were rare in occurrence (Table 1).

Internal mycoflora of seeds. In total 18 fungal species were cultured from the internal surface of all the wheat cultivars, of which 8, 8, 11, 10, 4, 9, 2 and 6 species were associated with 'Raj-821', 'MACS-9', 'Ridley', 'HD-2012', 'Lal Bahadur', 'Khapli', 'Kalyansona' and 'Sonalika' respectively. But none of them was found to associate with all the wheat cultivars. *Rhizopus nigricans* ('Kalyansona'), *Aspergillus sydowi* ('MASC-9', 'Kalyansona'), *Curvularia lunata* ('Lal Bahadur', 'Sonalika') and *Alternaria tenuis* ('Raj-821', 'HD-2012') were not present only on the cultivars mentioned under brackets. While, most of the fungi were associated with anly a

Table 1

Distribution of fungi on the external surface of seeds of certain wheat (*Triticum aestivum*) cultivars

Fungi isolated	Wheat cultivars							
	RA	MA	RI	HD	LA	KH	KA	SO
Absidia sp.	—	+	+	—	—	—	+	—
Rhizopus nigricans Ehrenb.	+++	++	+++	+++	+++	+++	—	+
Mucor hiemalis Wehmer	+	—	+	+	—	—	—	—
Thielavia terricola (Gilm. et Abb.) Emmons	—	+	+	—	++	—	—	—
Chaetomium indicum Corda	+	—	—	—	—	+	—	—
C. homopilatum Omvik	—	—	+	—	—	—	—	+
C. globosum Kunze	+	+	+	—	+	+	+	+
C. spirale Zopf	—	—	—	—	—	—	+	—
Trichoderma viride Pers. ex Fr.	+	+	+	++	—	+	—	—
Aspergillus aculeatus Iizuka	+	+	—	—	+	—	—	+
A. nidulans Eidam	+++	+++	++	+++	—	+++	+	++
A. sydowi (Bain. et Sart.) Thom et Church	+	—	++	+	++	+	—	+
A. flavus Link	+	+	+	+	—	+	—	+
A. terreus Thom	++	++	+	—	+	+	+	+
A. niger van Tieghem	++	+++	+	++	+	++	+	+
Penicillium chrysogenum Thom	—	—	—	++	+	—	+	—
P. oxalicum Currie et Thom	—	+	+	—	—	+	—	—
P. humicola Oud.	—	—	+	+	—	+	—	—
Memnoniella echinata (Riv.) Galloway	—	—	+	—	+	—	—	—
Cladosporium epiphyllum (Pers.) Martius	++	—	—	+	+	++	+	+
× *Curvularia tetramera* (McKinney) Boedijn	— —	+	+	+	—	+	—	+
C. lunata (Wakker) Boedijn	+	++	++	+++	+	—	++	++
C. pallescens Boedijn	+	+	—	—	+	+	—	+
Helminthosporium sp.	—	—	+++	+	—	+	—	—
Alternaria tenuis Nees	+	+++	+	++	++	++	+++	++
Fusarium nivale (Fr.) Cesati	—	—	++	—	—	+	+	+
Black sterile colonies	+	—	+	+	—	+	—	—

Denotions: RA = 'Raj-821' LA = 'Lal Bahadur'
MA = 'MACS-9' KH = 'Khapli'
RI — 'Ridley' KA = 'Kalyansona'
HD — 'HD-2012' SO = 'Sonalika'
× = *Bipolaris tetramera* (McKinney) Shoemaker

+ = Rare
++ = Subdominant
+++ = Dominant
— = Absent

few cultivars. *Aspergillus terreus* and *Alternaria tenuis* were dominant while, *Rhizopus nigricans*, *A. sydowi* and *Curvalaria lunata* were the subdominant species associated internally. The remaining species were rare in occurrence (Table 2).

Table 2

Distribution of fungi on the internal surface of seeds of certain wheat (*Triticum aestivum*) cultivars

Fungi isolated	Wheat cultivars							
	RA	MA	RI	HD	LA	KH	KA	SO
Rhizopus nigricans Ehrenb.	++	+	+	+	+	+	—	+
Chaetomium globosum Kunze	+	—	—	+	—	—	—	—
Trichoderma viride Pers. ex Fr.	—	+	+	—	—	—	—	—
Aspergillus nidulars Eidam	+	—	+	++	—	++	—	—
A. sydowi (Bain. et Sart.) Thom et Church	+	—	+++	+	+	++	—	+
A. flavus Link	+	+	+	—	—	—	—	—
A. terreus Thom	++	++	++	++	—	—	—	++
A. flavipes (Bain. et Sart.) Thom et Church	—	+	—	—	—	+	—	—
A. niger van Tieghem	—	—	—	+	—	—	—	—
A. ochraceous Wilhelm	—	—	—	+	—	+	—	—
A. tamarii Kita	—	—	+	—	—	+	—	—
Penicillium chrysogenum Thom	+	—	—	+	—	—	—	+
Spicaria sp.	—	—	—	+	—	—	—	—
Memnoniella echinata (Riv.) Galloway	—	—	+	—	—	+	—	—
Curvularia lunata (McKinney) Boedijn	+	++	+	+	—	+	+	—
Helminthosporium sp.	—	—	—	—	+	—	—	—
Alternaria tenuis Nees	—	+	++	—	++	++	++	++
Fusarium nivale (Fr.) Cesati	—	+	+	—	—	—	—	+

The seeds of 'Kalyansona' were inhabited by only a few fungal spores whereas, 'Ridley' cultivar was more susceptible, and higher numbers of fungal spores were isolated from this cultivar. The remaining cultivars studied were in between the two extremes (Fig. 1).

Effect of culture filtrate on seed germination. The germination of all the wheat cultivars was adversely affected by the culture filtrates. The seeds of 'Kalyansona' and 'Lal Bahadur' were not very susceptible whereas, 'Khapli' and ‚Ridley' cultivars were highly susceptible to all the culture filtrates. Considerable decrease in seed germination of 'Raj-821', ‚MACS-9', 'HD-2012', and 'Sonalika' was noted after treatment with culture filtrates. The inhibitory effect of the culture filtrates of the test fungi was in following sequence: *Aspergillus niger* → *Alternaria tenuis* → *Rhizopus nigricans* → *Aspergillus sydowi* → *A. terreus* →

and *Curvularia lunata* (Table 3). The values obtained for the seed germination in the filtrates of the different species differend significantly.

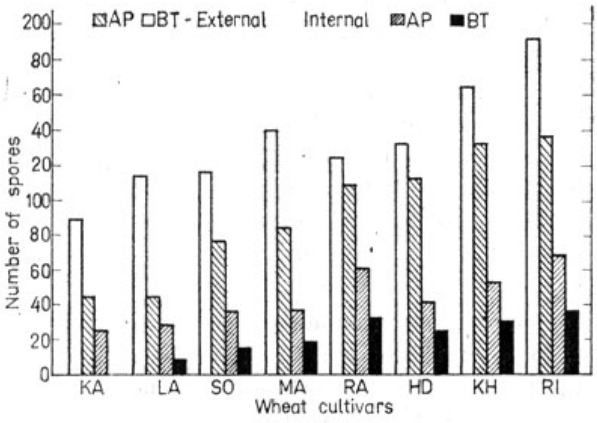

Fig. 1. Percentage association of fungal spores on and in the seeds of different wheat cultivars: KA — 'Kalyansona', LA — 'Lal Bahadur', SO — 'Sonalika', MA — 'MACS-9', RA — 'Raj-821', HD — 'HD-2012', KH — 'Khapli', RI — 'Ridlej', AP — Agar plare metod, BT — Blotter Technique

Table 3

Effect of certain fungal metabolites on seed germination (%)

Wheat cultivars	Fungi used						Control (distilled water)
	RN	AS	AT	AN	AL	CL	
'Raj-821'	71	69	78	46	51	81	95
'MACS-9'	63	78	81	61	70	85	96
'Ridley'	60	62	71	50	54	80	92
'HD-2012'	66	71	79	40	58	85	98
'Lal Bahadur'	82	85	92	71	76	90	100
'Khapli'	58	70	76	43	53	83	94
'Kalyansona'	85	86	90	77	80	94	100
'Sonalika'	70	76	84	59	66	87	100

F values

	F (Calculated)	F (Table) 5%	F (Table) 1%
1. Columns	69.14 *	2.32	3.26
2. Rows	18.52 *	2.24	3.10

* Significant at 5% and 1% levels.

Denotions: RN = *Rhizopus nigricans*, AS = *Aspergillus sydowi*, AT = *A. terreus*, AN = *A. niger*, AL = *Alternaria tenuis*, CL = *Curvularia lunata*

DISCUSSION

The variation in the number and type of fungi associated with different types of seed and various factors affecting the seed mycoflora is well known (Mishra, Kanaujia 1973). It might be expected that the mycoflora of different cultivars of wheat should be similar. As evident from the results of the present investigation the variation in the number and type of fungi associated with different cultivar has been observed. The resistant and susceptible behaviour of 'Kalyansona' and 'Ridley' cultivars respectively is possibly due to the selective nature of seed coats (Mishra, Kanaujia 1973), presence of certain antifungal substances therein (Srivastava, Mishra 1971), and their defensive nature against seed infection (Ark, Thompson 1958).

Quantitative as well as qualitative decrease in internal mycoflora is probably due to the inhibitors present in the seed coats (Srivastava, Mishra 1971) and the biochemical nature of the seed. As a result, out of five dominant and subdominant species on outer surface of the seeds (Table 1) only *Aspergillus terreus* and *Alternaria tenuis* were able to colonize internally (Table 2).

An adverse effect of the cultural filtrates on the seed germination is possibly due to the inhibitory action of certain toxic substance(s) present in the filtrates, which is in agreement with earlier work done by Martin et al. (1956) and Srivastava, Mishra (1972).

Acknowledgements

My sincere thanks are due to Dr. R. R. Misra, Department of Botany, North Eastern Hill University, Shillong for guidance and to Dr. R. J. Das for his valuable suggestions.

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Studia nad niektórymi grzybami zasiedlającymi nasiona
VI. Grzyby towarzyszące różnym odmianom hodowlanym pszenicy

Streszczenie

Badano grzyby towarzyszące ośmiu odmianom pszenicy. Z zewnętrznej oraz wewnętrznej powierzchni ziarniaków wyizolowano 27 gatunków. Z grzybów dominujących tylko *Aspergillus terreus* i *Alternaria tenuis* były zdolne do kolonizowania z zewnątrz. Filtraty grzybów testowych w różnym stopniu ograniczały kiełkowanie ziarniaków wszystkich odmian pszenicy.