

OCCURRENCE OF PREHARVEST SEEDBORNE FUNGI OF CROPS AND THEIR CELLULOLYTIC AND PECTOLYTIC ENZYME ACTIVITY

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Thirty seven spp of preharvest endophytic seedborne fungi were isolated from 18 crops seed. Most of them belong to Dematiaceae of Hyphomycetes with the exception of *Chaetomium* spp belonging to Pyrenomycetes and *Fusarium* spp belonging to Tuberculariaceae. *Alternaria tenuissima*, *A. alternata*, *Cladosporium herbarum*, *Curvularia lunata*, *Epicoccum nigrum*, *Memnoniella echinata* and *Fusarium moniliforme* were observed on most of the crop seeds. Maximum number of spp of such fungi were recorded on paddy (26), jowar and marigold (20), bajra and ragi (18) and minimum number on Bengal gram (9) and black gram (7). *A. alternata*, *C. herbarum*, *C. lunata*, *E. nigrum*, *M. echinata* and *F. moniliforme* were of common occurrence on most of the crops seed, possess high cellulolytic and pectolytic enzyme activity.

Keywords: Crop seeds; Cellulolytic and Pectolytic Enzyme Activity; Preharvest fungi.

Introduction

Host of the literature is available on the seedborne storage fungi and their impact¹⁻⁴ but only stray reports of observation of preharvest seedborne fungi are documented to date⁵⁻¹¹. In the present paper, the endophytic preharvest seedborne fungi of paddy, some millets, pulses, oil yielding crops, spices and asteraceous garden plants have been surveyed along with assaying cellulolytic and pectolytic enzyme activities of some common spp.

Material and Methods

The seeds of paddy (*Oryza sativa*) var Sita, Jowar (*Sorghum vulgare*) var. Local, bajra (*Pennisetum typhoides*) var Local, Setaria (*Setaria italica*) var. Local, ragi (*Eleusine coracana*) var. RAU 3, pea (*Pisum sativum*) var Pusa Prabhat, lentil (*Lens culinaris*) var. Pant 406 Bengal gram (*Cicer arietinum*) var Rajendra, green gram (*Phaseolus aureus*) var. Pusa Vishal, black gram (*Phaseolus mungo*) var. Pant Urd 30, mustard (*Brassica campestris*) var. Rajendra Sarso1, turnip (*Brassica rapa*) var. Pusa Kanchan, cauliflower (*Brassica oleracea* var *botrytis*) var Snow ball, sunflower (*Helianthus annuus*) var. Surya, marigold (*Tagetes patula*) var Local, coriander (*Coriandrum sativum*) var Pant Hariyali and ajwain (*Trachyspermum ammi*) var. Local were collected 15 to 21 days before normal harvesting in sterilized polyethylene pockets and temporarily stored at 3-5°C in

a refrigerator till the time of proceeding for isolation of fungi from them. The fungi from the seeds mentioned ahead were isolated after surface sterilization of five hundred seeds of each crop with 0.1% aq. mercuric chloride solution for 1 min. Such treated seeds were thoroughly washed with sterilized distilled water, plated 10-20 seeds per sterilized moist blotter and incubated at 28 ± 1°C for 7-10 days. The fungi appeared on the seed were taken for preparing temporary slides in cotton blue, cultured on potato dextrose agar medium and identified with the help of standard texts¹²⁻¹⁶. The frequency of preharvest seedborne fungi was calculated as -

Frequency $\frac{\text{Number of seeds harbouring particular fungus}}{\text{Total number of seeds taken for isolation of fungi}} \times 100$

It was recorded in Table 1.

The metabolite of test fungi i.e. *A. alternata*, *C. herbarum*, *C. lunata*, *E. nigrum*, *M. echinata* and *F. moniliforme* prepared in a synthetic medium¹⁷ with the composition of pectin 1%, asparagin 0.2%, potassium dihydrogen phosphate 0.3% and hydrated magnesium sulphate 0.5% in distilled water as solvent, was used for the study of activity of cellulolytic (except C₁) and pectolytic enzyme activity. The fungi were transferred to 50 ml of autoclaved medium in 200 ml flat bottom flasks as disc of 4mm diameter growing on potato dextrose agar medium for 7 days at 25 ± 1°C. After 7 days of incubation

at $25 \pm 1^\circ\text{C}$, the metabolite was filtered and centrifuged at 5000 rpm for 10 min and used for testing enzyme activity. **Cx enzyme:** The activity of this enzyme was estimated viscometrically using 5 ml of 1% solution of carboxy methyl cellulose as substrate and 2ml of the metabolite as enzyme source in a reaction mixture. The Cx activity was calculated as,

$$\text{Cx activity (\%)} = \frac{V_0 - V_t}{V_0 - V_w} \times 100, \text{ where } V_0 = \text{initial viscosity}$$

V_w = viscosity of distilled water and V_t = viscosity of reaction mixture after 30 min

Protopectinase (PP) enzyme: The activity was determined by potato disc maceration method¹⁸.

Polygalacturonase (PG) enzyme: The activity was measured by recording the loss in viscosity of 5 ml of 1% solution of sodium polypectate as substrate by 2 ml of the metabolite in 30 min period. The activity (%) was calculated as noted for Cx enzyme.

Pectin depolymerase (DP): The activity was measured by recording the loss in viscosity of 1% solution of apple pectin as substrate. The activity was calculated as for PG.

C_1 enzyme: The activity was detected by column clearing technique¹⁹.

Results and Discussion

Altogether 37 spp of fungi were isolated from preharvest crops seed. Most of these belong to Dematiaceae (Hyphomycetes, Deuteromycotina) (Table 1) with the exception of two spp of *Chaetomium* (Pyrenomycetes, Ascomycotina) and four spp of *Fusarium* (Tuberculariaceae, Deuteromycotina). *Alternaria tenuissima*, *A. alternata*, *Cladosporium herbarum*, *Curvularia lunata*, *Epicoccum nigrum*, *Memnoniella echinata* and *Fusarium moniliforme* were observed on most of the crops seed. Other fungi were recorded sporadic on preharvest seeds. Maximum number of preharvest seedborne fungi were recorded in association with paddy (26) followed by jowar and marigold (20), bajra and ragi (18) and others. The minimum number was recorded with Bengal gram (09) and green gram (07).

The cellulolytic and pectolytic enzyme activities of the noted ten preharvest seedborne fungi appear very high (Table 2). Highest activity of Cx was observed with *H. sativum* while of C_1 with *F. moniliforme*, PG and DP with *H. gramineum* and PP with *A. alternata* (Table 2).

The occurrence of preharvest seedborne fungi points out their presence in nature during crop period which has been established earlier⁸ in course of such investigation on coriander. The isolated fungi were found endophytic to the seeds in preharvest stage. Other than this, the meteorological factors seem important. The crop

that matures in winter or late ranging temperature between $25-28^\circ\text{C}$, harbour more number of preharvest fungi than those that mature in the beginning of the summer (rabi crop: Setaria, Bengal gram and lentil). Ragi and black gram mature in the rains or so having high RH and warm temperature favouring survival of the fungi in nature and colonizing the crop seeds in the pre-mature stage. Also, the preharvest fungi are expected to possess weak parasitic tendency which has earlier been confirmed^{20,21} working on fungal colonization of senescent stems of dicot and monocot plants. Again, these fungi possess high activity of pectolytic and cellulolytic enzymes⁸ besides having the merit of dissolving cutin²², the outermost layer on the seed surface. Contrary to storage fungi of seeds, the preharvest seedborne fungi have to face the adverse effect of the sunlight. The black pigment of dematiaceous fungi might serve as protective screen²³ but this explanation does not suit to the occurrence of *Fusarium* spp. Some other factors might play role for this incidence. The occurrence of *Chaetomium* spp as preharvest endophytic fungi with seeds appears rare report.

The main cell wall constituents of the seed are cellulose and pectic substance as cementing material besides cutin as outermost surface layer. Cutin has been reported to be degraded by most of the fungi by cutinolytic enzyme²² while cell wall constituents are dissolved into soluble sugars²⁴. PP, DP and PG spearhead the attack on cell wall exposing it for the attack by cellulolytic enzyme and others²⁵. Secretion of high amount of these enzymes by the fungi (Table 2) stated above supports their ability of invasion and colonization of the seed in preharvest stage besides possessing their weak parasitic tendency as mentioned earlier.

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Table 1. List of endophytic preharvest fungi of crop seeds with their frequency.

S. No.	Preharvest fungi	Frequency on different crop seeds*																	
		*1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.	<i>Alternaria tenuissima</i> (Nees ex Fr) Wiltshire	9	7	3	3	7	-	-	-	6	6	5	2	-	11	15	10	5	-
2.	<i>A. alternata</i> (Fries) Keissler	11	6	5	-	10	3	2	3	7	5	-	-	3	8	12	13	6	6
3.	<i>A. longipes</i> (Ellis & Everh) Mason	-	-	-	-	-	-	-	3	-	-	2	-	-	-	-	-	-	5
4.	<i>A. dianthicola</i> Neergaard	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2
5.	<i>A. sonchi</i> J.J. Davis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4
6.	<i>Bipolaris tetramera</i> (McKinney) Shoemaker	5	3	-	-	5	-	-	-	-	-	-	-	-	10	-	6	-	-
7.	<i>B. oryzae</i> (Breda de Haan) Shoemaker	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8.	<i>Cladosporium chlorocephalum</i> (Fres) Mason & Ellis-	-	-	-	-	-	-	-	-	8	-	-	-	-	3	-	-	8	-
9.	<i>C. herbarum</i> (Pers) Link	7	8	6	4	6	4	3	-	-	8	2	-	8	16	8	7	7	8
10.	<i>C. cladosporioides</i> de Vries	3	6	-	-	7	3	-	-	-	-	-	-	-	15	-	8	-	-
11.	<i>C. oxysporum</i> Berk & Curt	6	-	5	5	10	2	3	4	5	4	2	3	8	12	6	10	5	5
12.	<i>Curvularia lunata</i> (Wakker) Boedijn	8	5	6	7	7	-	-	-	-	3	-	-	3	-	6	8	-	-
13.	<i>C. geniculata</i> (tracy & Earle) Boedijn	-	-	5	-	-	2	2	3	-	-	3	3	-	7	-	9	-	6

14. <i>C. pallescens</i> Boedijn	8	3	4	8	3	3	-	-	-	-	3	5	6
15. <i>C. tetramera</i> (McKinney) Boedijn	3	2	-	-	-	-	-	-	-	-	2	5	3
16. <i>Dictyoarthrinium quadratum</i> Hughes	2	-	-	-	5	-	-	-	-	-	-	-	-
17. <i>Epicoccum nigrum</i> Link	6	7	-	5	-	-	-	-	-	-	-	2	-
18. <i>E. purpurescens</i> Ehrenb ex Schlecht	3	-	7	-	3	-	-	-	-	-	-	6	-
19. <i>Helminthosporium gramineum</i> Rab ex Schlecht	9	-	9	-	-	6	1	-	-	6	6	6	5
20. <i>H. Sativum</i> Pammel et al.	10	2	3	6	-	-	4	-	-	3	5	4	6
21. <i>Drechslera bisepitata</i> (Sacc & Raun) Richard & Fraser	2	3	-	-	4	-	-	3	-	-	2	2	-
22. <i>Nigrospora oryzae</i> (Berk & Br) Petoh	3	3	2	-	2	3	4	3	2	4	2	3	4
23. <i>N. sphaerica</i> (Sacc) Mason	2	2	-	-	5	2	3	-	-	3	4	2	5
24. <i>Memnoniella echinata</i> (Riv) Galloway	5	8	5	3	1	-	-	-	-	2	2	3	4
25. <i>Periconia lateralis</i> Ellis & Everh	-	3	-	-	-	-	-	-	-	-	2	5	1
26. <i>Periconia minutissima</i> Cord	3	-	-	-	3	-	-	-	-	3	-	4	2
27. <i>Stachybotrys atra</i> Cord	-	5	-	-	-	-	4	-	-	2	-	6	2
28. <i>Siemphyllium botryosum</i> Wallroth	2	-	-	-	-	-	-	2	-	-	-	-	2

Table 2. Cellulolytic and pectolytic enzyme activity of important preharvest seedborne fungi.

Fungus spp	Cellulolytic enzyme		Pectolytic enzyme (%)		
	Cx (%)	C ₁ (in mm)	PP	PG	DP
<i>H. gramineum</i>	85.6	17	50.7	96.7	90.5
<i>A. alternata</i>	73.5	13	52.3	84.2	89.2
<i>F. moniliforme</i>	78.9	18	43.5	93.6	80.7
<i>A. tenuissima</i>	80.2	15	49.7	80.9	81.8
<i>H. sativum</i>	88.7	16	46.8	95.7	86.7
<i>C. lunata</i>	80.3	12	36.3	88.3	76.3
<i>C. pallescens</i>	72.4	11	32.5	81.5	72.6
<i>C. herbarum</i>	77.6	12	30.4	62.8	73.6
<i>C. oxysporum</i>	72.2	13	30.8	60.3	70.2
<i>E. nigrum</i>	85.3	14	33.3	90.4	77.1

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