https://doi.org/10.29321/MAJ.10.A03371

Madras agric. J. 61 (3 & 4) : 55 – 59 March & April, 1974

Production of Hydrolytic Enzymes by Seed-borne Fungi

BY

D. LALITHA KUMARI[1], P. VIDHYASEKARAN[2] and C. V. GOVINDASWAMY[3]

ABSTRACT

The seed-borne fungi developed well on the castor shell and penetrated into the endosperm and embryo. They grew more abundantly on the shell than on the endosperm. The shell contained more cellulose and pectin. The seed-borne fungi were found to produce cellulolytic and pectinolytic enzymes in culture.

INTRODUCTION

Though seeds are protected by a thick seed coat (husk, shell, etc.) made up of sclerenchymatous and collenchymatous cells consisting of cellulose and pectin, many fungi develop both ectophytically and endophytically on seeds during storage. The seed-borne fungi may produce hydrolytic enzymes to degrade the cellulose and pectin and utilise them for their growth. This possibility was investigated and the results are presented in this paper.

MATERIALS AND METHODS:

The most frequently encountered seed-borne fungi on castor (*Ricinus communis* L.) *Alternaria brassicicola* (Cohn) Schroeter, *Curvularia pallescens* Boedijn, *Helminthosporium tetramera* McKinney, *Mucor hiemalis* Wehmer and *Aspergillus flavus* Link were used in the present investigation. The castor seeds were sterilized with 0.1 per cent mercuric chloride and inoculated with the five test fungi. Uninoculated seeds were kept as control. The seeds were stored at 75 per cent relative humidity. At frequent intervals the stored seeds were aseptically dissected into the shell, endosperm and embryo with a sterile scalpel and the seed parts were planted on oats agar medium. The development of the inoculated fungi in the different parts of the castor seeds was observed.

The shells and endosperms of castor seeds were separately autoclaved and the seed-borne fungi were inoculated on to it. The intensity of the growth of the test fungi on the seed parts was graded after 15 days. The cellulose and pectin content of the shell and endosperm were estimated as per the method described by Asada and Matsumoto (1967). To assess the production of pectic enzymes by the fungi, the fungi were grown in a special pectin medium (Vidhyasekaran, *et al.*, 1966). After 15 days, the culture filtrate was obtained, and the presence of various pectic enzymes was assessed. Pectin methyl esterase (PME) by the titration technique and

1. Research Scholar, Louvaine University, Belgium, 2. Assistant Professor, Tamil Nadu Agricultural University, Coimbatore 641003 and 3. Dean, Agricultural College and Research Institute, Madurai.

TABLE 1. Development of the seed-borne fungi in different parts of the castor seed

Days after inoculation	Percentage of seed parts yielding the inoculated fungi														
	A. brassicicola		*C. pallescens*			*H. tetramera*			*M. hiemalis*			*A. flavus*		
	a	b	a	b	c	a	b	c	a	b	c	a	b	c
2	80	0	80	0	0	80	0	0	100	0	0	100	20	0
4	88	0	88	0	0	75	0	0	95	4	0	88	30	11
6	80	0	80	1	0	82	5	0	96	10	0	89	25	10
8	95	0	82	3	0	85	6	0	91	11	0	90	35	15
10	90	0	90	3	0	80	3	0	93	15	0	95	40	10
12	80	0	85	4	0	77	1	0	90	10	0	100	45	10
14	86	0	82	3	0	86	8	0	85	15	0	95	60	15
16	92	0	81	6	0	91	10	0	93	15	0	90	65	20
18	84	0	86	5	0	84	10	0	91	20	1	85	67	23
20	88	0	87	10	0	98	14	0	88	21	10	90	60	22
22	90	0	92	10	2	82	10	0	98	25	11	95	72	23
24	88	15	91	6	1	90	15	0	91	28	11	95	66	20
26	92	20	80	14	2	98	25	4	96	30	12	80	60	50
28	90	16	88	15	3	95	32	8	92	35	15	90	65	80
30	80	18	94	22	3	96	29	15	99	38	17	90	70	60

a = Shell b = Endosperm c = Embryo

TABLE 2. Production of pectolytic enzymes by the seed-borne fungi

1	2	3	4	5	6	7
A. brassicicola	12.6	31.5	0.0	95.0	235.0	5.45
C. pallescens	0.2	14.0	444.0	120.0	141.0	2.45
H. tetramera	2.6	31.0	31.0	69.0	0.0	5.45
M. hiemalis	1.2	66.0	200.0	0.0	60.0	20.00
A. flavus	3.4	26.5	50.0	191.0	250.0	4.05
Control (Uninoculated medium)	0.0	0.0	0.0	0.0	0.0	No maceration

1. Fungi
2. PME (Micro equivalents of NaOH required to maintain the original pH/hr/ml of the enzyme)
3. Endo PG (Time taken in minutes for 50 per cent reduction in viscosity)
4. Exo PG (1 unit = Change in absorbance of 0.001/ml of enzyme/hr.
5. PGTE (1 unit = Change in absorbance of 0.001 ml of enzyme/hr)
6. PTE (1 unit = Change in absorbance of 0.001 ml of enzyme/hr)
7. Macerating enzyme (Time taken in hr for maceration of potato discs)

endopolygalacturonase (Endo PG) by the standard viscometric assay were determined (Hancock *et al.*, 1964). Exopolygalacturonase (Exo-PG) and Polygalacturonate transeliminase (PGTE) were assessed by using thiobarbituric reagent (Ayers *et al.*, 1966). Pectin transeliminase was estimated by measuring the rate of increase in optical density at 235 mμ in the pectin solution at pH 5.2 (Albersheim, 1963). Macerating enzyme activity was determined by recording the time taken to macerate potato discs of 10 mm diameter and 30μ thickness.

The production of cellulololytic enzymes by the fungi was assessed by growing the fungi in Czapek-Dox liquid medium substituting filter paper cellulose for sucrose in the medium. After 15 days, the culture filtrates were obtained. Cx enzyme activity was

TABLE 3. Production of cellulolytic enzymes by the seed-borne fungi

	C_1 enzyme activity (1 unit = change in absorbance of 0.001/ml enzyme)	Cx enzyme activity (Percentage reduction of viscosity in 2 hours)
A. brassicicola	57.5	37.2
C. pallescens	41.0	32.1
H. tetramera	122.5	22.5
M. hiemalis	61.0	40.2
A. flavus	100.5	32.5
Control (Uninoculated)	0.0	0.0

assessed by the method of Norkrans (1950), while C_1 enzyme activity was estimated viscosimetrically (Vidhyasekaran *et al.*, 1966).

RESULTS AND DISCUSSION

The data in Table 1 revealed that all the fungi developed well on the castor shell, penetrated and reached the endosperm with increase in periods of storage. *A. brassicicola* failed to reach the embryo within 30 days of storage, while *A. flavus* reached the embryo within four days.

The fungi grew more abundantly on the shell than on the endosperm. *A. flavus* grew equally well both on the shell and on the endosperm.

The analysis revealed that the shells contained more cellulose (51.3 per cent) and pectins (30.9 per cent) and in endosperm they were very much less (1.6 and 11.8 per cent respectively).

Both *C. pallescens* and *A. flavus* produced all the six pectic enzymes while others produced at least five of them (Table 2).

A. brassicicola produced the maximum amount of PME and PTE while *C. pallescens* produced appreciable quantities of endo PG, exo PG and the macerating enzyme. *M. hiemalis* produced good amount of Exo PG, while *A. flavus* produced the maximum quantity of both the transeliminases.

The results in Table 3 indicate that while *H. tetramera* showed high C_1 enzyme activity, *M. hiemalis* produced the maximum amount of Cx enzyme.

The seed borne fungi prefer the shell to endosperm and the shell consists of mostly polysaccharides

such as cellulose and pectin. For the utilization of polysaccharides, the production of hydrolytic enzymes by the fungi is essential (Hasija, 1968). The results of the present study revealed that the seed-borne fungi produced appreciable quantities of the hydrolytic enzymes.

After macerating the shell by producing hydrolytic enzymes, the fungi enter into the endosperm. The macerating enzyme runs parallel to the endo-PG and Spadling (1969) reported that macerating enzyme of *Rhizopus stolonifer* is also endopolygalacturonase. In the endosperm the fungi may deplete the sugars (Vidhyasekaran and Govindaswamy, 1968) or reduce the oil content (Lalitha Kumari *et al.*, 1971). When the embryos are invaded by the seed-borne fungi, seed germination failures occur (Lalitha Kumari *et al.*, 1970 and 1973).

REFERENCES

ALBERSHEIM, P. 1963. Auxin induced product inhibition of pectin transeliminase as shown by ozonolysis. *Plant Physiol.* 38: 426 - 9.

ASADA, Y. and I. MATSUMOTO. 1967. Formation of lignin in the root tissues of Japanese radish affected by *Alternaria japonica*. *Phytopathology*. 57 : 1339 – 43.

AYERS, W. A., G. C. PAPAVIZAS and A. F. DIEN. 1966. Polygalacturonate trans eliminase and polygalacturonate production by *Rhizoctonia solani*. *Phytopathology* 56 : 1006 – 11.

HANCOCK, J. G., R. L. MILLAR and J. W. LORBEER. 1964. Pectolytic and cellulolytic enzymes produced by *Botrytis allii*, *B. cinerea* and *B. squamosa in vitro* and *in vivo*. *Phytopathology* 54 : 928 – 31.

HARIJA, S. K. 1968. Assimilation of sugars by *Curvularia pallescens*, *Alternaria citri* and *A. tenuis*. *Indian Phytopath.* 21 : 49 – 61.

LALITHA KUMARI, D., C. V. GOVINDASWAMY and P. VIDHYASEKARAN. 1970. Role of aflatoxins in groundnut seed spoilage. *Curr. Sci.* 39 : 308 – 9.

LALITHA KUMARI D., C. V. GOVINDASWAMY and P. VIDHYASEKARAN. 1971. Effect of seed-borne fungi on the physico chemica properties of groundnut oil. *Indian Phytopath.* 24 : 283 – 9.

LALITHA KUMARI, D., C. V. GOVINDASAWAMY and P. VIDHYASEKARAN. 1973. Isolation of seed-borne fungi from stored groundnut seeds and their role on seed spoilage. *Madras agric. J.* 60 : 77 – 80.

NORKRANS, B. 1950. Influence of cellulolytic enzymes from Hymenomycetes on cellulase preparations of diffent crystallinity. *Physiol. Plantarum.* 3 : 75 – 87.

SPADLING, D. H. 1969. Toxic effect of macerating action of extracts of sweet potatoes rotted by *Rhizopus stolonifer* and its inhibition by ions. *Phytopathology* 59 : 685 – 92.

VIDHYASEKARAN, P. and C. V. GOVIDASWAMY. 1968. Role of seed-borne fungi in paddy seed spoilage. III. Production of carbon dioxide, fatty acids and reduciug sugars. *Indian Phytopath. Soc. Bull.* 4 : 71 – 8.

VIDHYASEKARAN, P., G. MUTHUSWAMY and C. L. SUBRAMANIAN. 1966. Role of seed borne microflora in paddy seed spoilage. I. Production of hydrolytic enzymes. *Indian Phytopath.* 19 : 333 – 41.