

SHORT COMMUNICATION

Studies on cell wall degrading enzymes of *Fusarium pallidoroseum* in relation to disease development

T. T. BARIA* AND R. K. PATIL



J. Mycopathol, Res, 55(1) : 105-107, 2017;
ISSN 0971-3719

© Indian Mycological Society,
Department of Botany,
University of Calcutta,
Kolkata 700 019, India

This article is protected by copyright and all other rights under the jurisdiction of the Indian Mycological Society. The copy is provided to the author(s) for internal non-commercial research and educational purposes.

SHORT COMMUNICATION

Studies on cell wall degrading enzymes of *Fusarium pallidoroeseum* in relation to disease development

T. T. BARIA* AND R. K. PATIL

Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University,
Anand 388 110, Gujarat

Received : 17.10.2016

Accepted : 14.12.2017

Published : 24.04.2017

The activities of polygalacturonase (PG), polymethylgalacturonase (PMG) and cellulolytic enzymes (CX) were studied in mature and semi-mature fruits inoculated with *F. pallidoroeseum* along with healthy fruit. The enzymatic activities were higher in mature fruits i.e., PG (32.56%), PMG (10.15%) and CX (16.36%) than in semi-mature (13.27, 10.14 and 15.61%) fruits, respectively. The enzymatic activity of PG, PMG and CX were found to increase with time.

Key words: Citrus, enzymes, *Fusarium pallidoroeseum*, fruit rot, post-harvest

Citrus, one of the most important fruits of the world, is cultivated widely in the tropical and sub-tropical regions. It ranks third among the sub-tropical fruits of the world with different varieties. Acid lime (*Citrus aurantifolia* Swingle) belongs to the family Rutaceae.

The lemon is rich in many food ingredients, particularly citric acid. Different varieties contain the citric acid in various proportions ranging from 3.71 to 8.40 per cent. It is mainly due to its citric acid and Vitamin C contents that the lemon is widely used in medicine. It is valued for its juice which is mostly used as an accessory food. It increases the flavor and improves the taste of various dishes. It is often used in the preparation of salads and prevents discoloration of sliced bananas and apples. It is widely used in the preparation of lemonades, squashes, jams, jellies, pickles and marmalades. The lemon juice has a good keeping quality and it can be preserved for a long time with certain precautions. It contains 85.0 per cent moisture, 70 mg calcium, 1.0 per cent protein, 10 mg phosphorus, 0.9 per cent fat, 2.3 mg Iron, 0.3 per cent minerals, 39 mg vitamin C and 11.1 per cent carbohydrates, small amount of vitamin B complex, 1.7 per cent fibre, value per 100 gm's edible por-

tion calorific value 57. Al-Hindi *et al.* (2011) reported that cell wall degrading enzymes such as pectinases, xylanases, cellulases and amylases were always greater in healthy fruit and diminished as the disease progressed in the fruit infected with *Aspergillus*, *Fusarium*, *Rhizopus* inciting rots in many fruits.

As very meagre research work has been carried out on fruit rots of citrus and their management in India, with a view to extend the shelf life of citrus fruits and to reduce the losses caused by post-harvest diseases; it is felt worthwhile to carry out the investigations on *Fusarium* fruit rot of Citrus.

Semi-mature and mature fruits were surface sterilized and separately inoculated with *Fusarium moniliforme* by stem-end pin-prick method. The inoculated fruits were incubated at ambient temperature. On 1st, 2nd, 3rd and 4th day, extracts from semi-mature and mature fruits were obtained according to the procedure described by Bell *et al.* (1955).

Five gram of the rotted and healthy fruit tissues were macerated separately with the help of a pestle and mortar in distilled water (15 ml) and 0.5 N NaCl (15 ml). The ground tissues extract were strained through several layers of cheese cloth. The filtrates

*Corresponding author : bariatushar4@gmail.com

Table 1 : Impact of fruit maturity on synthesis of cell wall degrading enzymes by *Fusarium pallidoroseum*

Stage	Polygalacturonase				Polymethylgalacturonase				Cellulolytic enzymes			
	Minutes			Mean	Minutes			Mean	Minutes			Mean
	10	30	120		10	30	120		10	30	120	
Semi-mature	3.68	10.51	25.61	13.27	3.03	5.81	15.61	10.14	11.97	15.19	19.67	15.61
Mature	10.03	23.53	64.12	32.56	3.50	5.16	21.79	10.15	7.93	14.62	26.58	16.36
Uninoculated (Mature)	2.76	6.87	18.87	9.50	4.28	7.18	18.95	8.15	8.40	12.29	21.56	14.09
Mean	5.49	13.63	36.20		3.60	6.05	18.78		9.40	14.03	22.60	
Source	S. Em. ± C. D.				S. Em. ± C. D.				S. Em. ± C. D.			
Stage (S)	0.75	2.19			0.91	2.64			0.87	2.52		
Period (P)	0.75	2.19			0.91	2.64			0.87	2.52		
S X P	1.29	3.85			1.57	4.68			1.50	4.47		
C.V. %		8.20				19.23				11.34		

from semi-mature and mature fruits were separately centrifuged at 4000 rpm for 20 min. The supernatant were used for cell wall degrading enzyme study.

The compositions of the reaction mixtures for 2 ml of enzyme sample for the different enzymes are as follows:

Polymethylgalacturonase (PMG)

Five ml of one per cent pectin dissolved in buffer solution (pH 5.0), 1.8 ml of 0.1 M phosphate citrate buffer (pH 5.0) and 1.5 ml of distilled water.

Polygalacturonase (PG)

Five ml of one per cent sodium polypectate dissolved in buffer solution (pH 5.0), 1.8 ml of 0.1 M phosphate citrate buffer (pH 5.0) and 1.5 ml of distilled water.

Cellulolytic enzymes (CX)

Five ml of 1.2 per cent carboxymethyl cellulose (CMC) dissolved in 1.8 ml of 0.1 M phosphate citrate buffer solution (pH 5.0) and 1.8 ml of distilled water.

The enzyme activity was assessed by determining the loss in viscosity of the reaction mixture immediately at intervals of 10, 30 and 120 minutes at 30°C temperature. Each treatment was reported for four times.

The per cent enzyme activity was calculated by the following formula :

$$\frac{V_0 - V_t}{V_0 - V_w} \times 100$$

Where, V_0 = The flow time at 0 min
 V_t = The flow time after 10/30/120 min
 V_w = The flow time of distilled water

The activity of polygalaturonase (PG), polymethyl galacturonase (PMG) and cellulolytic enzymes (CX) were studied in Immature, semi-mature and mature fruits inoculated with *F. pallidoroseum*. The enzymatic activities of PG, PMG and CX were higher in mature fruits (32.56, 10.15 & 16.36 %) than in semi-mature (13.27, 10.14 & 15.61 %), respectively. The enzymatic activity of PG, PMG and CX were found increased with time duration. Highest reduction in viscosity was observed in PG at

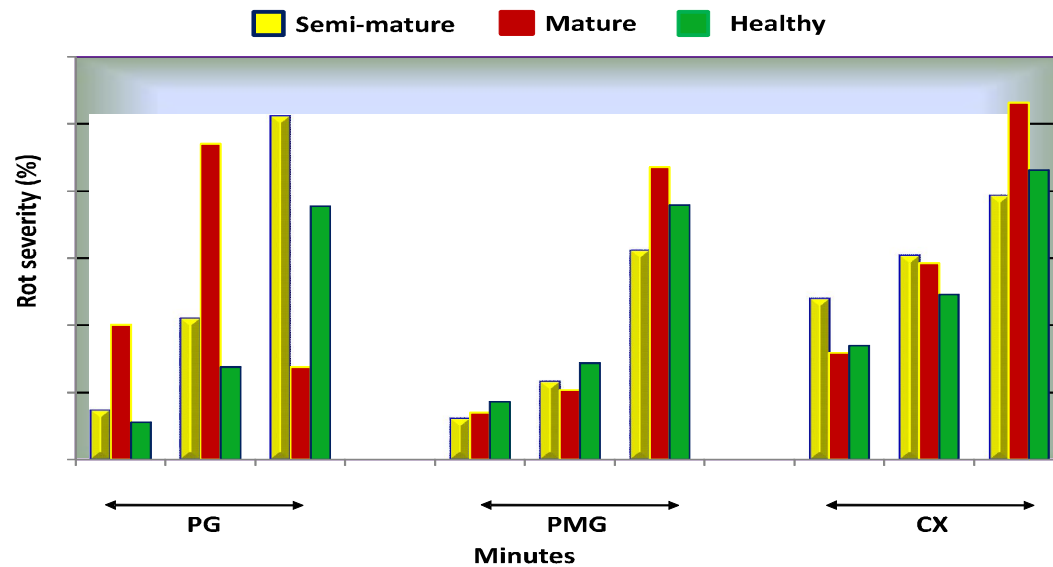


Fig. 1 : Effect of fruit maturity in relation to synthesis of PG, PMG and CX enzymes by *Fusarium pallidoroseum*

120 min, 30 min and 10 min with 13.27, 32.56 and 9.50 per cent, respectively (Table 1 and Fig. 1). The interaction effect between stage of fruit maturity and period found significant. The enzymatic activity (PG, PMG and CX) was lowest in healthy fruits as compared to inoculated fruits.

ACKNOWLEDGEMENTS

The authors are grateful to the Professor and Head, Department of Plant Pathology and Dean, Faculty of Agriculture, B. A. College of Agriculture, Anand Agricultural University, Anand for providing the necessary facilities during the course of investigation.

REFERENCES

- Al-Hindi R. R., Al-Najada, A. R. and Mohamed S. A. 2011. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *African Journal of Microbiology Research*, **5** :443-448.
- Bell, T. A.; Etchells, J. L. and Jones, I. D. 1955. A method for testing cucumber salt stock brine for softening activity. *U. S. Dept. Agr. Res. Serv.*, pp. 72-75.
- Patil, R. K. and Pathak, V. N. 1994. Effect of fruit ripeness in relation to synthesis and activity of cell wall degrading enzymes of mango rot pathogens. *Indian J. Mycol. Pl. Pathol.*, **24** : 156-157.
- Reddy, S. 2012. *Studies on Penicillium fruit rot (Penicillium citrinum) of citrus (Citrus aurantifolia Swingle) and its management*. M.Sc. (Agri.) thesis submitted to Anand Agril. Uni., Anand, Gujarat
- Singh, K. B. 2011. *Studies on Fusarium fruit rot (Fusarium moniliforme Sheldon) of banana (Musa paradisiaca L.) and its management*. M.Sc. (Agri.) thesis submitted to Anand Agril. Uni. Anand.