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Studies on activities of some hydrolytic enzymes of Tomato & Valencia Orange infected by Guignardiacitricarpa

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Abstract

Guignardiacitricarpa Kiely, the causal agent of the black spot disease of citrus fruits, was isolated in this laboratory from diseased mandarin oranges. The organism is also found to be present on different plants other than citrus. Infection of tomatoes&valencia oranges due to **Guignardiacitricarpa** brings about many physiological changes in the host plant tissues. It was observed that the infection was associated with the changes in some proteolytic, pectolytic& amylolytic enzyme activities. Therefore, it was considered worthwhile to study the changes in activities of some of these enzymes viz: protease, PG, PMG, amylase & invertase in tomatoes &valencia oranges infected with **Guignardiacitricarpa**. The activities of protease, PG & PMG were found increased while a significant decline in amylase & invertase activity was observed in infected tomato fruits. In case of valencia oranges, an increase in protease, PG, PMG, amylase & invertase was observed.

Key Words: Protease, PG, PMG, amylase, invertase, **Guignardiacitricarpa**.

Introduction

A number of microorganisms, including **Guignardiacitricarpa Kiely**, are known are known to cause spoilage of fruits, which are known to be affected in pre harvest & postharvest handling, preservation, transport & marketing [1]. Successful infection of a host by plant pathogen requires that the causative agent penetrates the host either by physical or chemical force or a combination of these two & thereby brings about many profound changes, after establishing itself in the host.Thus,studies on physiochemical & biochemical changes taking place in a host are likely to throw some light on the fundamental mechanisms involved in a given type of infection & may also help in understanding the mechanism of disease initiation & spread. Some possible ways & means of disease control may also be obtained by such studies. Considerable work on these lines has been reported in case of various plant pathogens[5, 7]. Hydrolytic enzymes such as pectolytic (PG & PMG), amylolytic (amylase & invertase) & proteolytic (protease) enzymes help the pathogen in penetrating & invading the host cell easily & effectively.

Keeping these observations in view, studies were undertaken to observe changes in the activities of some hydrolytic enzymes of tomato &valenciaoranges (mosambi) following infection with **Guignardiacitricarpa**.

Materials & Methods

Healthy & evenly matured fruits were surface sterilized by washing in ethyl alcohol & were inoculated by injecting 0.2 ml of spore suspension (2 x 10^6 spores/ml) of **Guignardiacitricarpa**. Inoculated fruits were wrapped in sterilized polyethylene bags & incubated for 10 days at 30^0C±2. Controlfruitswhich were inoculated with equivalent amount of sterile water, were also kept under identical conditions.

Enzyme assay

For measuring activityof enzymes, a 30 % extract was prepared in 0.2 M sodium phosphate buffer (pH – 7.0). Homogenate was then passed through a double layer of cheese cloth & clarified by centrifugation at 12,000 x g for 20 minutes. The supernatant was used as enzyme preparation.

Protease activity was measured following the method of Ettan& Bateman [7]. One unit of enzyme activity was defined as that amount of enzyme which liberates 1 mg TCA soluble peptide fragments per hour at 37^0C.

Activities of PG & PMG were measured by the method of Pressey &Avants [6]. One unit of PG & PMG activity was defined as the amount of enzyme which liberates 1 mg galacturonic acid per hour, under the assay conditions.

Amylase & invertase activities were measured following the methods of Mattoo& Modi [3]& Hatch &Glasziou [2] respectively. Reducing sugar liberated was determined by Nelson's method [4]. One unit of enzyme activity, in both the cases, was defined as that amount of enzyme which liberates 1 mg reducing sugars per 30 minutes at 37^0C.

Appropriate zero-time controls were run simultaneously in all the cases. Specific activity was expressed as units per mg protein. Proteins in enzyme preparations were estimated by the method of Lowry et al [5].

Results & Observations

Within 5 to 6 days after incubation brown & black spots were seen on the fruits. The spots grew in size & the portion of the fruit covered by the spot became soft; pulpy & water soaked. After 9 to 10 days of incubation whole fruits were decayed & were analysed at this level.

Activities of all the enzymes studied were generally affected by **Guignardiacitricarpa** infection. Changes in activities of hydrolytic enzymes studied in tomatoes &valencia oranges are shown in Tables: 1 & 2 (Figures: 1 & 2).

Although protease, PG & PMG activities in tomatoesincreased, amylase & invertase activities declined. In case of valencia oranges activities of all the enzymes studied were found elevated.Thedifference is statistically significant in all the cases.

Table: 1
Activities of hydrolytic enzymes of Tomato fruit infected with G. citricarpa

Enzyme	Healthy fruit		Infected fruit	
	U/g of seeds	**Specific activity**	U/g of seeds	**Specific activity**
Protease	0.0873	**0.2066 ± 0.0142**	0.7527	**1.0508 ± 0.1414***
PG	0.7008	**0.8565 ±0.1108**	1.1589	**1.6170 ± 0.081***
PMG	0.9381	**1.1466 ± 0.1121**	1.0581	**1.0581 ± 0.0893****
Amylase	0.7335	**0.8367 ± 0.250**	0.954	**0.4619 ± 0.170*****
Invertase	4.227	**6.889 ± 0.263**	1.449	**2.6960 ± 0.352***

*** p < 0.001, ** p < 0.01, *** p < 0.05**

Table: 2
Activities of hydrolytic enzymes of Valencia orange infected with G. citricarpa

Enzyme	Healthy fruit		Infected fruit	
	U/g of seeds	**Specific activity**	U/g of seeds	**Specific activity**
Protease	0.4691	**0.3515 ± 0.020**	0.8944	**0.6851 ± 0.06***
PG	0.240	**0.1797 ± 0.1125**	1.0221	**0.783 ± 0.221****
PMG	0.1556	**0.1166 ± 0.064**	0.8016	**0.614 ± 0.19****
Amylase	0.447	**0.3348 ± 0.166**	2.629	**2.014 ± 0.852****
Invertase	1.364	**1.022 ± 0.30**	2.385	**1.827 ± 0.425*****

*** p < 0.001, ** p < 0.01, *** p < 0.05**

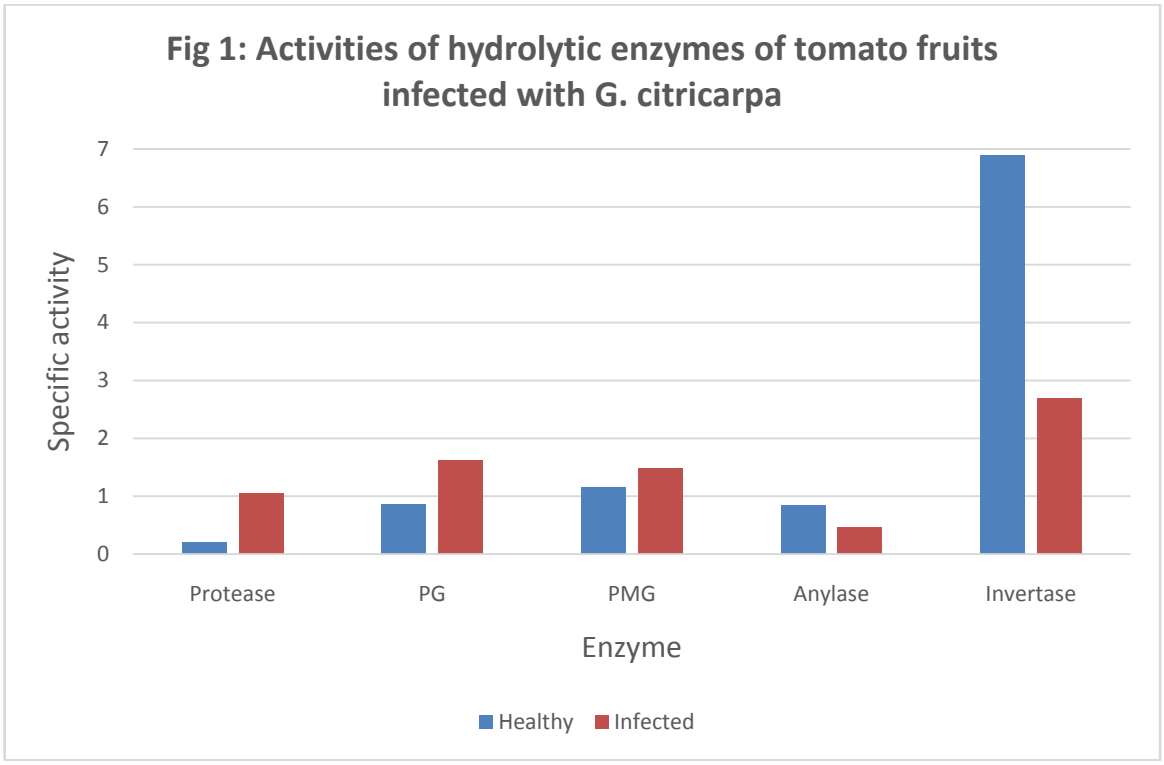
Fig 1: Activities of hydrolytic enzymes of tomato fruits infected with G. citricarpa
Specific activity
7
6
5
4
3
2
1
0
Protease
PG
PMG
Anylase
Invertase
Enzyme
Healthy
Infected

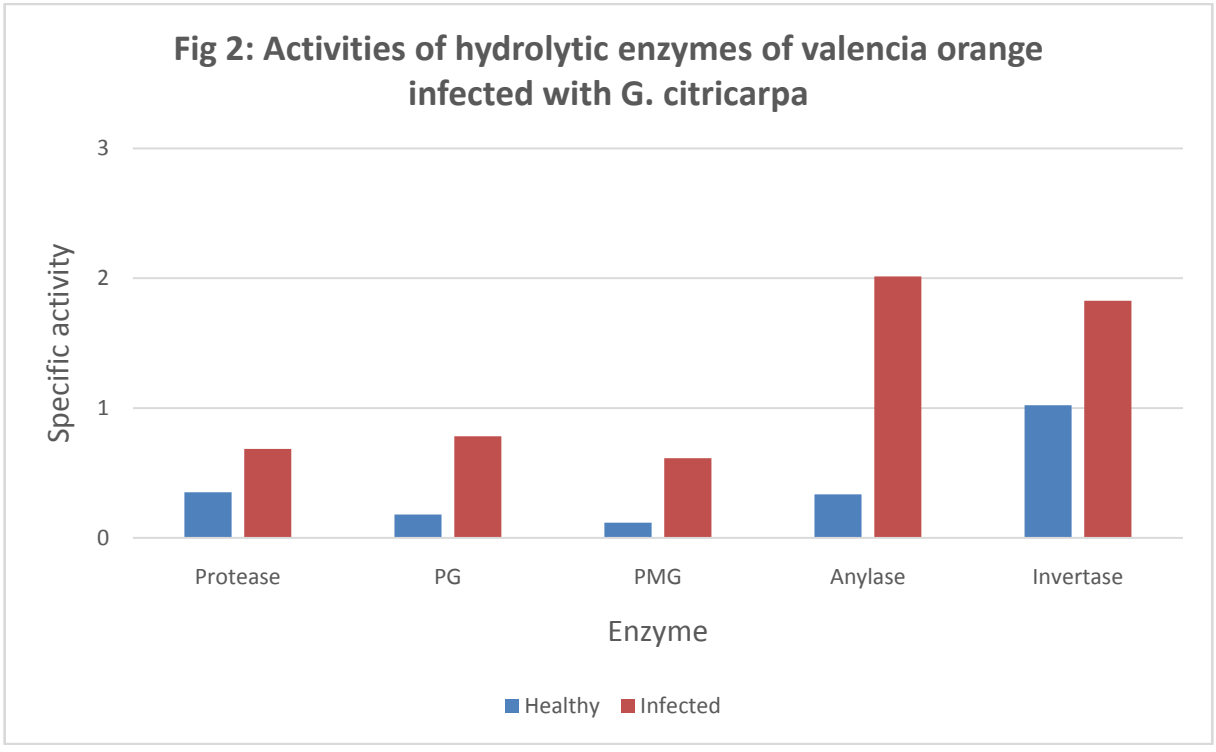
Fig 2: Activities of hydrolytic enzymes of valencia orange infected with G. citricarpa
Specific activity
3
2
1
0
Protease
PG
PMG
Anylase
Invertase
Enzyme
Healthy
Infected

Discussion

A number of workers have reported an increase in protease activity during fungal pathogenesis [7 – 10]. One of the reasons for this may be that besides cellulose & pectic substances, the middle lamella of plant cell wall contains proteins hemicelluloses [11, 12]. The infecting organism has to hydrolyse the proteins to break the cell wall barrier. According to Husain &Kelmen [13], proteolytic as well as other enzymes play an important role in pathogenesis. An increase in protease activity after fungal infection has been reported by Nelson [4] & Sharma & Wahab [8]. Similar results were shown by Patil & Shastri [14] in citrus fruits infected with **Alternariaalternata**.

Results on pectolytic enzymes indicated that both PG & PMG activities increased during infection in both the fruits. Barkai – Golan [15] has studied PG changes in citrus fruits infected with **Geotrichumcandidum** during stages of pathogenesis & reported an increase in PG activity in the infected tissues. Paynter & Jen also have reported an increase in PG & PMG activities in peaches infected with M. fructicola [16]. Similar elevated activities of both these pectolytic enzymes has been reported by Kunte& Shastri [17] in oranges infected with **Alternariaalternata**. It appears that the pectin of the tissue gets demethylated to pectic acids during pathogenesis & these acids in turn stimulates production of fungal PG & PMG.

Variable changes in amylase & invertase activities has been reported in diseased plant tissues. In mango fruits infected by **Aspergillus flavus**, a decrease in both amylase & invertase activities have been reported by Mazumdar & Modi [18]. Similar results were observed by Sharma & Wahab [8] in Luffa cylindrica fruits infected by **Phthiumaphanidermatum**.

Conclusion

In the present investigation, the proteolytic enzyme (Protease) as well as the pectolytic enzymes (PG & PMG) studied, shows an elevated level in both tomatoes&valencia oranges. The activities of amylolytic enzymes (Amylase & Invertase) were found decreased in infected tomato fruits while a significant increased activity of these enzymes was observed in infectedvalencia orangesas compared with healthy fruits.

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