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Localization of Metabolites and Enzymes in post harvested fresh and infected Apple and Guava fruits

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***Abstract*:** *Apple and Guava are an important fruit crops grown commercially in different agro climatic conditions of India for its diversified use. These fruits were respectively post harvested affected by Monilinia and Rhizoctonia solani fungi. Investigation was made on histochemical localization of different metabolites, like starch, insoluble polysaccharides, protein, peroxidase, acid phosphatase and polyphenoloxidase of fresh and infected apple and guava fruits. The purpose of the present investigation was to study the possible alteration in metabolic activity on affected fruits due to pathogens. Starch, insoluble polysaccharide and protein were observe in enhance quantity in fresh fruits as compare to infected fruits but relatively higher amount of enzymes were detected in the infected tissues, suggested altered metabolism of the host tissue due to pathogenesis.*

***Key words*:** *Monilia, Rhizoctonia solani, histochemical localization, metabolites, enzymes*

I. Introduction

Guava (*Psidium guajava*), family *Myrtaceae* is a large dicotyledonous shrub, or small evergreen tree native to Mexico, the Caribbean and Central-South America. The genus Psidium comprises approximately 150 species of small trees and shrubs but only a few produce edible fruits while the rest are wild with inferior quality fruits (Mani *et al*., 2011). It is very popular, evergreen fast growing tree relatively easy to grow and capable of high fruit yield with very little care. It is available almost throughout the year, but it mainly bears fruits twice a year, ripening during the rainy and winter seasons. All though fruit production during the rainy season is high, the quality of fruit is inferior to those produced in winter. Its cost of production is low as it does not require much fertiliser, irrigation and plant protection. Various cultivars have white, pink, or red, ovoid or pear-shaped berry fruits enveloping numerous, cream to brown, kidney-shaped or flattened seed (Orwa *et al.*, 2009).

Apple trees belong to the family of *Rosaceae*. These fruit trees are grouped under the name *Malus domestica* (Bondoux, 1992). However, the origins of the actual cultivated varieties are complex and remain uncertain (Jones and Aldwinckle, 1990). Apple fruits are oval or pear shaped. Its outer peel has different colours depending upon the cultivar type. Internally, its crispy, juicy pulp is off-white to cream in colour, and has a mix of mild sweet and tart flavour. Its seeds are bitter in taste, and therefore, inedible. Apples are rich in antioxidant phyto-nutrients flavonoids and polyphenols.

Postharvest diseases of fruits represent a very important source of wastage and causes economic losses. Roughly 70% of all the major crop diseases are caused by fungi (Deacon, 2006). Fungal diseases play a major role in the wastage of post harvested fruits and many fungicides are used during production and storage for the control. Among various fungal diseases of Apple and Guava *Monilinia* and *Rhizoctonia solani* respectively causing huge revenue losses due to reduction in crop yield. Qualitative histochemical analyses provide an insight into the biochemical phenomenon at cellular level. The present investigation deals with the histochemical localization of metabolites in fresh and *Monilinia* and *Rhizoctonia solani* infected Apple and Guava fruits.

II. Material And Methods

Histochemical studies were conducted on healthy and fungal infected fruits of Apple and Guava. The normal and infected fruits were collected from Eastern Rajasthan (Dist. Bharatpur) and adjoining areas, and their morphology was studied. Fresh hand cut sections of fruits were used for histochemical analysis. The metabolites, starch (Johansen, 1940), Insoluble Polysaccharides (Hotckiss,1948 and Mcmanus, 1948), proteins (Weime, 1959) and enzymes viz., polyphenoloxidase (Sexton and Hall, 1978), peroxidase (Isaac and Winch, 1947) and acid phosphatase (Gomori, 1952) were localized and documented. Their qualitative increase or decrease in localization was assessed in terms of intensity of stain. The degree of distribution of the stain in various tissues was recorded as low (+), moderate (++), high (+++) very high (++++) and nil (-).

III. Result And Discussion

Low intensity of starch was observed in hypodermis and cortex region of fresh seasonal and infected apple. Intensity of starch was also present in low quantity in fresh and infected guava fruit. Starch show less amount in infected fruits because fungus produce glucoamylase enzyme which hydrolyzes alpha- 1, 4 glycosidic bonds and form glucose as the end product (Mertens and Skory, 2007).

Insoluble polysaccharide intensity was moderate in fresh apple cortex region in compare to low intensity in cortex region of infected apple. Mesocarp region of guava showed high stain intensity of total insoluble polysaccharides but it was low in infected fruit. Similarly (Saud *et al*., 2000) reported the reduction of sugar content in fruit rot infected guava fruit. After infection insoluble polysaccharides decreased because fungi broke down the complex carbohydrates in their simple form, same results were also observed by the (Chauhan *et al*., 1981). Protein stain intensity was low in fresh and infected cortex region of apple fruit while it was high and moderate in mesocarp of fresh and infected guava fruit. (Singh and Sinha, 1983) found that the protein content was decreased in guava after infected with Aspergillus flavus and Aspergillus parasiticus. According to (Okolie *et al*., 2011) the prospecting reason for decrease of protein is the fungi utilize nearly all the nitrogen sources.

Polyphenol oxidase stain intensity was high in epidermis and cortex region of the fresh apple fruit while infected epidermis, hypodermis and cortex showed very high staining reaction for the enzyme. The polyphenol oxidase activity was moderate in mesocarp region of fresh guava while it was high in epicarp and mesocarp region of infected guava. (Tyagi *et al*., 1998, 2000) observed higher activities of PPO and PO in wheat infected with Alternaria triticina. (Sahoo *et al*., 2009) presented higher PPO activity and change in its isozyme pattern in taro inoculated with Phytophthora colocasiae.

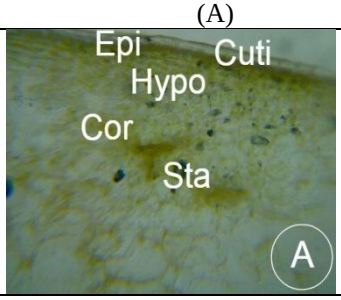
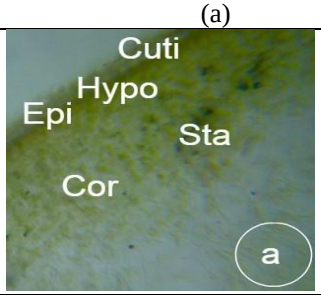
High stain intensity of peroxidase was seen in epidermis, hypodermis and cortex region of fresh apple but its intensity was very high in epidermis, hypodermis and cortex region of infected apple fruit. The stain intensity of enzyme was moderate in mesocarp region of fresh guava but very high intensity of stain was seen in mesocarp region of infected guava fruit. Peroxidases play a central role in the biosynthesis of plant cell wall components, including lignin, suberin, and cross linked extensions that are linked with plant defence responses to pathogen, particularly to fungi, (Almagro *et al*., 2009). The peroxidase enzyme has also been involved in deteriorative changes in flavour, texture and colour in raw and processed fruits and vegetables (Clement *et al*., 1996).

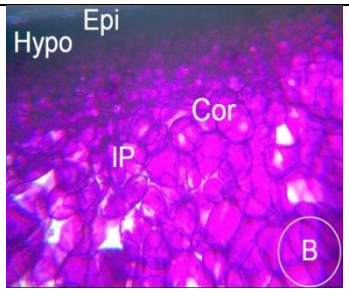
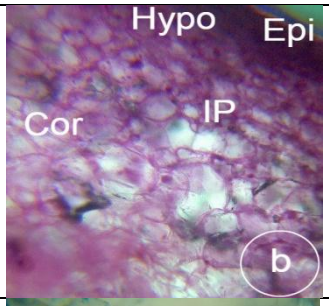
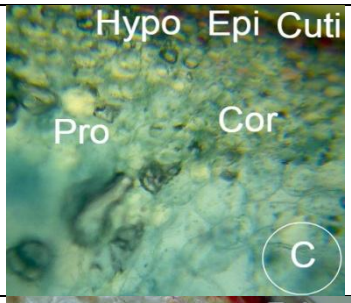
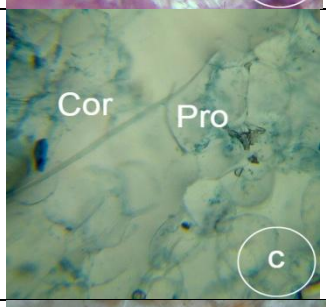
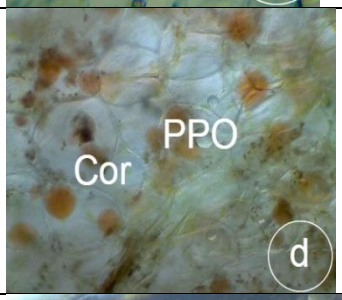
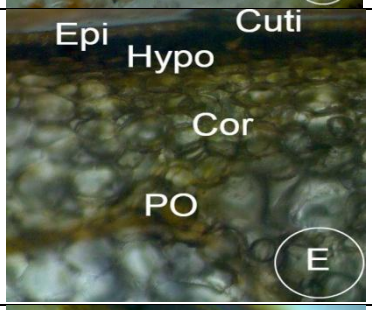
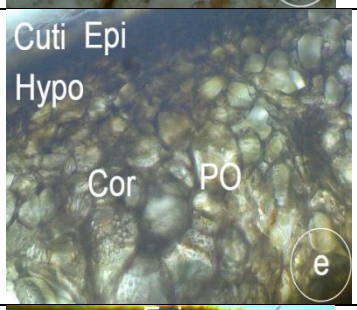
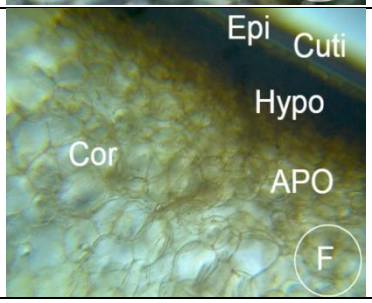
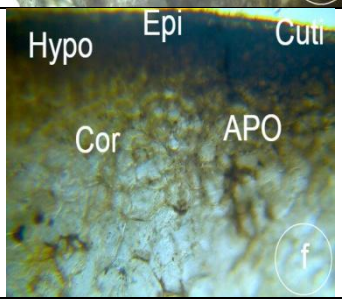
Acid phosphatase intensity was high in epidermis, hypodermis and cortex of fresh apple but in infected acid phosphatase intensity was very high. In guava acid phosphatase intensity was moderate in mesocarp of fresh fruit than infected where very high stain intensity of acid phosphatase was shown in mesocarp region. It is increase in infected fruit because acid phosphatase is associated with hydrolysis of storage compounds and transport of phosphatase which is necessary for large number of metabolic reactions, (Murray, 1980). Its occurrence in the infected plant part suggested a possible role in lysis of fungi. According to (Yadav *et al*., 2004) high intensity of acid phosphatise was observed in blighted stem, leaf and root of *Cuminum cyminum* infected with Fusarium oxysporum than healthy.

Table 1.1: Localization of Secondary Metabolites in Seasonal Fresh and Infected Apple and Guava Fruits

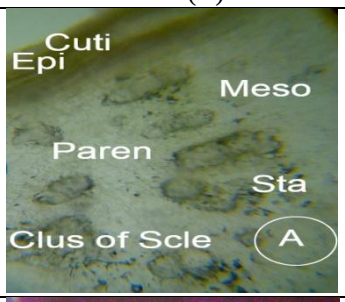
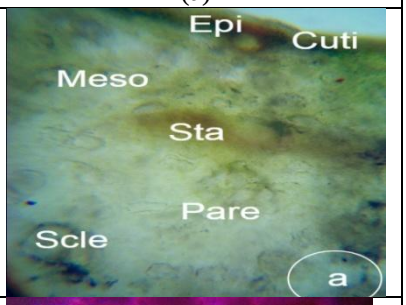
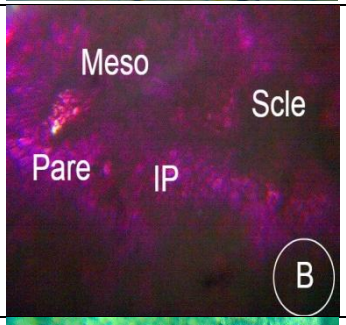
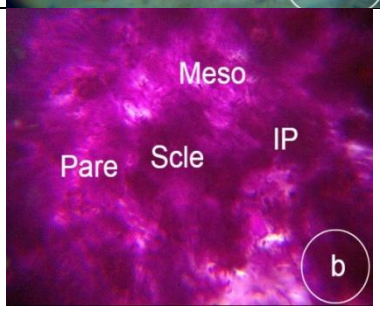
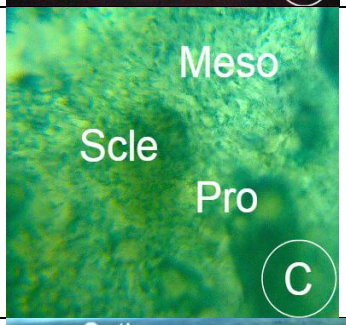
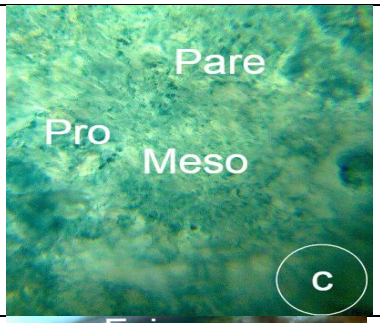
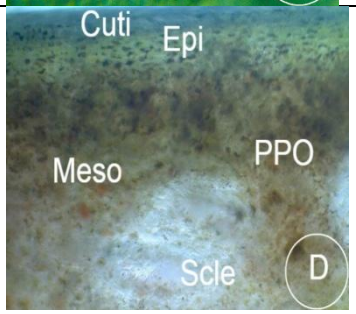
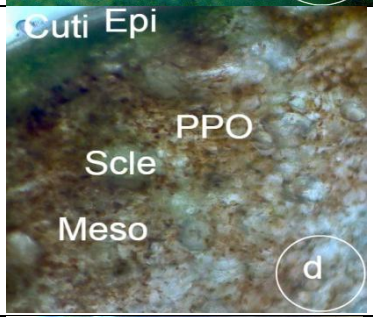
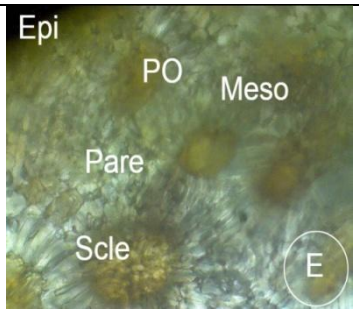
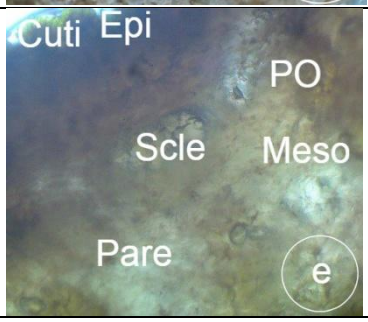
S. No	Secondary Metabolites	Apple Seasonal Fresh	Apple Sea. Infected	Guava Seasonal Fresh	Guava Sea. Infected
1	Starch	+	+	+	+
2	Insoluble Polysaccharides	++	+	+++	+
3	Protein	+	+	+++	++
4	Polyphenol Oxidase	+++	++++	++	+++
5	Peroxidase	+++	++++	++	++++
6	Acid phosphatase	+++	++++	++	++++

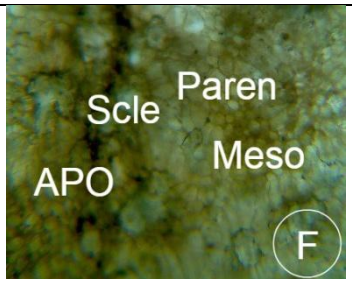
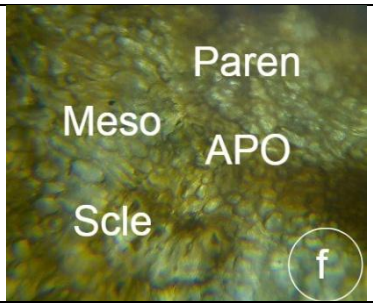
+ = Low, ++ = moderate, +++ = High, ++++ = Very High

Apple	Seasonal fresh (A)	Seasonal infected (a)
starch		

Insoluble polysaccarides		
Protein		
Polyphenoloxidase		
Peroxidase		
Acid phosphatase		

Figre2.1: Histochemical localization of Secondary Metabolites in Apple Fruit. A- Seasonal Fresh, a- Seasonal Infected, at 45x. Hypo= Hypodermis, Epi= Epidermis, Cuti= Cuticle, Cor= Cortex, Sta= Starch, IP= Insoluble Polysaccharides, Pro= Protein, PPO= Polyphenoloxidase, PO= Peroxidase and APO= Acid Phosphatase

Guava	Seasonal fresh (A)	Seasonal Infected (a)
Starch		
Insoluble polysaccharides		
Protein		
Polyphenol oxidase		
Peroxidase		

Acid phosphatase		

Figre2.2: Histochemical localization of Secondary Metabolites in Guava Fruit. A- Seasonal Fresh, a- Seasonal Infected at 45x. Epi= Epidermis, Meso= Mesoderm, Clus of Scle= Cluster of Sclarid, Paren= Parenchyma, Sta= Starch, IP= Insoluble Polysaccharides, Pro= Protein, PPO= Polyphenoloxidase, PO= Peroxidase and APO=Acid Phosphatase

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