DIFFERENTIAL LOCALIZATION OF METABOLITES IN LEAF GALL OF FICUS RELIGIOSA INDUCED BY PIPALDIPLOSIS-PIPALDIPLOSIS

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Abstract

The purpose of the present study was to understand the definite alteration of metabolic activity at cellular level in ***Ficus religiosa*** leaf galls induced by *Pipaldiplosis- pipaldiplosis*. A marked difference in the anatomy was observed between gall and normal tissue. Histochemical studies revealed highest activities of the enzymes and various metabolites in the hypertrophied cells. High content of metabolites viz. starch, cellulose, proteins, etc. were observed in gall tissues as compared to normal counter parts, similarly high enzymatic activities of acid phosphatase, polyphenol oxidase and peroxidase were observed in the leaf galls. A functional elaboration in the cells closer to the feeding site during cecidogenesis was evident. Their differential response of enzymes and metabolites at cellular level of the host proved advantageous to the gall forming insect.

Key words: **Metabolites, *Pipaldiplosis- pipaldiplosis*, *Ficus religiosa* enzymes**.

Introduction

Galls on *Ficus* have attracted the attention of naturalists from early times. They are essentially neoplastic growth and unique examples of complex interaction and mutual adaptation between plants and gall inducing agents. Among the galls induced by various agencies, the range and amplitude in form and structural specialization are more marked

among those galls induced by insects[2]. Galls on leaves of ***Ficus religiosa*** are widely distributed in India.

Insect and mite induced leaf galls on mango have been reported by many workers in the past Sharma (1976) has described external morphology and anatomy of many leaf galls on *Ficus* tree found in semi-arid regions of Rajasthan. *Ficus* Leaf galls have been reported on leaf caused by *Pipaldiplosis- pipaldiplosis* and it was found that there were changes in carbohydrate and amylase contents. The present communication deals with the histochemical changes of metabolites and enzymes in insect induced leaf galls of ***Ficus religiosa***.

Materials and methods

Normal and heavily galled ***Ficus religiosa*** leaves of equal sizes were collected from Sodala region of Jaipur, Rajasthan and their morphology was studied. Fully expanded un-injured leaves were selected, washed in running tap water and used for histochemical studies. Localization of metabolites and enzymes was done by different methodologies of histochemistry. Starch, cellulose and lignin, carbohydrates, proteins, lipids, tannis, Polyphenol oxidase, peroxidase and acid phosphatase were localized and documented. The stained preparations were observed under photolight trinocular microscope (Nikon) and photographed. Their qualitative increase or decrease was assessed in terms of intensity of metabolites as nil, low, moderate, high and very high.

Results and Discussion

Leaf galls were generally epiphyllous, but sometimes also hypophyllous, oval green or yellowish-green on veins. Often 10-15 galls (4-5mm long and 1-2 mm wide) on the single leaf were present. Numerous individual midges feed and pass a part of their life cycle lying within the large, The entire gall mass was composed of undifferentiated parenchyma. The leaf galls are remarkable for total inhibition of differentiation of normal tissues of the mesophyll. Result

of histochemical localization of metabolites and enzymes in leaf gall and normal leaf of ***Ficus religiosa*** are presented in Table 1 and Figs. 1, 2.

Starch

Starch was evident in both normal and gall tissues as black granules. High amount of starch was localized in gall parenchyma and pallisade tissue of normal leaf, while it was moderately localized in spongy parenchyma. High amount of starch content in gall parenchyma detected in present study could be correlated with the high concentration of total soluble sugars. Accumulation of starch in the gall is due to feeding and enzymatic activities of the cecidozoan. Starch is present in form of soluble polysaccharides.

Cellulose

Cellulose was localized as dark blue to black in colour. It was moderately present in gall parenchyma and high in pallisade tissue of normal leaf. It was slightly more in epidermal cells of gall tissue as compared to normal tissue. **Protein**

Protein was stained blue in colour. It was slightly more in gall tissue as compared to normal leaf. It was localized moderate in gall parenchyma and high in outer gall cortex. Similar view has been expressed by Bhatnagar and Kant (1995) Protein is supposed to increase, because of more auxin, cytokinin and phenolics. A higher peroxidase activity has also played a major role in accelerating protein synthesis.

Lipid

Lipid was stained as yellow in colour. High intensity of lipids was observed in outer cortex and moderate intensity in inner cortex of gall. It was absent in spongy and pallisade tissue in normal leaf while it was present in low intensity in vascular region. Presence of lipids in gall cortex could be correlated with continuous wounding caused by feeding activity of

cecidozoan. Abundance of lipid globules in the diseased host suggested that they played a definite role in the metabolic pathway of the host due to fungal infection.

Lignin

Lignin was stained as yellow orange in colour. High intensity of lignin was observed in pallisade tissue and moderate in spongy parenchyma of normal leaf. It was localized in moderate intensity in cell walls of outer cortical cells of gall. The fact that lignins are utilized by acridids implies it on an adaptive strategy for the cecidozoan. Presence of lignin was evident in vascular tissue probably infection with pathogenic agent have delayed the process of lignification in diseased tisssue.

Polysaccharides

Total insoluble polysaccharides were stained pink in colour. They were present in almost all cells of tissue but observed slightly more in gall as compared to normal leaf. It was localized in high intensity in outer cortical cells of gall, while moderately in inner cortical region. In normal leaf pallisade cells and epidermis showed high intensity of polysaccharides. A high amount of total insoluble polysaccharides in gall could probably help the life activity of insect.

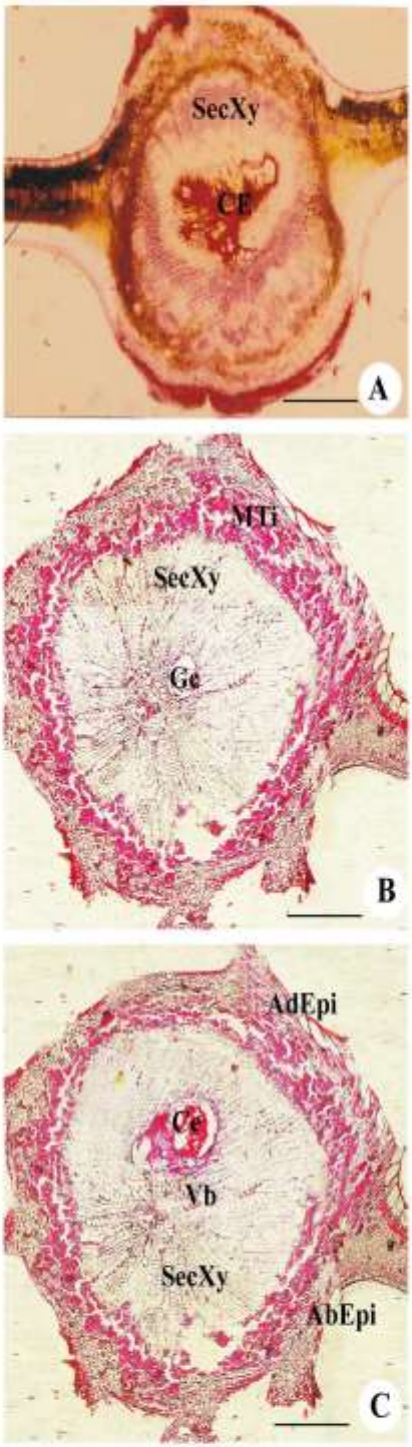
Enzymes

Some enzymes activities were observed in the normal leaf and leaf gall of ***Ficus religiosa***. Enzymes like peroxidase, polyphenol oxidase and acid phosphatase were stained brown to black in colour. High peroxidase activity was observed in outer cortex of gall and in pallisade cells of normal leaf. Polyphenol activity was observed more in gall parenchyma and pallisade cells of normal leaf. Polyphenol oxidase and peroxidase activity have a role in increased growth and metabolism by stimulating RNA synthesis, there by leading to an enhanced protein synthesis.

Acid phosphatase activity was more in outer cortex of gall and pallisade tissue of normal leaf. It was observed less in inner cortical region of gall and spongy parenchyma of normal leaf.

A higher activity of acid phosphatase has also been reported earlier. In the area of high metabolic activity, rapid cellular differentiation and vascularization. Increase of acid phosphatase in gall tissue after insect attack could be due to release of enzymes from lysosome so as to help in the nourishment.

Feeding activity of insect in galls leads to an increase synthesis of enzymes like polyphenol oxidase, peroxidase and acid phosphatase.



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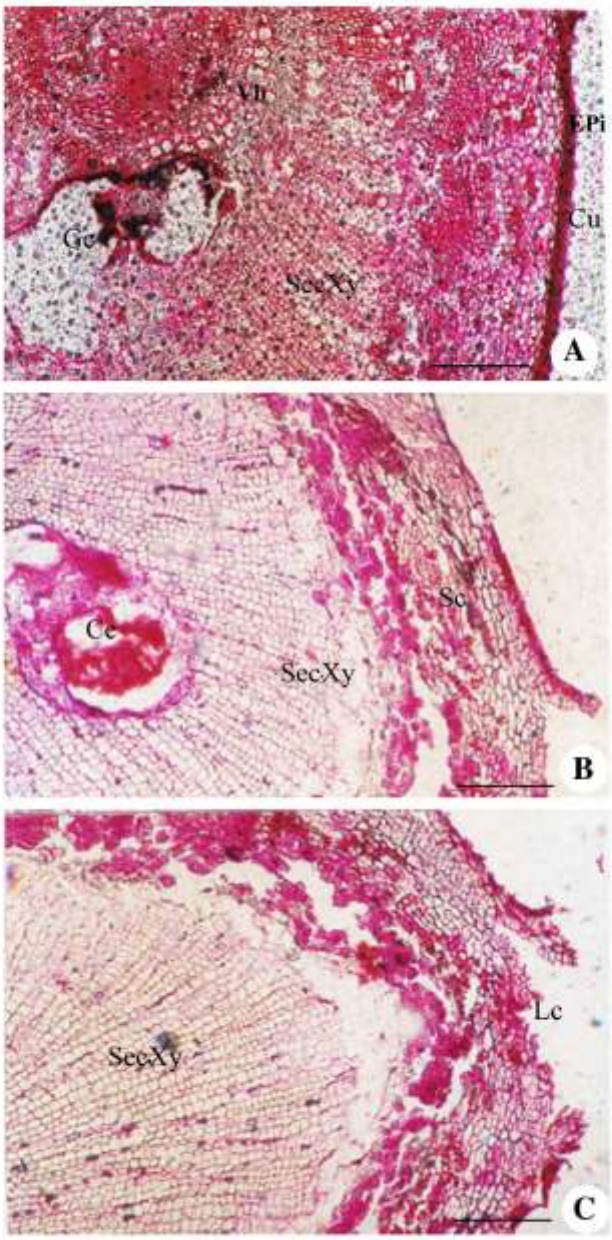

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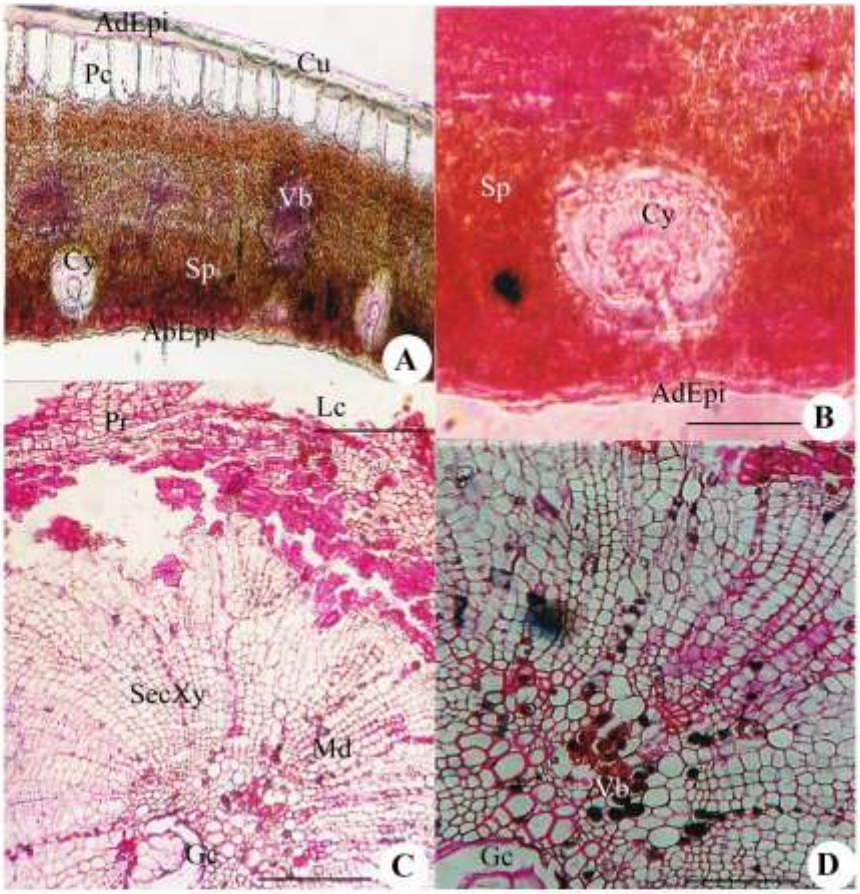
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PLATE-4

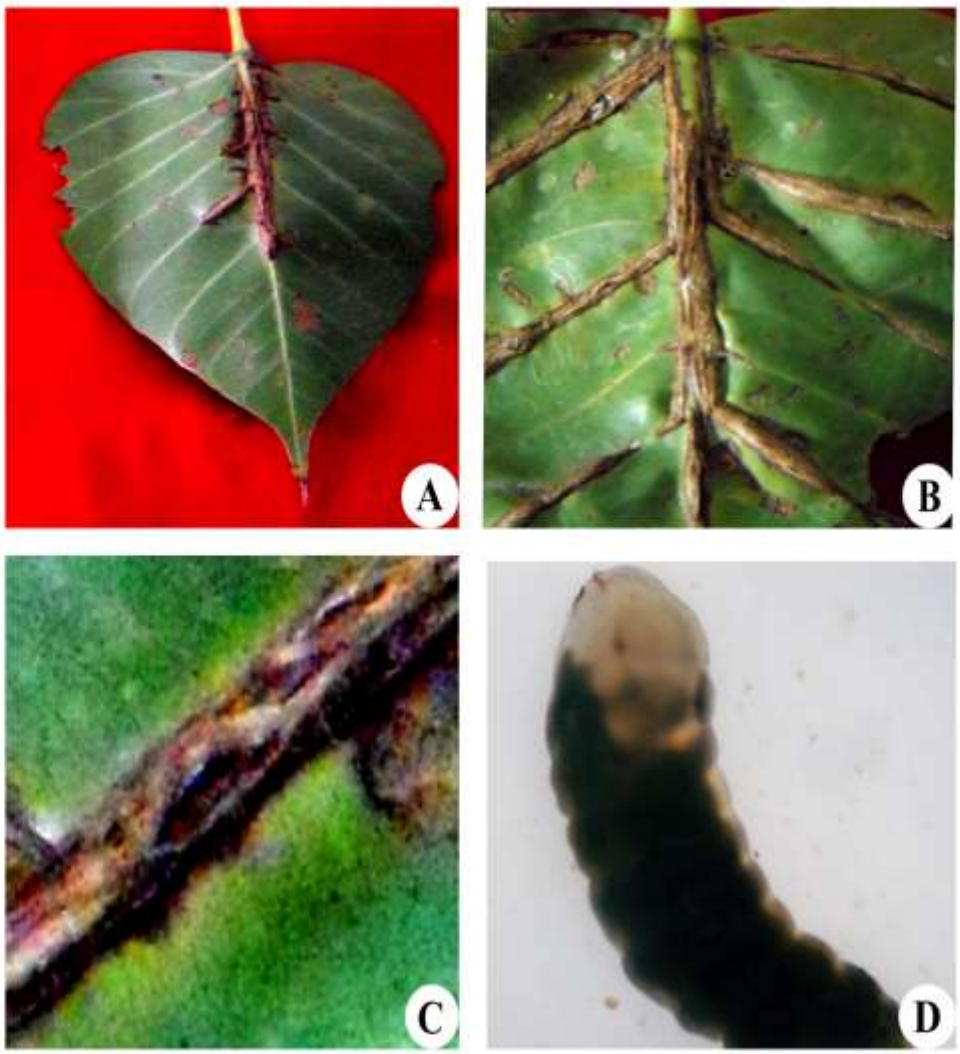

PLATE-3

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Table 1: Histochemical localization of metabolites and enzymes in *Ficus religiosa* normal leaf and gall induced by *Pipaldiplosis- pipaldiplosis*

Metabolites	Normal/gall	Epidermis	Pallisade tissue	Region showing localization in	
				Spongy parenchyma	Gall parenchyma
Starch	Normal	L	H	L	-
	Gall	M	-	-	H
Cellulose	Normal	L	H	L	-
	Gall	L	-	-	M
Protein	Normal	-	H	M	-
	Gall	L	-	-	H
Total insoluble Polysaccharides	Normal	H	H	M	-
	Gall	H	-	-	H
Lipid	Normal	L	-	-	-
	Gall	-	-	-	H
Polyphenol oxidase	Normal	L	H	M	-
	Gall	L	-	-	H
Acid phosphate	Normal	-	H	L	-
	Gall	-	-	-	H
Peroxidase	Normal	-	H	M	-
	Gall	L	-	-	H

Figure1. Localization of various metabolites and enzymes in leaf gall of *Ficus religiosa* (A) Normal leaf showing starch in palisade tissue tissue, spongy parencghyma (X-140). (B) Leaf gall showing starch in epidermis, Gall parenchyma (X-350). (C) Normal leaf showing cellulose in palisade tissue, spongy parenchyma (X-140). (D) Leaf gall showing cellulose in a gall parenchyma (X-350). (E) Normal leaf showing protein in palisade tissue (X-140). (F) Leaf gall showing of protein in gall parenchyma and outer cortex (X-350). (G) Normal leaf showing lipid in vascular bundle (X-140) (H) Leaf gall showing lipid in gall parenchyma.(X-350).

St= Starch, Epi= Epidermis, Gp= Gall parenchyma, Ce= Cellulose, Pro= Protein, Lp= Lipid

Figure2. Localization of various metabolites and enzymes in leaf gall of *Ficus religiosa* (A) Normal leaf showing carbohydrate in palisade tissue and epidermis(X-140). (B) Leaf gall showing carbohydrate in epidermis and gall parenchyma (X-350). (C) Normal leaf showing polyphenol oxidase in palisade tissue (X-140). (D) Leaf gall showing polyphenol oxidase in gall parenchyma (X-350). (E) Normal leaf showing acid phosphatase in palisade tissue and parenchyma (X-140). (F) Leaf gall showing in gall parenchyma (X-350). (G) Normal leaf showing peroxidase in spongy parenchyma (X-140). (H) Leaf gall showing peroxidase in gall parenchyma (X-350).

Car= Carbohydrate, Epi= Epidermis, Gp= Gall parenchyma, Ppo= Polyphenol oxidase, Po= Peroxidase, Ac= Acid phosphatse