J. Phytol. Res. 21(1) : 57-62, 2008

DIFFERENTIAL LOCALIZATION OF METABOLITES IN LEAF GALLS OF *MANGIFERA INDICA* INDUCED BY *AMRADIPLOSIS ALLAHABADENSIS* GROVER

KAMAL SINGH MARMIT, VIDYA PATNI and SUMAN LATA SHARMA
Plant Pathology, Tissue Culture and Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302004. India

The purpose of the present study was to understand the definite alteration of metabolic activity at cellular level in *Mangifera indica* leaf galls induced by *Amradiplosis allahabadensis*. 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.

Keywords : *Amradiplosis allahabadensis*; Enzymes; *Mangifera indica;* Metabolites.

Introduction

Galls on mango 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[1]. 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 *Mangifera indica* are widely distributed in India.

Insect and mite induced leaf galls on mango have been reported by many workers in the past[3-7]. Sharma[7] has described external morphology and anatomy of leaf galls on mango tree found in semi arid regions of Rajasthan. Mango galls have been reported on leaf[3-6, 8,9], shoot[10-12] and flowers[4,13] of *Mangifera indica* caused by *Amradiplosis brunneigallieda* 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 mango.

Materials and Methods

Normal and heavily galled *Mangifera indica* leaves of equal size 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[14], carbohydrates[15,16], proteins[17], lipids[18], tannis[19], polyphenol oxidase[20], peroxidase[21] and acid phosphatase[22] were localized and documented. The stained preparations were observed under photoiight 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 mostly between veins. Often 10-15 galls (1-2 mm long and 1-2 mm wide) were present on the single leaf. Numerous individual midges feed and pass a part of their life cycle lying within the large, axial crevices (gall chamber) of the galls. 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 *Mangifera indica* 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

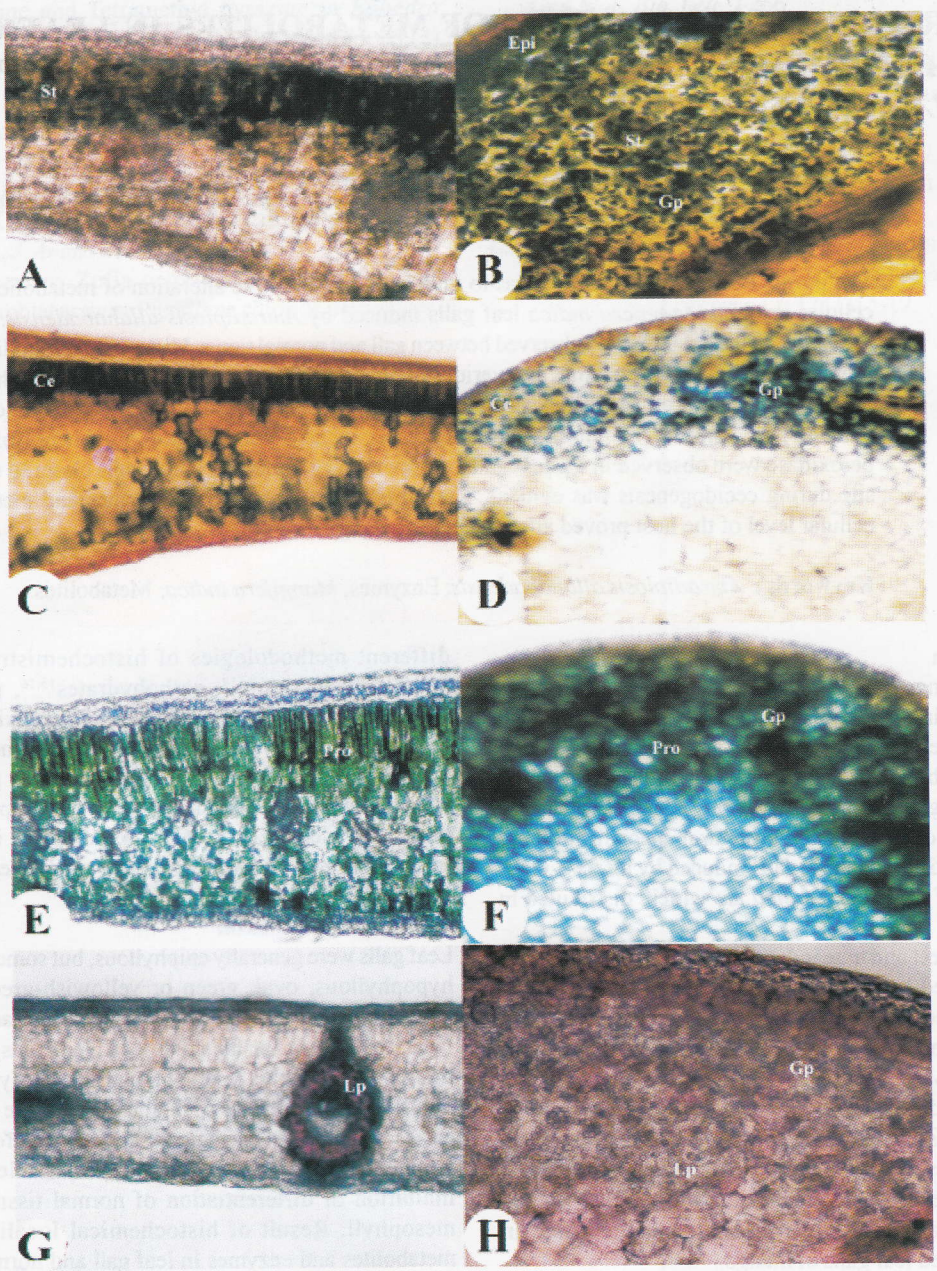

Fig.1. Localization of various metabolites and enzymes in leaf gall of *Mangifera Indica* (A) Normal leaf showing starch in palisade : tissue, spongy parencghyma (X-140); (B) Leaf gall showing starch in epidermis, gall parenchyma (X-350); (C) Normal leaf showing cellulose in palisade tissue, spongy parenchyma (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 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).

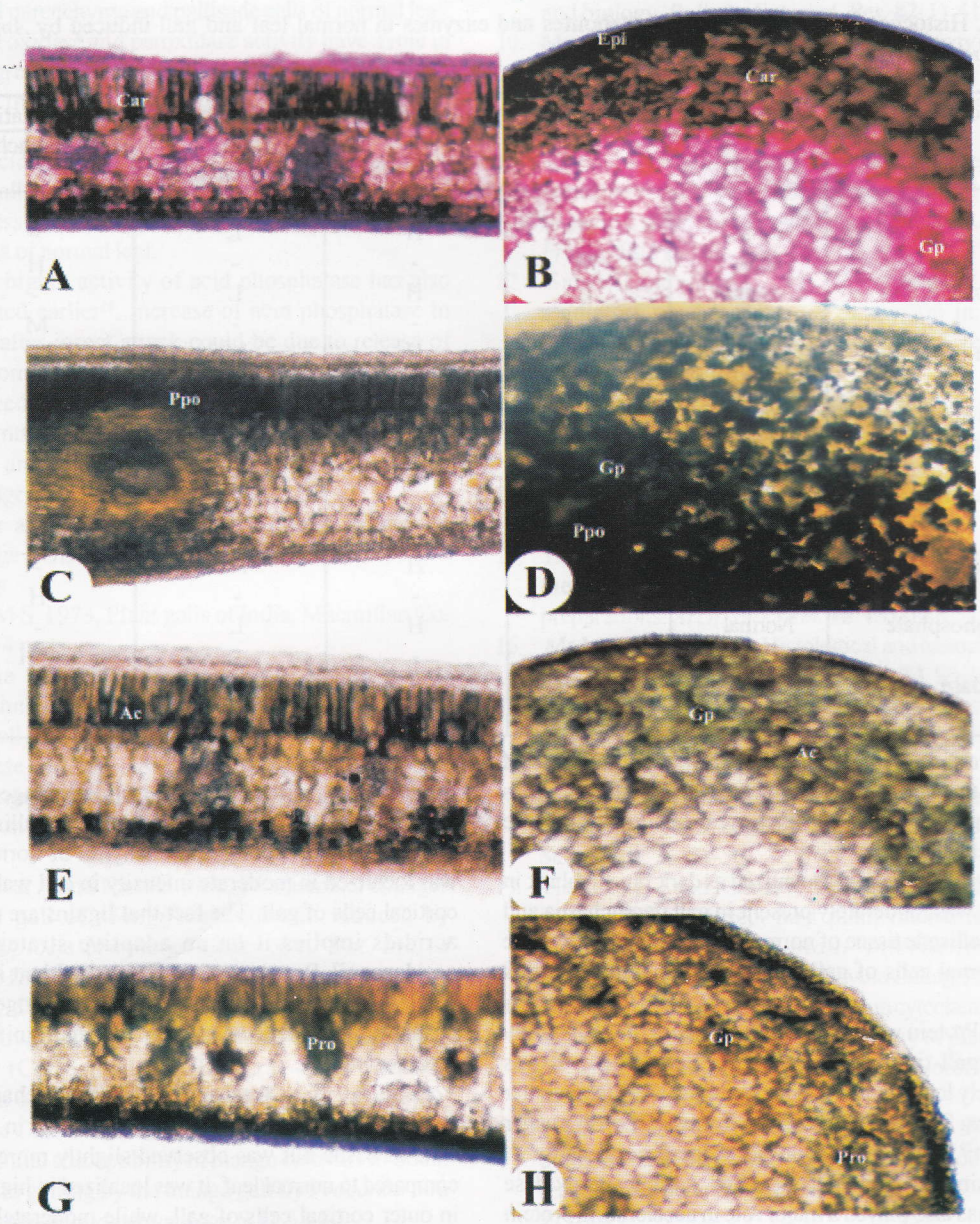

Fig.2. Localization of various metabolites and enzymes in leaf gall of *Mangifera indica* (A) Normal leaf showing carbohydrate in palisade tissue and epidermis(X-140); (B) Leaf gall showing carbohydrate in epidermis and gall parenchyma (X-350); (C) Normal leaf showing polyphenol oxidase in palisade tissue (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=

Table 1. Histochemical localization of metabolites and enzymes in normal leaf and gall induced by *Amradiplosis allahabadensis* Grover.

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

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[4].

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 moderately localized in gall parenchyma and high in outer gall cortex. Similar view has been expressed by Bhatnagar and Kant[23]. 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[24].

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 matobolic pathway of the host due to fungal infection[25,26].

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[27]. Presence of lignin was evident in vascular tissue due to infection because the pathogenic agent probably 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 was 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. 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 enzyme activities were observed in the normal leaf and leaf gall of *M. indica*. 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[28]. 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[29].

Acknowledgement

The senior author (Kamal Singh Marmit) wishes to acknowledge RGNF for Junior Research Fellowship.

References

1. Mani M S 1973, Plant galls of India, Macmillan Co. Ltd. 354 pp.
2. Sharma S L 2000, Zoocecidia of mango plant. Biotechnology and plant pathology. *Current trends*, Printwell Publishers, Jaipur 257-267.
3. Verghese and Rao G S P 1988, Spatial distribution of the mango leaf gall, procontarinia matteriana kief. And Cocc. on mango cv. Dasheri. *Ind. J. Horti.* **42** 139-143.
4. Karnawat A and Kant U 1990, Biochemical changes in leaf gall of *Mangifora indica* L. induced by *Amardiplosis brunneigallieda. Acta Bot. Indica* **18**(2) 312-313.
5. Singh S D 1992, On the anatomy and morphology of full grown larva of *Amradiplosis allahabadensis* grover (Cecidomyiidae : Deptera). *Uttar Pradesh Journal of Zoology* **12**(2) 101-106.
6. Githure C W, Schoeman A S and Mcgeoch M A 1998, Differential susceptibility of mango cultivars in South Africa to galling by the mango gall fly *Procontarinia matteiana. Africa entomology* **6**(1) 33-40.
7. Sharma S L 1976, Studies on some insect and mite induced plant galls *in vitro* and *in vivo*. Ph.D. thesis, University of Rajasthan, Jaipur.
8. Rao S B R and Rao B R S 1986, *Mangoma spinidorsum gen.* Et. sp. n associated with Mango leaf galls. **76**(3) 389-392.
9. Harris K M and Schreiner I H 1992, A new species of gall midge (Deptera : Cecidomyiidae) attacking mango foliage in guam, with observation on its pest status and biology. *Bulletin Entomol. Res.* **82**(1) 41-48.
10. Manobrullah M and Singh R 1997, Efficiency of foliar spray of important insecticides against the mango shoot gall psyllid *Apsylla cistellata.* (Homoptera : Psyllidae). *J. Entomol. Res.* **21**(4) 377-380.
11. Kumar K K 1990, Studies on the varietal susceptibility of mango cultivar from different geographical regions to psyllid shoot gall *Apsylla cistellata. Int. J. Plant Protec.* **18**(1) 93-95.
12. Singh G and Singh G 1987, Effect of plant growth regulators on the shoot gall formation in mango caused by *Apsylla cistellata* in India. *Tropi. Pest Managem.* **33**(2) 176-179.
13. Grover P and Vasantika 1985,Organoid galls by species of Dasineura. *Cecidologia international* **6**(1-3) : 93-95.
14. Johansen D A 1940, In : *Plant microtechniques*. Mc. Graw Hill Book Co. New York, 523 pp.
15. Hotchkiss R D 1948, A microbial reaction resulting in the staining of polysaccharide structure in fixed preparations. *Arch. Biochem.* **16** 131-141.
16. Mc Manus J F A 1948, Histological and histochemical uses of periodic acid. *Stain Technol.* **23** 99-108.
17. Weime R J 1959, Studies on agar electrophoresis *Arcia nitgraphens* New York Brussels and Elsevier Amsterdam, 1965 pp.
18. Chiffelle T L and Putt F A 1951, Propylene and ethylene glycol as solvents for Sudan IV and Black. *Stain Tech.* **26** 51-56.
19. Haridass E T and Kumar N S 1986, Some techniques in the study of insect host plant interaction. In : *Dynamics of insect plant interaction* (ed. Ananthkrishnan, T.N.). E.R.I. Loyola College, Madras.
20. Sexton R and Hall J L 1978, Enzyme cytochemistry in electron microscopy and cytochemistry of plant cells. *In* : Amsterdam Elsevier (ed. Hall, J.L.) North Holland Botanical Press, pp. 63-148.
21. Issac W E and Winch N H 1947, Guaicol. hydrogen peroxide and benzidine hydrogen peroxide colour reactions in bean (*Phaseolus vulgaris* L.) *J. Pomol.* **27** 23-27.
22. Gomori G 1952, Microscopic histochemistry, Principle and practice, University of Chicago Press, Chicago.
23. Bhatnagar K and Kant U 1995, Histochemical profile of blighted seed and stem of Cumin (*Cuminum cyminum*) induced by *Alternaria burnsii. J. Indian Bot. Soc.* **74** 235-238.
24. Stahmann M A and Demorest D M 1973, Changes in enzymes of host pathogen with special reference to peroxidase interaction. *In : Fungal pathogenicity and*

plant response (eds. Byrde, RJW and CV cutting) London., NY Academic press, p 405-422.

25. Kahl G 1982, Molecular biology on wound healing. The conditioning phenomenon. *In : Molecular biology of plant tumors*. Kahl, G. and Schell, G.S. (Eds.), Academic Press, New York, pp. 211-267.
26. Kant U, Qureshi M A and Jain P 1992, *In vitro* studies of nematode induced galls of economically important plants. *In* : *Vistas of seed Biology* (Eds.) Singh, T. and P.C. Trivedi 91-77 pp.
27. Bernays E A, Chamberlain D J and Woodhead S 1983, Phenols as nutrients for a phytophagour insect. *Anacridium melanorhodon. J. Insect. Physiol.* **29** 535-539.
28. Maheshwari D K and Chaturvedi S N 1983, Histochemical localization of acid phosphatase in two fungus galls. *Ind. Phytopathol.* **36** 1167-1170.
29. Debnath M, Sharma S L, Sharma S and U Kant 2002, Differential metabolic changes in midge induced leaf gall of *Mangifera indica. J. Ind. Bot. Soc.* **81** 293-299