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HISTOCHEMICAL STUDIES ON LEAF GALL AND NORMAL LEAF OF PONGAMIA PINNATA (L.)

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Galls are irregular plant growth arising from a reaction between plant hormones and growth regulating chemicals produced by parasites. *Pongamia pinnata* is an oil yielding plant, its oil contain a large percentage of C16 and C18 fatty acids, thus making it highly suitable for biodiesel production. But the galls on its leaves adversely affect its economic value. In the present study investigations were carried out on histochemical localization of different metabolities in leaf gall induced by *Aceria pongamiae* (Acarina : Eriophyidae) and normal leaf. The gall and normal tissue showed histochemically differential behaviour in terms of metabolites.

Keywords : Gall; Histochemical; Metabolities; *Pongamia pinnata.*

Introduction

Pongamia pinnata (family fabaceae) is a nitrogen fixing tree, found in costal area of India, Malaysia, Indonecia, Taiwan, Bangladesh, Srilanka, Northern Australia and Florida. In India it is present abundantly in Rajasthan, Gujarat, Madhy Pradesh, Utter Pradesh, Himachal Pradesh, Bihar and Maharashtra. Its root, bark, leaves, sap and flower have medicinal properties and its oil is known to be used for the treatment of rheumatism and human and animal skin diseases. It is generally not grazed by animals and it can withstand harsh climates. It can be planted on arid and semi arid zone, and near sea shores to prevent water streams. It also helps in controlling soil erosion and binding sand duens. *Pongamie* seeds contain 30-40% oil. It's non edible oil commonly used to fuel lamp and stoves in different parts of India. Its oil, as a biofuel, has physical properties very similar to conventional diesel. So it is a clean fuel (ecofiriendly), than conventional diesel. Insect galls of higher plants are generally thought to be caused by the introduction of chemical substances produced by the causative insect[1,2]. However, authorities differ as to whether each species of gall-maker releases a different cecidogen (gall inducing compound) or if there is one related group of compounds common to most gall-makers[3-5]. A number of specific inorganic chemicals have been reported to produce gall-like plant growths[6,7]. The insect damage the plant leaves and decreases seed production.

Material and Method

The leaf gall and normal leaf of *Pongamia pinnata* were collected from Keola Deo National Park, Bharatpur and their morphology was studied. Fresh hand cut sections of leaf were used for histochemical analysis.

The metabolites, starch[8], cellulose[8], Proteins[9], lipid[10], lignin[11], tannins[12] and suberin[12] were localized and documented. Their qualitative increase or decrease was assessed in terms of intensity of metabolities as; Low (+), Moderate (++), high (+++) and very high (++++).

Results and Discussion

Results obtained for localization of metabolites in normal and leaf gall tissue are presented in Table 1 and Fig. 1-2.

Starch- Starch, the most important carbohydrate reserve in plants is localized as blue to black granuels. Starch granules were observed in palisade parechyama cells while it was in very high quantity in spongy perenchyma. In gall tissue more starch granules were observed in mesophyll regions and nutritive zone and outer layer of gall (Fig.1. C and D).

Cellulose - Cellulose was stained dark blue to black. In normal leaf low quantity of cellulose was observed in mesophyll and vascular region and high quantity was observed in epidermis. While in gall tissues localization of cellulose was observed more in nutritive zone and vascular tissue followed by mesophyll region and epidermis. Higher intensity of cellulose in the nutritive zone could be corrected to the feeding habit of cecidozoan[13] (Fig.1. E and F).

Protein - Protein was stained blue in colour. It was present throughut the various tissues of normal and galled part except in thick walled tisues like xylem and sclerenchyma.

Table 1. Histochemical localization of metabolities in different regions of normal and gall tissue of *Pongamia pinnata.*

S.No.	Metabolites	Normal/Gall	Region localizaed	Intensity
1.	Starch	Normal	Palisade and spongy parenchyama	+++
		Gall	Outer layer of gall nutritive zone, spongy parenchyama	+++
2.	Cellulose	Normal	Epidermis, Mesophyll, vascular region	++ +
		Gall	Nutritive zone, vascular region, Mesophyll and epidermis	++++ ++
3.	Protein	Normal	Mesophyll, vascular region, Epidermis, Spongy parenchayama	++ + +++
		Gall	Epidermis and nutritive zone, Palisade parenchyama, Spongy parenchyma	++++ ++ +
4.	Lipid	Normal	Epidermis, Palisade parenchyma, Spongy parenchayama	+ +++ ++
		Gall	Epidermis, Vascular regions, Nutritive zone	+ +++ ++++
5.	Lignin	Normal	Epidermis, Spongy parenchyama Palisade parenchyama	++ +++
		Gall	Outer layer of gall, vascular region, mesophyll, Nutritive zone	+++ ++++
6.	Tannins	Normal	Epidermis, Palisade parenchyama, Spongy parenchyama, Vascular region	+ ++++ +++
		Gall	Outer layer of gall, Nutritive zone, Vascular region, Spongy parenchyama	++++ ++
7.	Suberin	Normal	Epidermis, Vascular region, mesophyll region	+++ ++
		Gall	Epidermis, Mesophyll region, Nutritive zone, Vascular region	+++ ++++

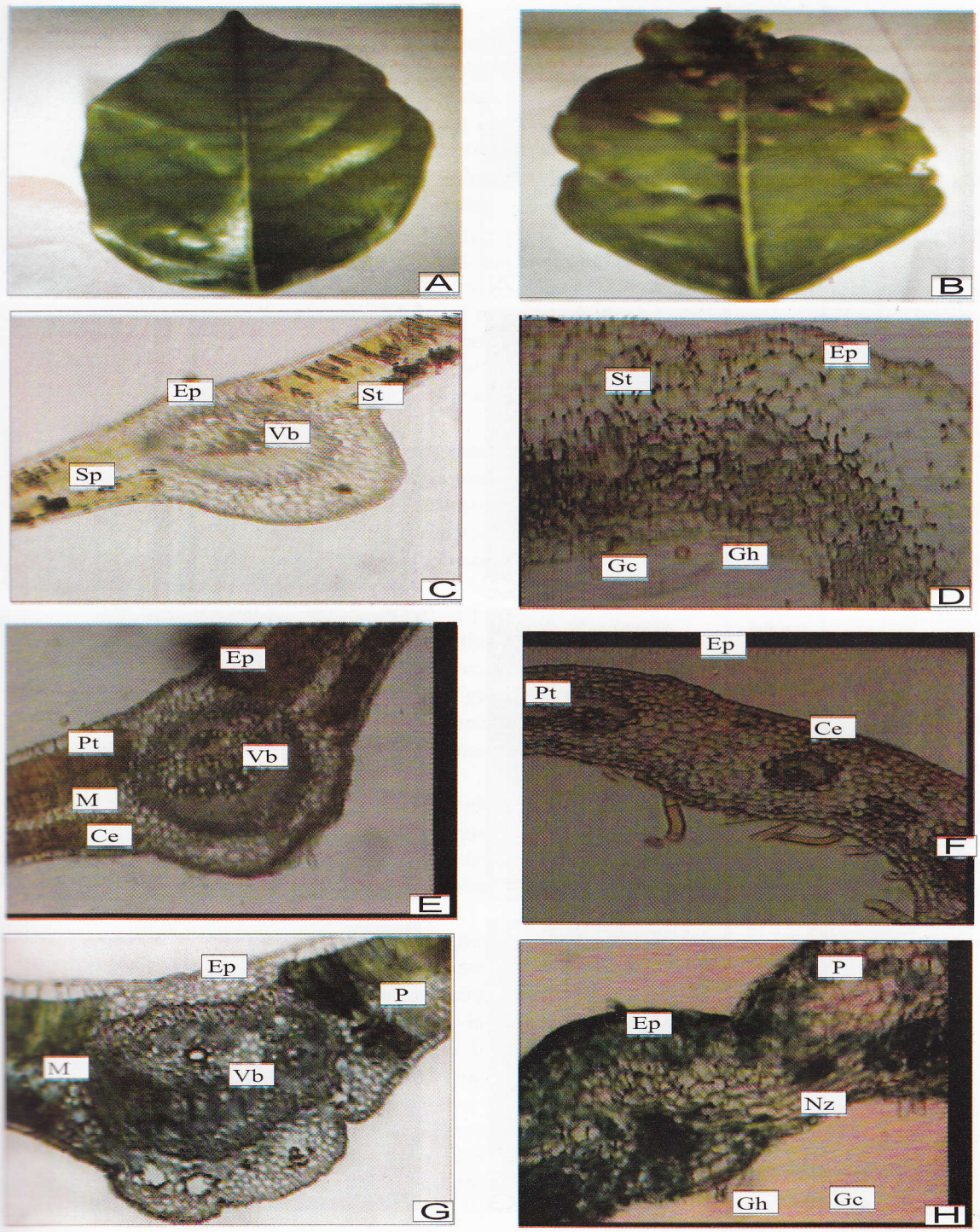

Fig.1. Localization of various metabolites in normal and leaf gall of *Pongamia pinnata,* A,C,E and G- normal leaf, B,D,F and H-leaf gall, C and D- localization of starch, E and F- localization of cellulose,G and H-localization of protein. (M= Mesophyll, Vb= Vascular bundle, Ep= Epidermis, Pt= Palisade tissue, Nz= Nutritive zone, St= Starch, Ce= Cellulose, P=Protein, Sp= Spongy parenchyma , Gc= Gall cavity, Gh= Gall hair)

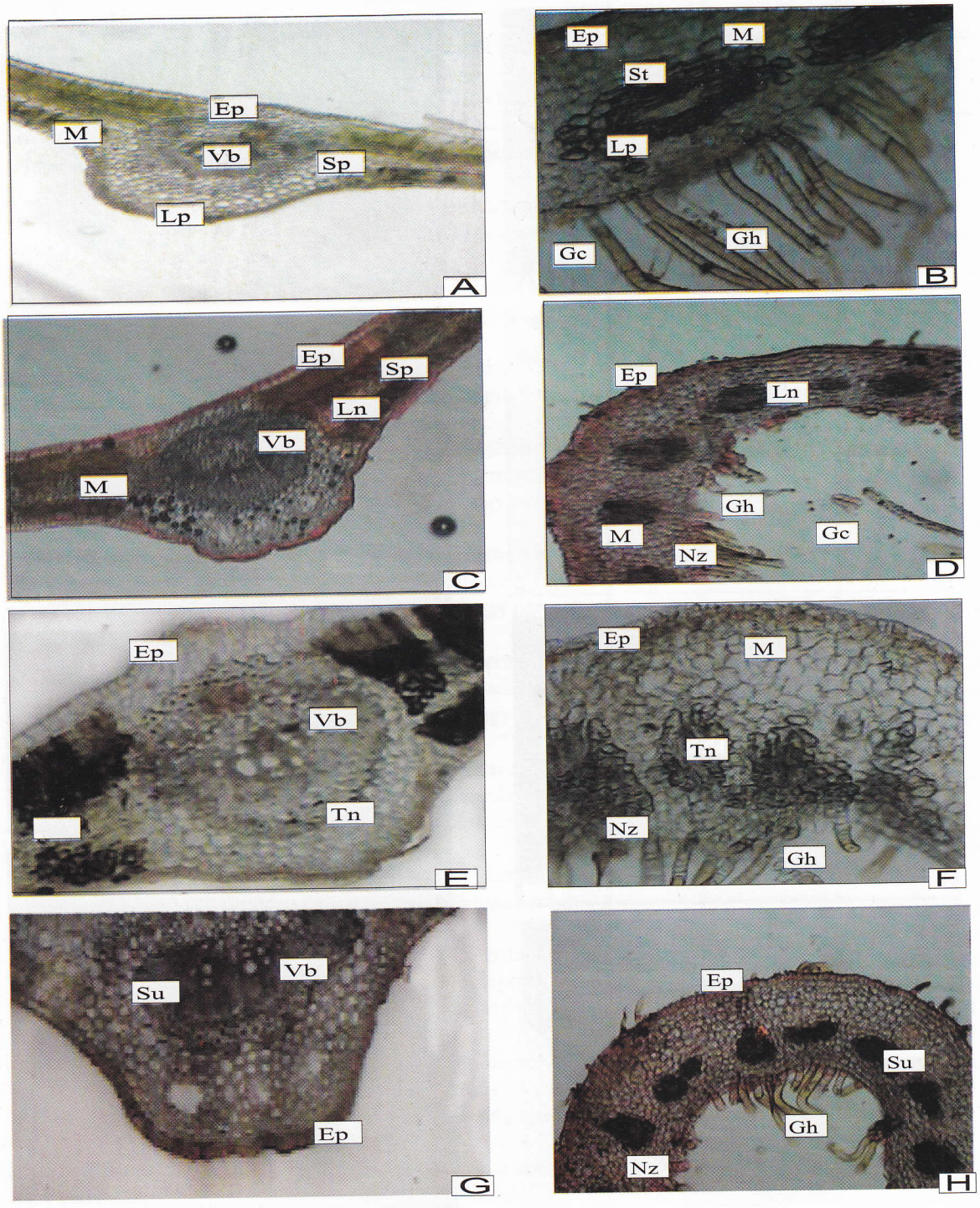

Fig.2. Localization of various metabolites in normal and leaf gall of *Pongamia pinnata,* A,C,E and G - normal leaf, B,D,F and H-leaf gall, Aand B- localization of lipid, C and D- localization of lignin,E , F-localization of tannin and G and H - localization of suberin.
(M= Mesophyll, Ln= Lignin, Vb= Vascular bundle, Ep= Epidermis, Lp= Lipid, Tn= Tannin, Su=Suberin, Sp= Spongy parenchyma, Nz= Nutritive zone, Gc= Gall cavity, Gh= Gall hair)

It was present in low quantity in the epidermis and moderate quantity in vascular bundle and spongy parenchyama showed high amount of protein in normal leaves. Very high quantity of protein was present in nutritive zone and gall parenchyama and moderate amount was observed in epidermis of gall tissues. Higher peroxidase activity also play a major role in accelerating protein synthesis[4] (Fig.1. G and H).

Lipid - Lipid appear as yellowish to pinkish granules. It was mostly localized in the mesophyll tissue of normal leaf and less staining reaction for lipid was observed in the cell wall of epidermis. The leaf gall tissue showed very high contents of lipid in the cells of nutritive zone[13]. High quantity of lipid was persent in vascular region of gall tissue (Fig.2. A and B).

Lignin - Lignin was stained pinkish brown in the tissues. Lignin was observed in the cells of epidermis and mesophyll region of normal tissue. It was localized intensively in nutritive zone[14]. High amount of lignin was observed in gall parenchyama outer layers of gall tissue and vascular region (Fig.2. C and D).

Tannin- Tannins were stained red orange or brown in colour. In normal leaf tannins were observed in the mesophyll and vascular region and low amount was present in epidermis. Outer layer of gall showed very high localization of tannins. Nutritive zone, vascular and mesophyll region of gall showed high amount of tannins (Fig.2. E and F).

Suberin - In gall tissues suberin was observed in very high amount in vascular region and nutritive zone and moderate in epedermis and mesophyll region. While in normal tissue intensity of suberin was higher in epidermis and vascular region than mesophyll (Fig.2. G and H).

The above resume demonstrates a wide degree of variation both is normal and gall as the nutritive zone function as source of nutrition to the larva. The intensity of biochemicals were higher in gall tissue mainly in vascular bundles, mesophyll tissue and nutritive zone. It show the higher metabolic rates in the gall regions by the influence of galling agent.

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References

1. Malpighi 1975, *Antome plantarum.* Plant growth hormones in pinyon insect galls. *Marcellia* **39** 125-134.
2. Plumb G H 1953, Formation and development of the Norway Spruce gall caused by *Adelges abietis* L. Conn. *Agric. Exp. Sta. Bull.* 566. New Haven Conn.
3. Boysen, Jensen P 1948, Formation of galls by *Mikiola fagi. Physiol. Plant.* 1 95-108.
4. Miles P W 1968, Insect secretions in plants. *Ann. Rev. Phytopath.* **6** 137-164.
5. Steriling C 1952, Ontogeny of the phylloxera gall of grape leaf. *Am. J. Bot.* **39** 6-15.
6. Levine M 1950, The growth of normal plant tissue *in vitro* as affected by chemical carcinogens and plant growth substances -1. The culture of carrot tap-root meristem. *Am. J. Bot.* **37** 445-458.
7. Mani M S 1964, Ecology of plant galls. Dr. W Junk, Publishers, The Hague. 434p.
8. Johansen D A 1940, Plant microtechnique, McGraw-Hill Book Co., Inc. New York and London, pp. 491.
9. Werine 1959, Studies on agar electrophoresis. Arcia nitrographens, NY Brussels and Elservier Amsterdam 1965, pp.
10. Chiffele TI and Putt FA 1951, Propylene and ethylene glycol as solvents for Sudan IV and Black *B. Stain Tech.* **26** 51-56.
11. Haridass E T and Suresh Kumar N 1985, Some techniques in the study of insect-host plant interactions. In : *Dynamics of insect Plant interactions* (ed.) T.N. Ananthakrishnan, Entomology Research Institute, Loyola College, Madras.
12. Sexton R and Hall J L 1978, Enzyme cytochemistry in Electron microscopy and cytochemistry of plant cells (ed.) J. L. Hall, (Amsterdam El-sevier North Holland Botanical Press), pp. 63-148.
13. Singh S, Patni V and Arora D R 2005, Localization of metabolities and enzymes in Leaf gall of *Ficus racemosa* induced by *Pauropsylla depressa. J. Mycol. Pl. Pathol.* **35**(2) 241-246.
14. Debnath M, Sharma SL, Sharma S and Kant U 2002, Differential metabolic change in midge induced Leafgall of *Magifera indica. J. Ind. Bot. Soc.* **81** 293-299.