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HISTOCHEMICAL STUDIES ON LEAF GALLS AND NORMAL LEAF OF *ALSTONIA SCHOLARIS*(L.) R. BR.

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Galls are localized outgrowth of various host-organs in which host cells are stimulated to excessive growth by parasite. *Alstonia scholaris* (L.) R. Br. is a very beautiful ornamental tree, which is commonly known as pagoda tree because of its pagoda like growing habit. But the galls on its leaves adversely affect the looks and thus the economic value and also cause a lot of damage to the host tissue. In the present study, investigations were carried out on histochemical localization of different metabolites and enzymes in leaf gall induced by the Homopteran, *Pauropsylla tuberculata* Crawf. and normal leaf. An alteration in localization of metabolites and enzymes due to cecidogenesis was observed. Relatively higher amount of metabolites detected in gall tissue suggest altered metabolism of the host tissue due to pathogenesis.

Keywords: *Alstonia scholaris;* Gall; Histochemical; Metabolites.

Introduction

Alstonia scholaris (L.) R.Br. is an economically and medicinally important tree species distributed throughout the tropical and sub-tropical regions of the world. Its bark is useful in fevers, abdominal disorders, dysentery, dyspepsia, leprosy, skin diseases, tumours, ulcers, asthma, bronchitis, cardiopathy, helminthiasis, agalactia, debility. It is also grown as ornamental and avenue tree. Leaf galls of *Alstonia scholaris* are extremely complex structure and present intriguing problems in morphogenesis, physiology, pathology and ecology. These are essential neoplastic growths and unique examples of complex interaction and mutual adaptation between plant and gall inducing agent[1]. Among the gall induced by various agencies, the range and amplitude in form and structural specialization is more marked among these galls induced by insect[2]. Qualitative histochemical analysis provide an insight into the biological phenomenon at cellular level. The present investigation deals with the histochemical localization of metabolites in leaf gall of *Alstonia scholaris* induced by the cecidozoan *Pauropsylla tuberculata* Crawf. .

Material and Methods

The normal leaves and leaf galls of *A. scholaris* were collected from Jaipur and adjoining areas and their morphology was studied. Fresh hand cut sections of leaf gall and normal leaf of *A. scholaris* were used for histochemical analyses.

The metabolites and enzymes localized and the methodology used are as follows : Starch, cellulose and lignin[3], carbohydrates[4,5], proteins[6], lipids[7], tannins[8], polyphenol oxidase[9], peroxidase[10] and acid-phospatase[11]. 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 (+++), very high (++++).

Results and Discussion

Leaf galls of *A. scholaris* are globose, conical or obtusely conical on the adaxial side of the leaf and truncated conical on the abaxial side. These are covering growth pouch galls ,about 2.5mm in diameter at tip and 3 mm in base, the cone on the underside of the blade about 5-6 mm long, pale green when young and yellowish when mature, glabrous hard scattered irregularly in large numbers on the leaf and sometimes as covering galls on petiole also. The ostiole is very narrow in immature galls but widens out as the development proceeds and opens on the summit of the truncated cone. The results of histochemical analysis are shown in table 1 and Fig. 1 and 2.

Starch - Starch, the most important carbohydrate reserve in plants is localized as blue to black granules. Starch was localized in very high quantity in gall parenchyma specially in the region between mechanical zone and epidermis. It was conspicuously absent in the nutritive zone. In normal leaf starch was localized in palisade tissue in high quantity (Fig. 1 a_1 and a_2).

Carbohydrates- Carbohydrates were stained magenta-pink in colour and were observed in slightly more intensity

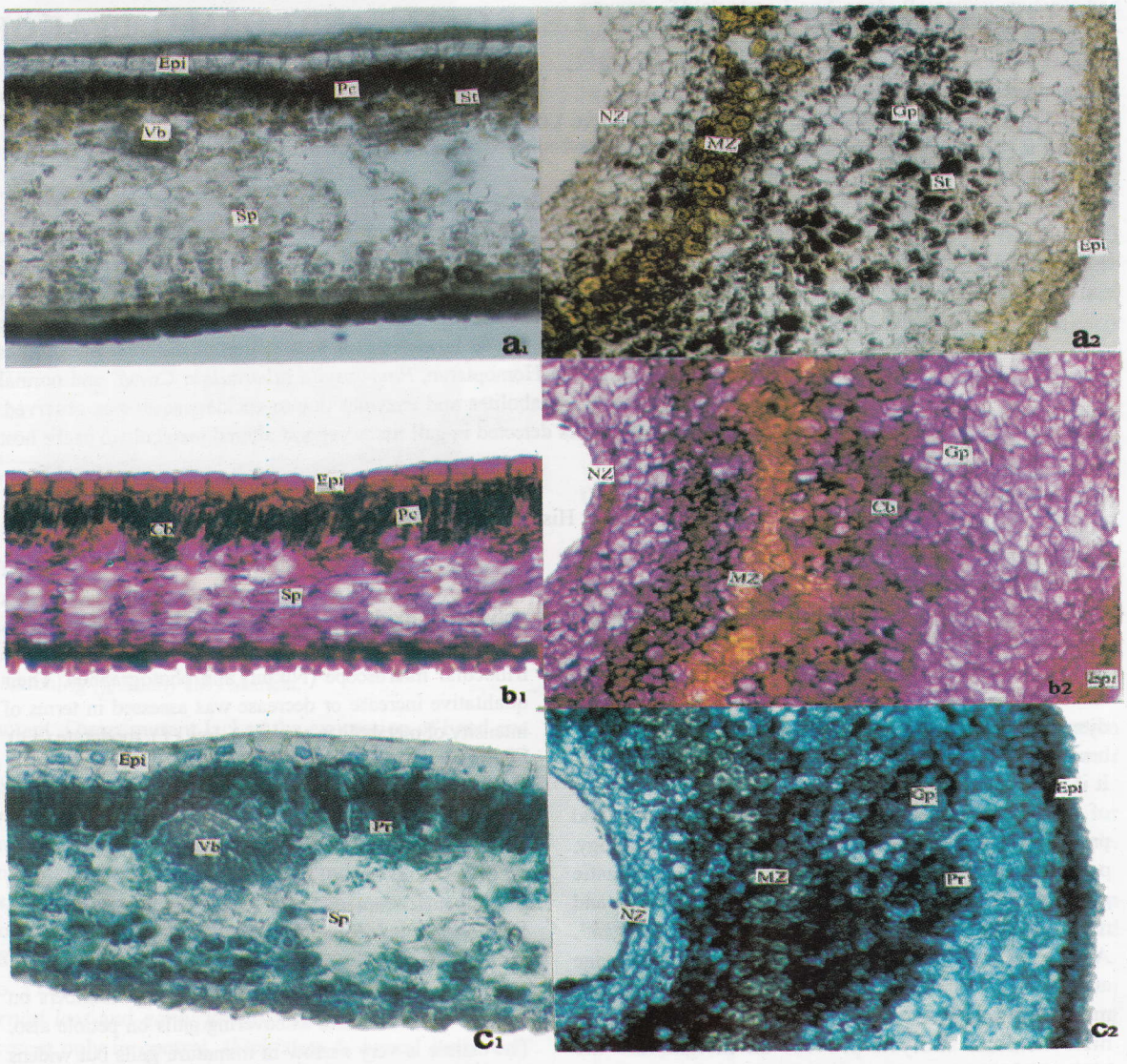

Fig.1. Localization of metabolites in different regions of leaf gall and normal leaf of *Alstonia scholaris*.
a_1 = T.S. of normal leaf showing high localization of starch in palisade cells (x 325)
a_2 = T.S. of gall showing very high localization of starch in gall parenchyma (x 130)
b_1 = T.S. of normal leaf showing very high and high localization of carbohydrates in palisade cells and epidermis respectively(x 325)
b_2 =T.S. of gall showing intense localization of carbohydrates in gall parenchyma and nutritive zone (x 130)
c_1 = T.S. of normal leaf showing very high localization of protein in palisade cells (x 325)
c_2 = T.S. of gall showing very high and high localization of protein in gall parenchyma and nutritive zone(x130) (Epi = epidermis, Pc = palisade cells, Sp = spongy parenchyma, Vb=vascular bundle, Gp = gall parenchyma, NZ= nutritive zone, MZ= mechanical zone, St = starch, Cb= carbohydrates, pr= proteins)

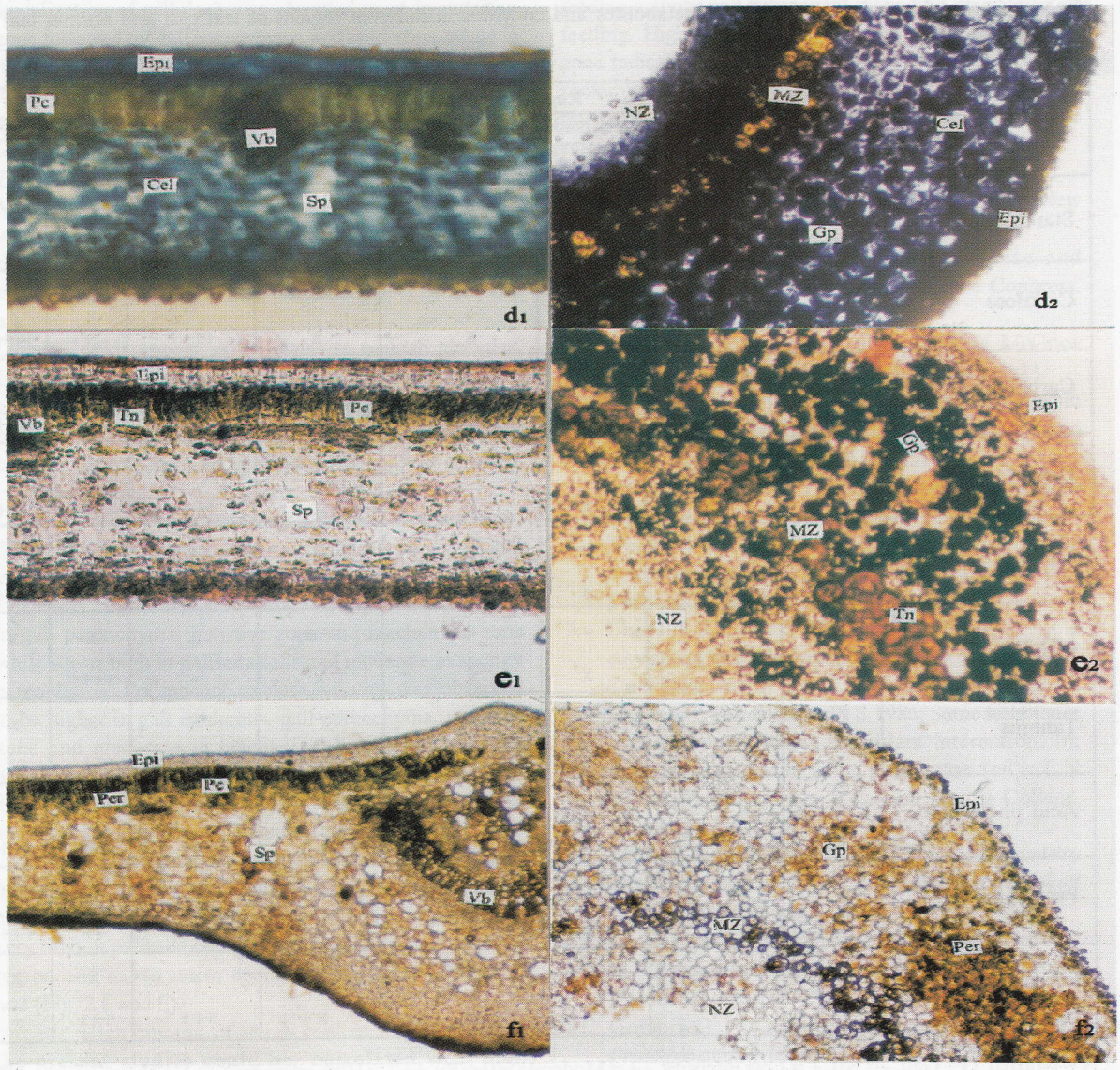

Fig. 2. Localization of metabolites and enzymes in different regions of leaf gall and normal leaf of *Alstonia scholaris*.
d_1 =T.S. of normal leaf showing moderate localization of cellulose in epidermis and spongy parenchyma (x 325)
d_2 =T.S. of gall showing very high localization of cellulose in gall parenchyma and nutritive zone (x 130)
e_1 =T.S. of normal leaf showing moderate localization of tannins in epidermis (x 325)
e_2 =T.S. of gall showing very high localization of tannins in mechanical zone(x 130)
f_1 =T.S. of normal leaf showing very high peroxidase activity in palisade cells (x 325)
f_2 =T.S. of gall showing high peroxidase activity in gall parenchyma and mechanical zone (x 130)
(Epi = epidermis, Pc = palisade cells, Sp = spongy parenchyma, Vb=vascular bundle, Gp = gall parenchyma, NZ= nutritive zone, MZ= mechanical zone, Cel=cellulose, Tn=tannins, Per = peroxidase)

Table 1. Histochemical localization of metabolites and enzymes in different regions of leaf gall and normal leaf of *Alstonia scholaris*(L.)R.Br.

Metabolites	Normal Gall	Regions showing localization						
		Epidermis	Palisade	Spongy Parenchyma	Vascular Bundle	Gall Parenchyma	MZ	NZ
Starch	N	+	+++	+	+	x	x	x
	G	+	x	x	x	++++	+	-
Cellulose	N	++	-	++	++	x	x	x
	G	++	x	x	x	++++	+	++++
Carbohydrates	N	+++	++++	++	++	x	x	x
	G	+++	x	x	x	++++	++	+++
Protein	N	+	++++	++	+	x	x	x
	G	++	x	x	x	++++	++	+++
Lipids	N	++	++	+	+	x	x	x
	G	+++	x	x	x	++	-	++++
Lignin	N	-	-	-	+++	x	x	x
	G	-	x	x	x	-	++++	-
Tannins	N	++	+	+	+	x	x	x
	G	++	x	x	x	+	++++	-
Acid phosphatase	N	-	+++	++	-	x	x	x
	G	+	x	x	x	+++	+++	+++
Peroxidase	N	++	+++	+	+	x	x	x
	G	+++	x	x	x	+++	++	+++
Polyphenol oxidase	N	+++	++++	++++	+++	x	x	x
	G	++	x	x	x	+++	+++	+

N = Normal, G = Gall, - = Nil, + = Low intensity, ++ = Moderate intensity, +++ = High intensity, ++++ = Very high intensity, x = absent, N = Normal leaf, G = Gall, MZ = mechanical zone, NZ = Nutritive zone.

in gall as compared to normal leaf. These were observed in very high intensity in gall parenchyma and in high intensity in nutritive zone and gall epidermis. In normal leaf these were in very high intensity in palisade cells, high in epidermis and moderate in spongy parenchyma and vascular region (Fig. 1 b_1 and b_2).

Protein - Protein was stained blue in colour. It was present throughout the various tissues of normal and galled part except in thick walled tissues like xylem and sclerenchyma. Gall parenchyma and palisade tissue of normal leaf showed very high abundance of protein. It was present in lesser quantity in gall epidermis and very less in the epidermis of normal leaf. Spongy parenchyma showed moderate amount of protein. Increase in protein could be because of more auxin, cytokinin and phenolics. Higher peroxidase activity also play a major role in accelerating protein synthesis[12] (Fig. 1 c_1 and c_2).

Cellulose- Cellulose was stained dark blue to black.

Localization of cellulose was observed more in nutritive zone followed by gall parenchyma and epidermis, while in normal leaf moderate quantity was observed in epidermis, spongy parenchyma and vascular region. Higher incidence of cellulose in the nutritive zone could be correlated to the feeding habit of cecidozoan[13] (Fig. 2 d_1 and d_2).

Tannins- Tannins were stained red orange in colour. Mechanical zone of gall showed high localization of tannin while it was in low intensity in gall epidermis and gall parenchyma and moderate in epidermis and low in palisade cells and spongy parenchyma of normal leaf (Fig. 2 e_1 and e_2).

Lipids- Lipids appear as yellowish to pinkish globules. High and very high intensity of lipids was observed in gall epidermis and nutritive zone and moderate in gall parenchyma, respectively. Moderate intensity of lipid was observed in epidermis and palisade cells and low intensity in spongy parenchyma of normal leaf.

Lignin- Lignin was stained pinkish brown in the tissues. It was localized intensively in mechanical zone of gall and vascular region of normal leaf.

Enzymes- High acid phosphatase activity was observed in gall parenchyma, nutritive zone and mechanical zone while it was high in palisade cells and moderate in spongy parenchyma. Polyphenol oxidase activity was observed to be higher in gall epidermis, gall parenchyma, nutritive zone and moderate in mechanical zone. Similarly the polyphenol oxidase activity was high in palisade cells of normal leaf while it was moderate in epidermis and low in spongy parenchyma and vascular bundle.

High intensity of peroxidase activity was observed in gall parenchyma and mechanical zone, moderate in epidermis and low in nutritive zone. It was very high in palisade cells, high in epidermis and vascular region and moderate in spongy parenchyma of normal leaf (Fig. 2 f_1 and f_2).

High amount of starch content in gall tissue in the present studies could be correlated with high concentration of total soluble sugar. Occurrence of starch in gall parenchyma suggest localization of starch in the form of soluble saccharides diffusing through cortical cells[14]. A significant increase of protein in gall tissues could be due to wounding which is known to accelerate protein synthesis[15]. Feeding activity of cecidozoa in galls leads to increased synthesis of enzymes. Increase in tannins and lignin is attributed to hyperactivity of polyphenol oxidase and peroxidase[16]. Tannins are involved in defence mechanism, wound healing and regulation of growth regulators. Hyperactivity of these enzymes suggests a triggering of their synthesis as a result of feeding activity and subsequent wounding on account of feeding. High acid phosphatase activity in the nutritive zone indicates a higher metabolic activity in the cells in proximity of the cecidozoa suggesting its role in energy transfer mechanism by bringing about hydrolysis of some relevant substances.

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