J. Phytol. Res. 21(2) : 191-196, 2008

LOCALIZATION OF METABOLITES AND ENZYMES IN LEAF OF *SALVADORA PERSICA*

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Salvadora persica (L) is an medicinally important plant. The whole plant is used for preparation of medicine in Ayarveda, Homoeopathy and Unani system of medication. The medical properties of the plant are associated with their chemical constituents. The present study deals with leaf galls caused by unknown *Itonididae* (Diptera). Histochemical studies of normal and gall tissue have been done. The gall tissue showed relatively higher amount of metabolites. Thus, the normal and gall tissue showed histochemially differential behaviour in terms of metabolites. These results indicate that the higher amount of metabolites favour the gall formation due to the insect attack.

Keywords : Enzymes; Histochemical; Metabolites; *Salvadora persica;* Starch.

Introduction

Salvadora persica (family Salvadoraceae), popularly known as "Khara Jhal/Pilu" is a perennial shrub/tree found in saline and drought conditions of Rajasthan, western Uttar Pradesh, Gujarat and Tamil Nadu. Its green branches and roots are used as chewing sticks known as Meswak. The whole plant is used for preparation of medicines in Ayurveda, Homoeopathy and Unani system of medication. The roots have steam-distillable oil that has 90% Benzylisothiocyanate, a compound responsible for decreasing dental caries and used in tooth paste preparation[1]. Leaves has anti-corrugate and astringent properties. Leaf decoction is used in asthma and cough and its juice is given in scurvy[2]. The 2-3 years old fruits are crushed in water and given orally against snake bite for inducing vomiting[3]. The leaf galls of *S. persica* were selected to study the metabolic changes induced by unknown Itonididae (Diptera).

Material and Methods

The normal leaf and leaf galls of *Salvadora persica* were collected from Keola Dev National Park, Bharatpur and their morphology was studied. Fresh hand cut sections of leaf were used for histochemical analysis. The metabolites, starch and cellulose[4], carbohydrates[5-6], proteins[7], lipids[8], lignin[9] and tannins[10], and enzymes *viz.*, polyphenol oxidase[11], peroxidase[12] and acid phosphatase[13] were localized and documented. Their qualitative increase or decrease in localization was assessed in terms of intensity of staining. The degree of distribution of the stain in various tissues was recorded as low (+), moderate (++) and high (+++).

Results and Discussion

Results obtained for localization of metabolites and enzymes in normal and leaf gall tissues are presented in Table 1 and Figs. 1-3.

Starch - Strach granules were observed in palisade parenchyma cells in normal leaf tissue. In gall tissue more starch granules were observed in mysophyll regions and nutritive zone[14] (Fig. 1C and D).

Cellulose - The inner lining of the leaf gall cavity also exhibited a positive reaction for cellulose. It was present in high quantity in nutritive zone and moderate in gall parenchyma[15] (Fig. 1E and F).

Total insoluble Polysaccharides- Very light stain was observed in the cell walls of epidermis, palisade and spongy parenchyma regions. Strong reaction was observed in mesophyll and nutritive zone[16] (Fig. 1G and H).

Lipids - Lipids were mostly localized in the mesophyll tissue of normal leaf and less staining reaction for lipids was observed in the cell walls of epidermis. The leaf gall tissue showed high contents of lipids in the cells of nutritive zone[17] (Fig. 2A and B).

Lignin - Feable staining was observed in epidermis and mesophyll region. Lignin was observed in the cells of epidermis, mesophyll and vascular region of gall. It was surrounding the nutritive zone[18] (Fig. 2C and D).

Proteins - Normal leaf tissue showed the presence of proteins in epidermis and mesophyll region. Very intense staining showed the presence of more proteins in gall regions and also in hairs[19] (Fig. 2E and F).

Tannins - In normal leaf tannins were observed in the mesophyll and vascular region. Tannin filled cells were

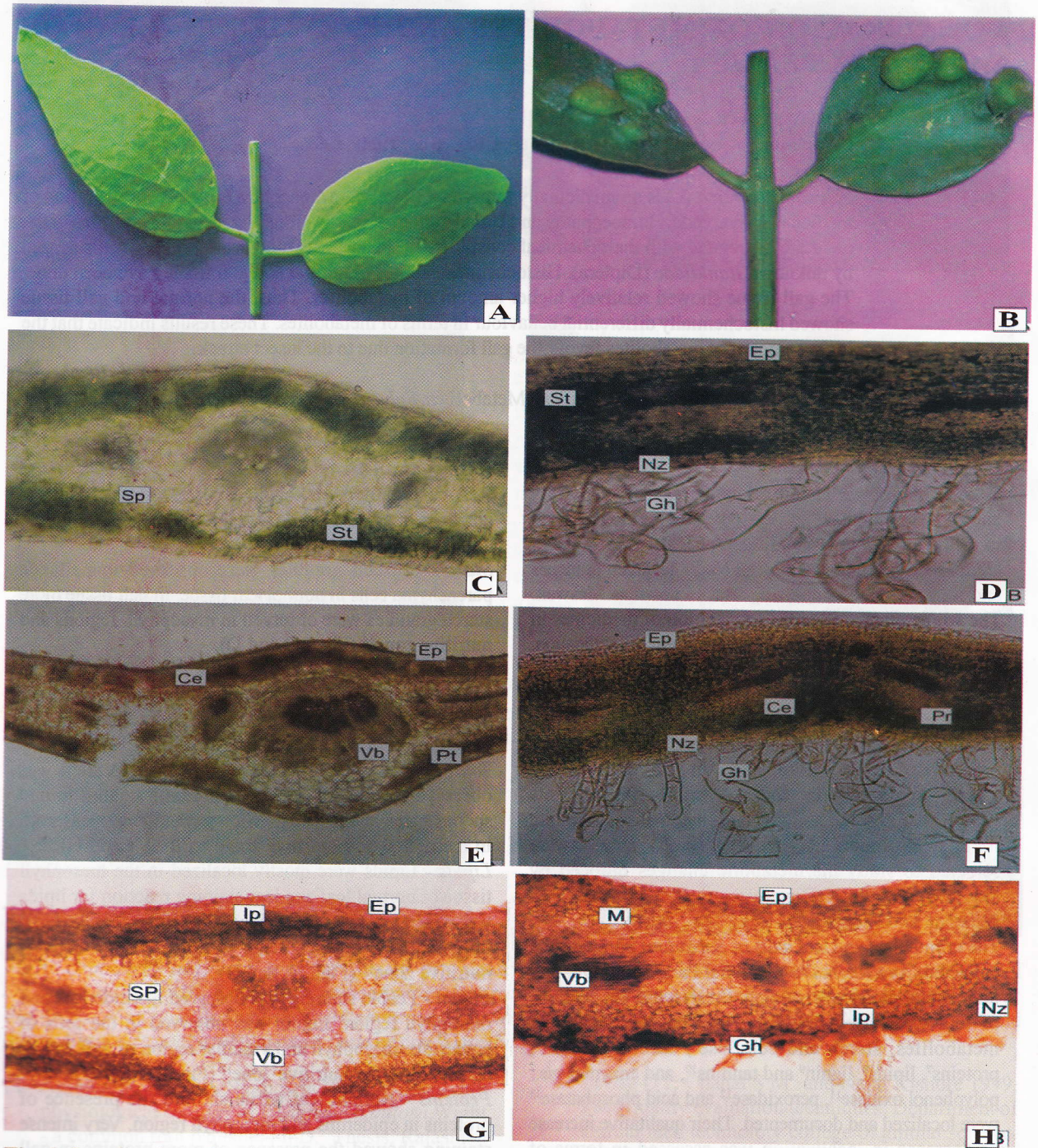

Fig. 1. Localization of various metabolites in normal and leaf gall of *Salvadora persica,* A, C, E, G-normal leaf, B,D,F,H- leaf gall, C and D- localization of starch, E and F- localization of cellulose, G and H- localization of total insoluble polysaccharides.
(Ce=Cellulose, Ep= Epidermis, Gh= Gall hair, Ip=Insoluble polysaccharide, M= Mesophyll, Nz= Nutritive zone, Pr= Parenchyma, Pt= Palisade tissue, Sp=Spongy Parenchyma, St=Spongy tissue, Vb Vascular bundle) C x=200, D x=130, E x=200, F x=200, G x = 370, H x=200

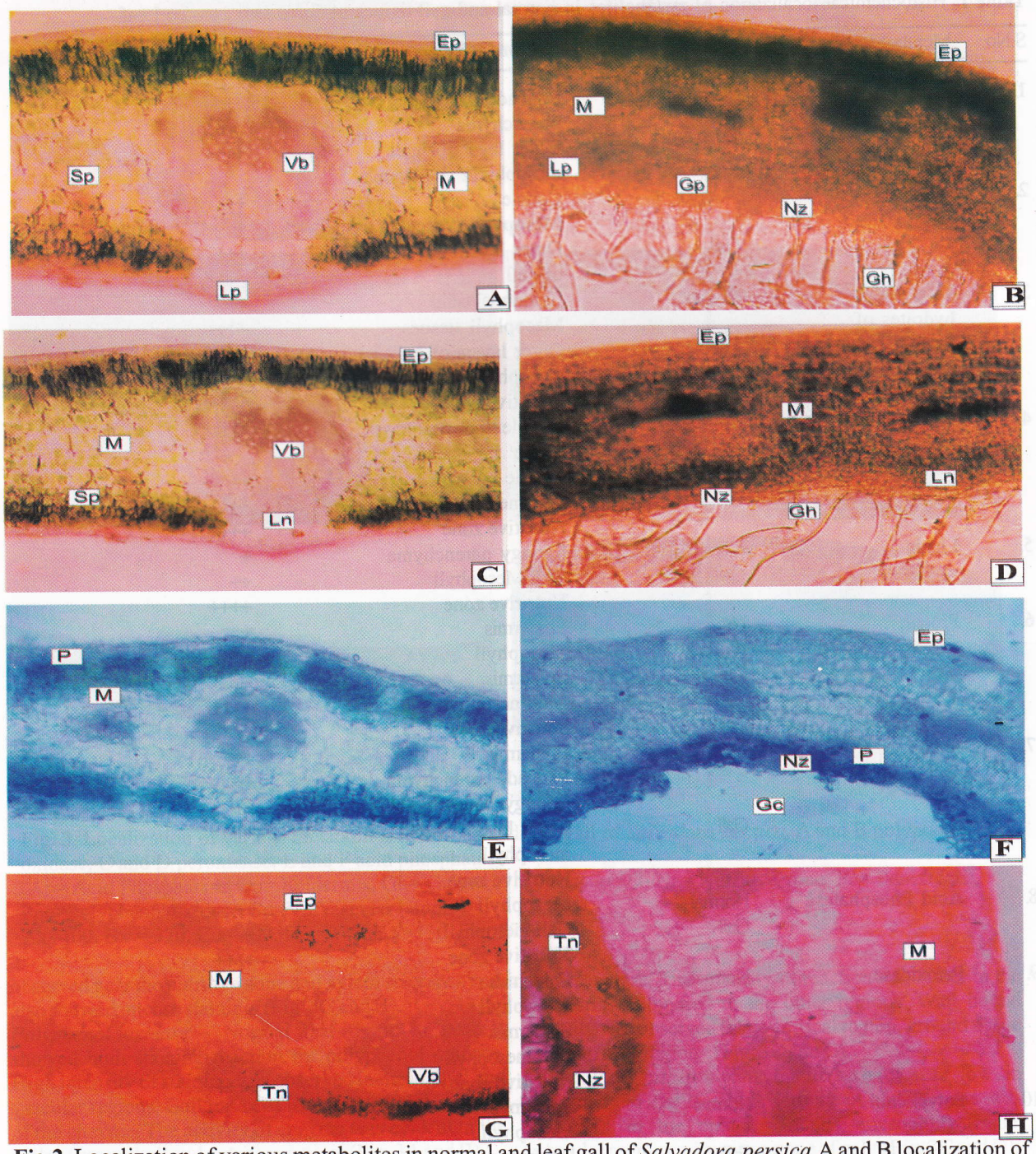

Fig.2. Localization of various metabolites in normal and leaf gall of *Salvadora persica*, A and B localization of lipids, C and Dlocalization of lignin, E and F localization of proteins, G and H localization of tannin.
(Ep= Epidermis, Gc= Gall cavity, Gh= Gall hair, Gp= Gall parenchyma, Lp= Lipid, Ln= Lignin,M= Mesophyll, Nz= Nutritive zone, P=Protein, Pr= Parenchyma, Pt= Palisade tissue, Sp=Spongy Parenchyma, Tn=Tannin, Vb=Vascular bundle)
Ax=370, Bx=130, C x=370, D x=200, E x=370, F x=370, G x = 370, H x=370

Table 1. Histochemical localization of metabolites in normal and gall tissues of *Salvadora persica* Leaf.

S.No.	Metabolite	Normal/Gall	Regions localized	Intensity
1.	Starch	Normal	Palisade and spongy parenchyma	++
		Gall	Outer layers of gall mesophyll/Parenchyma	++++
2.	Cellulose	Normal	Cuticle	++
			Mesophyll, Vascular tissue	+
		Gall	Outer layers of gall	+++
3.	Total carbo-hydrates of insoluble Polysaccharides	Normal	Epidermal cells	++
			Mesophyll tissues	++++
		Gall	Outer layers of gall mesophyll	++++
			Nutritive zone	++++
4.	Lipids	Normal	Cuticle and mesophyll tissue	+
		Gall	Cuticle	++
			Gall mesophyll	++
			Nutritive zone	++++
5.	Lignin	Normal	Spongy parenchyma	+
		Gall	Gall Mesophyll	++
			Nutritive zone	++++
6.	Protein	Normal	Epidermis	+
			Mesophyll	++++
		Gall	Epidermis	+
			Gall mesophyll and nutritive zone	++++
7.	Tannin	Normal	Epidermis	++
			Palisade parenchyma	++
			Spongy parenchyma	++
		Gall	Outer layers of gall mesophyll	++
			Nutritive zone	++++
8.	Acid Phosphatase	Normal	Mesophyll	++
		Gall	Gall mesophyll	+
			Nutritive zone	++++
9.	Peroxidase	Normal	Epidermis	+
			Mesophyll	+
		Gall	Epidermis	+
			Gall mesophyll	+++
			Nutritive zone	++++
10.	Polyphenol oxidase	Normal	Epidermis	+
			Mesophyll	+
		Gall	Epidermis	+
			Gall mesophyll outer layers	++++
			Nutritive zone and inner layers of gall mesophyll	+++

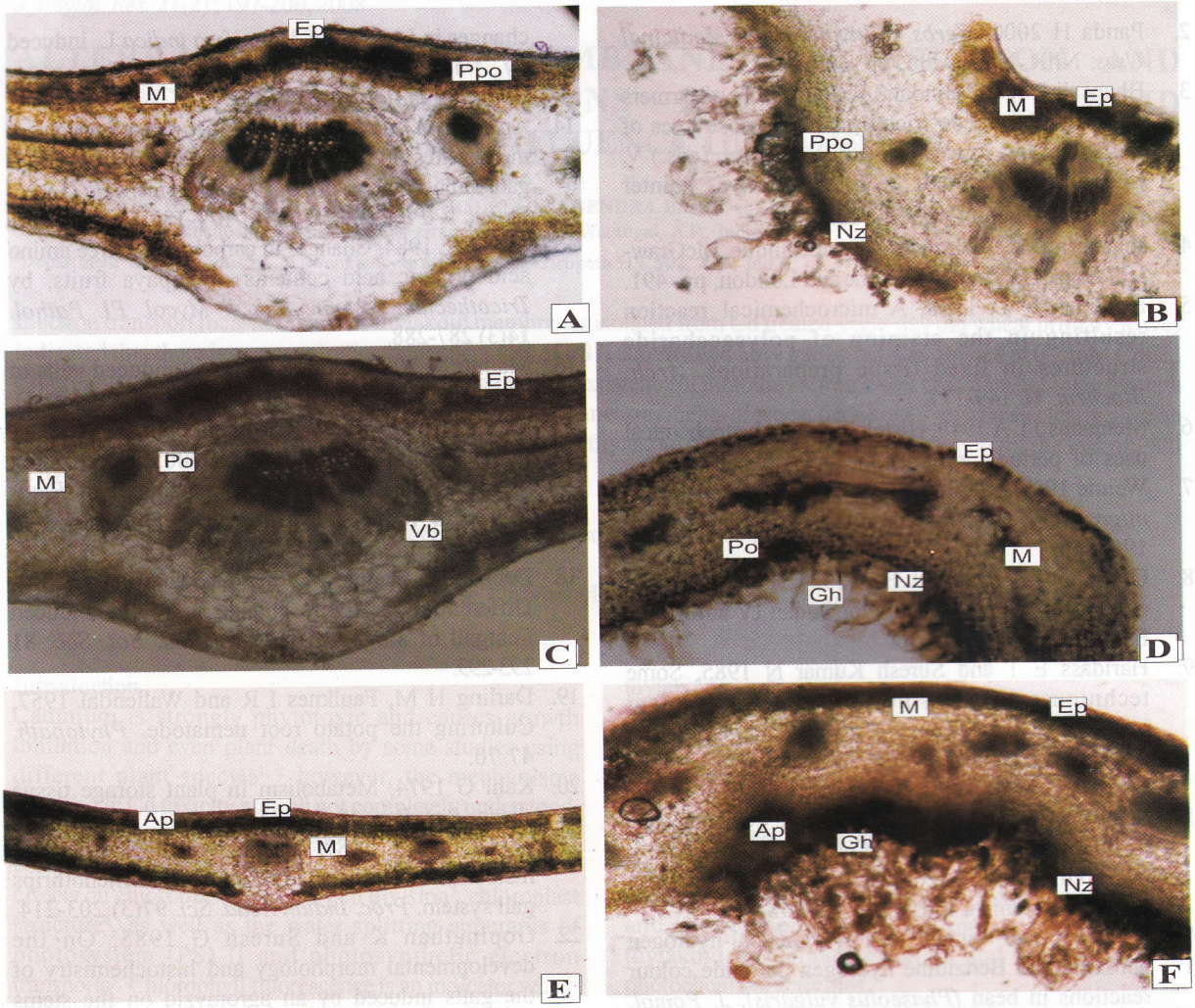

Fig. 3. Localization of various metabolites in normal and leaf gall of *Salvadora persica,* A and B polyphenol oxidase, C and D peroxidase, E and F acid phosphatase
(Ap= Acid Phosphatase, Ep= Epidermis, Gh= Gall hair, M= Mesophyll, Nz= Nutritive zone, Po= Peroxidase, Ppo=Polyphenol oxidase, Sp=Vb Vascular bundle)
Ax=400, Bx = 370, C x=400, D x=370, E x=130, F x=370.

observed near the nutritive zone and xylem sclerenchyma also showed intense staining[20] (Fig. 3G and H).

Polyphenol oxidase - Normal leaf tissue in general showed high staining reaction for polyphenol oxidase. High intensity of staining was observed in mesophyll and vascular regions. Gall tissue of leaf showed high activity of the enzyme. The activity of enzyme was evident in the cells of mesophyll and nutritive region[21] (Fig. 3A and B).

Peroxidase - High peroxidase activity was evident in the normal leaf tissue, mainly in the cells of palisade and spongy region. Vascular bundles showed high intensity of the stain. Leaf gall tissue showed a very strong reaction of peroxidase. The intensity of reaction was lesser in the epidermis compared to the other regions. Wall bound activity was observed in the cortical region, ground tissue and nutritive region[22] (Fig. 3C and D).

Acid Phosphatase - Enzymatic activity was low in normal Leaf tissue. High activity of the enzyme was observed in the epidermis cells, mesophyll tissue, vascular region and nuritive zone of gall[23] (Fig. 3C and D).

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