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Localization of Metabolites and Enzymes in Stem Galls of *Terminalia arjuna*

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Abstract : Histochemical localization of metabolites and enzymes was studied in stem gall of *Terminalia arjuna* (Linn.) induced by unknown *Itonididae* (*Diptera)*. These studies revealed higher activity of various metabolites in gall tissue, especially near the nutritive zone. Relatively higher amount of metabolites sugested altered metabolism of the host tissue due to Pathogenesis. A functional elaboration in the cells closer to the feeding site of the cecidozoan during cecidogenesis was evident. Also, a different response of metabolites and enzymes at cellular level of the host proved advantageous to the insect toward gall formation. Most of the plants of *Terminalia arjuna* were found to be heavily galled during the months of March to June.

Key words : Enzymes; Histochemical; Stem gall; Metabolites, *Terminalia arjuna*.

Introduction

Terminalia arjuna is a tree (family combretaceae). The bark is used in certain herbal combinations as a powerful, soothing tonic for the heart. It is good for both the physical heart as a muscle, as well as for the emotions associated with the heart. Arjuna is used for loneliness, sadness and frustration. It strengthens the emotions to decrease excessive response to stress and trauma. It helps in strengthening the body's natural rejuvenative processes, hastening the replacement of dead or weak cells with fresh, vital ones. In proper combinations, Arjuna helps to stabilize an erratic heart beat.

The Vedas and Puranas refer various materials of medical importance including herbs, plants and trees. Modern research has discovered that Arjuna has antioxidant properties and may be clinically helpfull in cardiovascular health.

The anti bacterial activity of crude drug form the tree bark of *T. arjuna* was tested against bacteria. The bark of *T. arjuna* is astringent and is used in fevers, fractures and contusions; it is also taken as cardiac tonic. Clinical evaluation of this botanical medicine indicates that it can be of benefit in the treatment of coronary artery disease, heart failure, and possibly hypercholesterolemia. It has also been found to be antibacterial and antimutagenic (Perumalsay and Ignacimuthu, 2001; Sivalokanathan *et al.*, 2004). *Terminalia's* active constituents include tannins, triterpenoid, saponine (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoides (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCS), phytosterols, calcium, magnesium, zinc and copper (Sivalokanathan *et al.*, 2004).

The possible alteration in metabolic activity caused by insect attack was studied

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by histochemical localization of metabolites and enzymes in stem galls of *T. arjuna* caused by unknown *Itonididae (Diptera)*. Galls are globose, sub-globose or oval, single or compound and indehiscent. Young galls are brownish yellow in colour. But mature galls turn dark brown. Galls develop on lateral branches. In case of severe infection the whole branch is replaced by an agglomerated mass of galls. Each gall possesses a single, centrally placed larval cavity. A small ostiole communicates the larval cavity with the exterior of the gall. The ostiole lies at the tip of the gall and is clearly visible in very young galls (Fig.1A, B).

Materials and Methods

The normal stem and stem galls of *Terminalia arjuna* were collected from western Uttar Pradesh (Dist. Mathura), Eastern Rajasthan (Dist. Bharatpur) and adjoining areas, and their morphology was studied. Fresh hand cut sections of stem were used for histochemical analysis. The metabolites, starch and cellulose (Johansen, 1940), carbohydrates (Hotckiss, 1948;

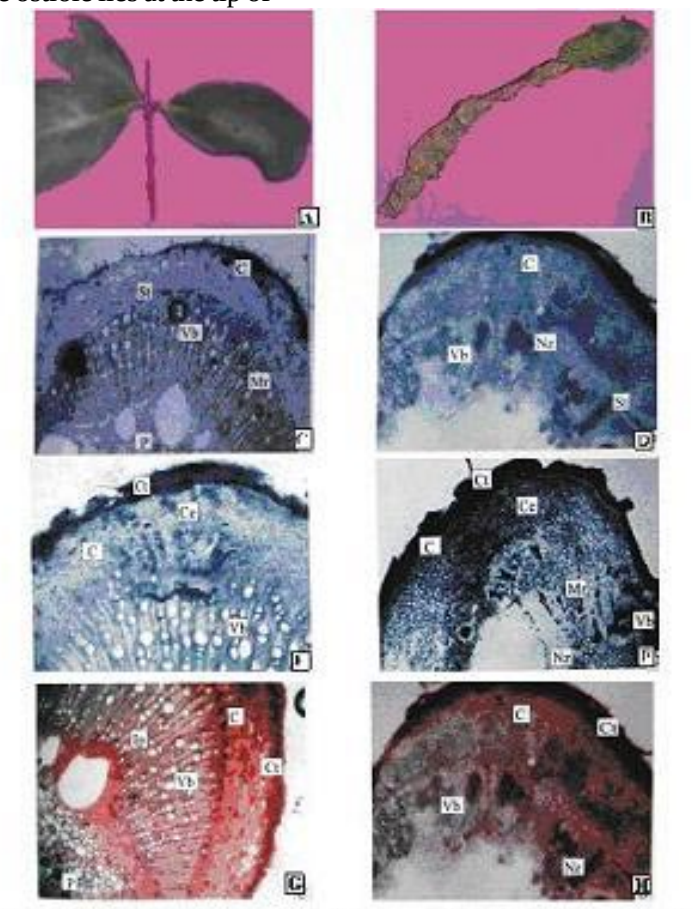

Fig. 1 : Localization of various metabolites in normal and stem gall of Terminalia arjuna A,C,E,G-normal stem, B,D,F,H- stem gall, C and D- localization of starch, E and F-localization of cellulose, G and H-localization of total Insoluble polysaccharides.

(C=Cortex, Ce=Cellulose, Ct=Cork tissue, IP=Insoluble polysaccharide, Mr=Medullary rays, Nz=Nutritive zone, P=Pith, St=Starch , Vb=Vascular bundle); Cx 370, Dx 200, Ex 370, Fx 150, Gx 370, Hx 200

Mcmanus, 1948), proteins (Weime, 1959), lipids (Chiffelle and Putt, 1951), lignin (Johansen, 1940) and tannins (Haridass and Suresh Kumar, 1985), and enzymes *viz.*, polyphenol oxidase (Sexton and Hall, 1978), peroxidase (Isaac and Winch, 1947) and acid phosphatase (Gomori, 1952) were localized and documented. Their qualitative increase or decrease in localization was assessed in terms of intensity of stainin. The degree of distribution of the stain in various tissues was recorded as low (+), moderate (++) and high (+++).

Observations and Discussion

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

Observations and Results

Starch- In cortex, medullary rays and pith cells of normal stem high intensity of starch was observed. It was present in high quantity in nutritive zone, more in vascular region and less in cortex parenchyma cells of stem gall tissues (Fig. 1C and D). Presence of starch near the gall cavity suggests that the insect may be utilizing starch as food material as such. Starch present in nutritive zone suggests a possible diffusion of soluble saccharides produced by starch hydrolysis (Karnawat and Kant, 1990).

Cellulose- Cork and cortical cells of normal stem tissues showed the presence of cellulose. Strong staining reaction for cellulose was observed in cork, sclerenchyma, medullary rays and feable staining was observed in nutritive zone of the stem gall tissues (Fig. 1E and F). High amount of cellulose in gall tissue has been reported in *Prosopis cineraria* rachis gall (Arora and Patni, 2001).

Total insoluble polysaccharides - Cork, cortical region, phloem cells and pith region of normal stem showed the positive reaction to the stain. Cork and nutritive region of stem gall showed very strong reaction to the stain. Cortical region of stem gall showed feable presence of total insoluble polysaccharides (Fig. 1 G and H).

Lipids - In normal stem tissues lipids were mostly localised in the cork tissue. Feable staining reaction for lipids was observed in the cells of xylem and pith region. The stem gall tissues showed strong reaction in outer cork region, cortex and cells of the nutritive zone (Fig. 2A and B). Abundance of lipids in the nutritive region could be related to the continuous wounding as a result of feeding activity of the cecidozoa which alters the metabolic pathway. These lipids are, in turn utilized by the insects for survival. Similar findings have also been reported by several workers (Vyas, 1984; Debnath *et al*., 2002).

Lignin - In normal stem cork, cortex, parenchymatous cells and vascular regions showed positive stain. The cells of vascular region took green stain signifying the presence of cellulose together with lignin in normal stem tissues. Very high intensity of lignin was observed in all stem gall regions, mainly in nutritive zone and cork cells (Fig. 2C and D).In general, parenchymatous cells showed less amount of lignin because infection with any pathogenic agent might have delayed the process of lignification in cortical and pericycle region (Darling *et al.*, 1957).

Proteins - Almost all the tissues in normal stem showed positive staining. High intensity of proteins were observed in cortical zone. The nutritive zone and vascular region of stem gall showed very high intensity of proteins where as it was moderate in cortical and epidermal cells (Fig. 2 E and F). In increased incidence of proteins, the maximum amount was noticed in nutritive tissues which helps the cecidozoan in its growth and development. Wounding is known to accelerate protein synthesis (Kahl, 1974).

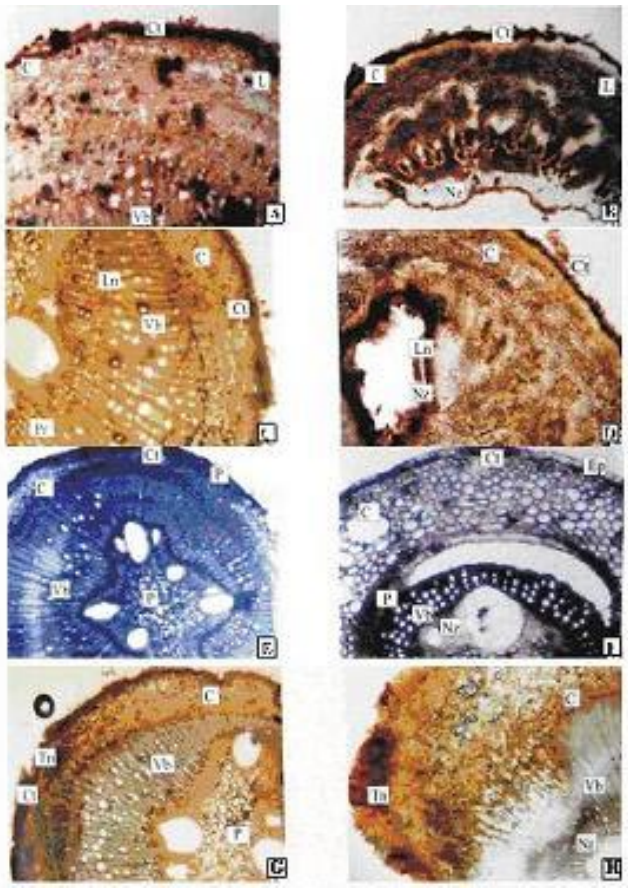

Fig.2. Localization of various metabolites in normal and stem gall of *Terminalia arjuna* A,C,E,G-normal stem, B,D,F,H- stem gall, A and B- localization of Lipids, C and D- localization of Lignin, E and F-localition of Proteins, G and H-localization of Tannins.

(C=Cortex, Ct=Cork tissue, L=Lipid, Ln=Lignin, Vb=Vascular bundle, Nz=Nutritive zone, P=Protein, Tn=Tannins); Ax=130, Bx=130, Cx=200, Dx=130, Ex=370, Fx=400, Gx=370, Hx=200

Tannins - In normal stem tannins were observed in cork, cortex, vascular and pith regions. High amount of tannins were observed in cortical region and tannin filled cells were observed near the nutritive zone of stem gall. Vascular region of stem gall showed high intensity of tannins (Fig. 2G and H). An increased amount of tannins in gall tissues could be attributed to the higher incidence of polyphenol oxidase and peroxidase activity. Similar observation was made by Gopinathan (Gopinathan, 1987) in thrips induced leaf gall of *Mimusops.*

Polyphenol oxidase - Cork, cortex and vascular region of the normal stem showed high staining reaction for the enzyme. High intensity of enzyme activity was seen in nutritive region of stem gall tissues (Fig. 3A and B).

Peroxidase - Very high activity of peroxidase enzyme was seen in cortex, vascular region and pith cells of normal stem. It was moderate in cortical region and very high in nutritive zone of stem gall tissue. The intensity of enzyme action was lesser in ground tissue as compared to other regions of stem gall (Fig. 3C and D). Higher activity of these

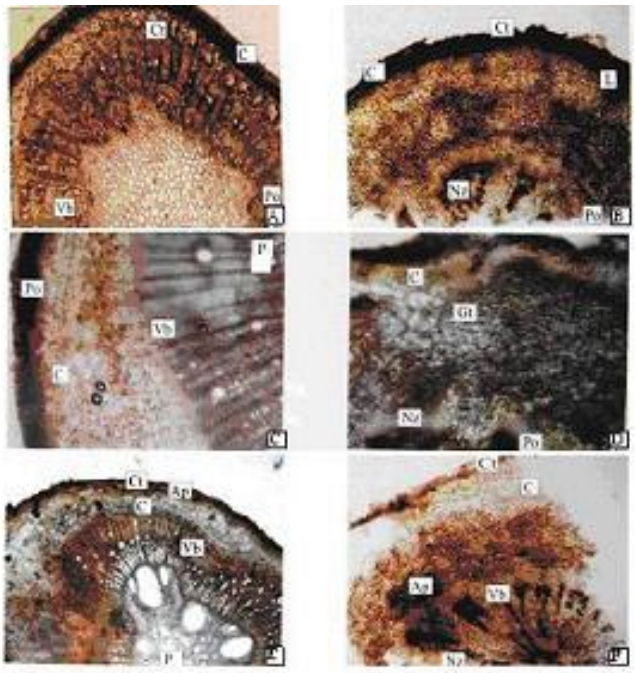

Fig.3. Localization of various metabolites in normal and stem gall of *Terminalia arjuna* A,C,E normal stem, B, D, F stem gall; A and B showing activity of Polyphenol oxidase; C and D showing activity of peroxidase; E and F showing activity of Acid phosphatase.
(Ap=Acid phosphatase, C=Cork, Ct=Cork tissue, Gt=Ground tissue, Nz=Nutritive zone, Po=Peroxidase, Po=Polyphenol oxidase, Vb=Vascular bundle); Ax=370, Bx=130, Cx=370, Dx=200, Ex=370, Fx=130

enzymes in gall tissues has been observed in several insect induced galls (Debnath *et al.*, 2002; Gopinathan, 1987). An increased activity of polyphenol oxidase leads to the stimulation of auxin activity in plants which is well substantiated by maximum hyperplasy seen in gall tissues.

Acid Phosphatase - Presence of moderate enzyme activity was observed in cork and feable presence of the enzyme was observed in cortex, vascular region and pith cells of normal stem tissues. Strong activity was shown in stem gall tissues, mainly in the nutritive region where it was very high. Cork and cortex tissues showed low activity of the enzyme in stem gall (Fig. 3E and F). This enzyme might be directly or indirectly linked with energy transfer mechanism by bringing about hydrolysis of some suitable substances, for example, sugar phosphate for making available phosphate groups. An increase in rate of growth of plant tissues is likely to be advantageous to insect feeding on them primarily due to an increased mobilisation of metabolites. High activity of acid phosphatase in nutritive regions has also been reported by Gopinathan and Suresh (Gopinathan and Suresh, 1985) and Gopinathan and Ananthakrishnan (Gopinathan and Ananthakrishnan, 1986).

Table 1 : Histochemical localization of metabolites in normal and gall tissues of *Terminalia arjuna* stem

S.No.	Metabolite	Normal/Gall	Regions localized	Intensity
1	Starch	Normal	Cortex	++
			Vascular region	++
			Pith	+
		Gall	Cortex	++
			Vascular region	++
			Nutritive zone	++++
2	Cellulose	Normal	Cork, Cortex	+
		Gall	Cork	++
			Nutritive zone	+
			Vascular region	+
3	Total carbo-hydrates of insoluble polysaccharides	Normal	Cortex, Cork	+
			Vascular region	+
			Pith	+
				+
		Gall	Cortex, Cork	+++
			Nutritive zone	++++
4	Lipids	Normal	Cork	+++
			Xylem	+
			Pith	+
		Gall	Cortex	++
			Cork	++
			Nutritive zone	+++
5	Lignin	Normal	Cortex	+
			Vascular region	++
		Gall	Cork	++
			Nutritive zone	+++
6	Protein	Normal	Cortex	++
			Vascular region	+++
		Gall	Cortex	+
			ritive zone	++++
7	Tannin	Normal	Cork, Cortex	+
			Vascular region	++
			Pith	++
		Gall	Cortex	+++
			Nutritive zone	+++
8	Acid Phosphatase	Normal	Cortex, Cork	+
			Vascular region	+
			Pith	+
		Gall	Cortex	+
			Nutritive zone	++++
9	Peroxidase	Normal	Cortex	+
			Vascular region	++
			Pith	+
		Gall	Cortex	+
			Nutritive zone	+++
10	Polyphenol oxidase	Normal	Cork, Cortex	++
			Vascular region	+++
		Gall	Cortex	+
			Nutritive zone	+++

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