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HISTOCHEMICAL INVESTIGATIONS OF LEAF GALLS OF *TECTONA GRANDIS* LINN. INCITED BY AN UNKNOWN MIDGE FROM SITAMATA FOREST—RAJASTHAN

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Histochemical investigations were made on leaf galls of *Tectona grandis* Linn. caused by an unknown gall midge. The galls were discoid, clothed with multicelled thick walled acute trichomes and exhibited marked difference in the histology between gall and normal tissues. Distribution of various metabolites and activities of enzymes provide a clear pointer alteration that the chemicals of insect origin and their interactions with the chemicals of the host plant, those of normal existance and those induced as a response of midge invasion, mediate and regulate gall insect host relationships during cecidogenesis.

Keywords: Enzymes; Histochemistry; Metabolites; *Tectona grandis;* Trichomes.

Introduction

This paper deals with leaf galls on *Tectona grandis* collected from Sitamata forest cum sanctuary, Rajasthan. This forest is occupied by Aravalli system joined in by Malwa plateau called the Deccan trap. It covers the older formations like Pre Aravalli gneisses, Aravalli and Vindhyans. The Teak (*Tectona grandis*) is the main tree species and forms about 70% of the total area. The leaf gall is wholly hypophyllous but with a conspicuous epiphyllous chlorotic depression. A gall arises as a hemispherical covering growth with median larval cavity. A cup shaped meristmatic zone develops around the larval cavity. The sclerotic zone extends down as a central pillar like axis (Fig. 1). Jayaraman[1] has studied the anatomy of this gall. The present observation is to understand the changes in metabolites and enzymes which lead to the development of this neoplastic growth.

Material and Methods

Gall and normal tissues from leaves of *Tectona grandis* Linn. were collected from Sitamata forest, in the months of October and November. The specimen was deposited in the Department of Botany, M.L.V. College, Bhilwara. The following standard histochemical methods were used to localize the proteins, (Mercuric Bromophenol)[2], Lipids (Sudan III dye)[3], Starch (IKI reaction)[4], Tanin (Lugol's Iodine)[5], Polyphenol oxidase[6], Peroxidase[7], and Acid phosphatase[8]. Their qualitative increase was assessed in terms of intensity of staining and the degree of distribution of the stain in the concerned tissues. Adequate controls were also run.

Results and Discussion

Histochemical intensity of various metabolites and activity of enzymes in normal and gall tissues is shown in Table-1. Gall tissues showed a higher concentration of proteins in comparison to normal tissues. In normal leaf tissues high concentration of proteins was localized to mesophyll tissues, hairs and epidermal cells. Large amount of proteins was localized near nutritive zone and meristematic zone in mature and old gall tissues. The normal tissues exhibited a very sparse stain for lipids but in gall tissues lipids droplets were localized in cell layers close to the larval cavity, mechanical region and trichomes respectively. Starch was observed in the vascular bundles of the normal leaf tissues but in gall tissues it was localized around the nutritive region, meristematic zone

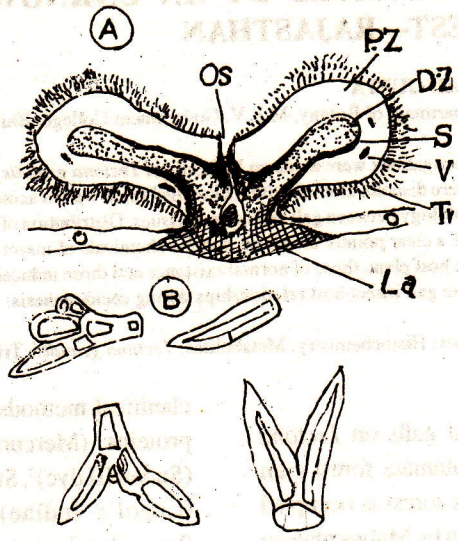

Fig. 1-A Vertical section of a mature gall.
Fig. 1-B Some representative samples of trichomes of the gall region.
(DZ - Dark cell zone (Mechanical zone); SZ = Sclerenchyma zone; VS = Vascular strand; Tr = Trichomes; PZ = Parenchyma zone; La = Larva; OS = Ostiole)

and upper part of the multicellular glandular hairs. Tannins were localized in stalked glandular hairs and trichomes in normal tissues, but in the gall region very high intensity was observed in trichomes as well as mechanical zone and vascular region. The Enzymatic activity of polyphenol oxidase, peroxidase and acid phosphatase were definitely higher in the cells around gall cavity (nutritive zone) as compared to the gall sclerenchyma zone.

Due to feeding activity of the midge the leaf galls of *Tectona grandis* Linn. arise as a hemispherical covering growth with a median larval cavity. The cells abutting the meristematic zone differentiated into a thin layer of sclerenchyma zone and this zone extends down as a central pillar like axis inside the stalk. The surface of the gall is densely clothed with short, stumpy, multicellular (few unicellular) branched hairs with extremely thick lignified walls with canals like pits (Fig. 1-B). In general, the gall tissues, especially those of the nutritive zone, meristematic zone and the associated surface growths (trichomes) showed a hyperactivity of proteins, essentially help the insect in its growth and development. This increased intensity of proteins can be correlated with increased peroxidase activity[9]. Increased protein accumulation in response to wounding has been reported[10]. Westphal[11], Kant *et al*[12], Kanika and Kant[13] also reported high concentration of protein in gall tissues of some insect and mite induced galls. Lipids, too occur

Table 1. Histochemical localization of metabolites in normal and gall tissues of *Tectona grandis* Linn. leaf gall.

S.No.	Metabolites	Normal/gall	Regions localized	Intensity
1.	Protein	Normal	Epidermis	+
			Multicelled epidermal hair	++
			Mesophyll	+++
		Gall	Multicelled trichomes	++
			Meristmatic zone	++++
			Sclerenchyma zone	++
			Nutritive zone	++++
2.	Lipids	Normal	Mesophyll tissue	++
		Gall	Multicelled trichomes	++
			Nutritive region	+++
			Sclerenchyma region	+
3.	Starch	Normal	Epidermal hair	+
			Bundle sheath	+
			Vascular bundle	+++
		Gall	Meristmatic zone	++
			Nutritive zone	++++
			Hair	++
4.	Tannin	Normal	Stalked glandular trichomes	+++
			Acute multicelled hair	++
		Gall	Nutritive zone	++
			Sclerenchyma zone	+++
			Trichomes	+++
			Vascular region	++
5.	Peroxidase	Normal	Mesophyll	++
			Trichomes	+
		Gall	Trichomes	+
			Nutritive zone	+++
			Mechanical zone	+++
6.	Polyphenol oxidase	Normal	Palisade zone	++
		Gall	Nutritive zone	++++
			Sclerenchyma zone	++
			Trichomes	++
7.	Acid Phosphatase	Normal	Mesophyll	++
			Hairs	++
		Gall	Nutritive zone	+++
			Mechanical zone	++
			Trichomes	++

+ = Very low intensity; ++ = Low Intensity; +++ = High Intensity; ++++ = Very high Intensity.

in the nutritive tissues, could be related to the continuous wounding as a result of feeding activity of cecidozoa. Feeding zone which in turn was utilized by the midge to maintain their life activities over a considerable period. The lipids that occur in the nutritive cells are essentially a mixture of non-saturated triglycerides and oil bodies[14]. The increase in

lipids in gall tissues in nutritive cells has also been reported in several gall systems[13-16]. With the maturation of leaf gall the accumulation of starch in the nutritive tissues and meristematic zone occur during the later stages of ontogeny suggests that starch hydrolysed into soluble polysaccharides was mobilized to the regions of midge feeding site where they were converted into monosaccharides, the form used by feeding larvae[17].

Tannins represented an important class of quantitative defense substances reducing the digestibility of plant carbohydrates and protein. High tannins localization in trichomes and sclerenchyma zone suggested that formation of phenolic substances was under the control of gall maker to protect themselve from the other predatory organism as well as defensive response of gall system[18].

Increased activity of peroxidase and polyphenol oxidase was evident throughout the gall tissues in all stages of gall development, particularly in the nutritive cells and in the cells, far away suggesting the synthesis of these enzymes being hastened by the wounding or feeding stimulus of midge within a gall[15]. The enzyme acid phosphatase in the gall tissue remained confind to the nutritive region in all the stages of gall development, indicates a higher metabolic activity in the cells very near to the site of insect incidence as found in thrips galls[13-15].

Increased activity of these oxidative enzymes in gall tissues particularly in the feeding zone leads to higher auxin activity, increase in growth and metabolism and accelerated protein synthesis. Thus there is mobilization and accumulation of active metabolites in the gall tissues which are then utilised by the gall inducer as reported by several workers during cecidogenesis[13,16,18].

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