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Development of specialized tissues in the foliar galls of *Quercus leucotrichophora* induced by cecidomyiid: Synchronization of nutritive and enemy hypotheses

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ABSTRACT

Based on scanning and transmission electron microscopy and light microscopy this paper describes the structural and histochemical features of leaf galls of Quercus leucotrichophora A. Camus of Himalayan region of India. These galls are induced by Cecidomyiid (unknown Itonideae). These studies emphasize that the development of a gall is not totally depend on both, insects' nutrition and the mode of protection it needs from the enemies. In the development process some tissues develop to nourish the larva like parenchymatous and nutritive tissues. Nutritive tissues showed deposition of granular food grains. On the other hand sclerenchymatous layers and trichomes protect the insects' progeny from the external environment. These barriers are required to let successfully complete the life cycle of the insect. Outer and inner sclereids make the gall tougher till the pupal stage of the cecidomyiid. Afterwards helps in the dispersal of the adult insect and complete the life cycle. Thus at some stages development of specialized tissues wholly controlled by the nutritive stage of insect(nutritive hypothesis), simultaneously some tissues develop to protect insects' progeny from herbivores and parasites (enemy hypothesis). Synchronization of these factors at times helps in the development of a complex anatomy of the cecidomyiid leaf galls of Q. leucotrichophora.

Keywords: Sclerenchyma, insect, protection, life cycle.

INTRODUCTION

Insect induced galls are formed as a consequence of mechanic and chemical stimuli produced by inducing insects which stimulate, via saliva enzymes and/or hormones, tissue hypertrophy and hyperplasia on host plants, thus originating distinct structures with high specificity due to the gall maker insect host plant interaction [1]. Almost all the gall inducing 'insects' are host, organ and tissue specific [2] and they take advantage of the fundamental property of the plant to react, whereby even the differentiated tissues could be converted into meristematic state. The host's response to the feeding or ovipositional stimulus is something unique so that the plant morphogenetic responses are altered. Therefore, gall formation is the climax stage inthe insect-host plant relationship, as this principally involves a highly specialized host preference and selection on the part of the insect and a number of adaptive phenomena on the part of the host plant. Different gall-insect systems have been studied by various researchers[3][4]. The gall making habit is found in six orders of insects: Coleoptera (beetles),Lepidoptera (moths

and butterflies), Homoptera (aphids), Thysanoptera (thrips), Diptera(midge), and Hymenoptera (sawflies and wasps). The order Diptera has the largest number of gall making insects. Felt [5] listed 701 species of which 682 were in cecidomyiidae. The Hessian fly, chrysanthemum midge, pea midge and clover leaf midge are the best known of the cecidomyiid. Many insect groups and an estimated 13,000 species include plant galls, within which the insect feeds and involve active differentiation and growth of plant tissues [6].

Several hypotheses for the adaptive nature of galls have been proposed including defense from natural enemies, protection from the external environment, expansion of the surface area available for consumption, and enhancement of the nutritional quality of plant tissue [7]. In its broadest form, the nutrition hypothesis for the nature of galls states that insects control the nutrient levels in galls for their own benefit [8]. The microenvironment hypothesis states that gall tissues act to protect the gall-inducer from unfavorable abiotic conditions, particularly desiccation [9,10]. The enemy hypothesis maintains that galls protect gall-inducers from attack by natural enemies. Galls do provide some protection against attack by non-specialist predators and pathogens [11]. Structural and physiological significance of different plant galls have been critically reviewed [12, 13].

An imbalance arising because of the stress induced by the physical action (wounding, sucking) and salivary secretions either triggers new growth because of synthesis of growth promotors or enhances the vulnerability of plant cells to growth promoters that are already present at that site. Such an imbalance results in a 'combined' function of different growth promoters (e.g. auxins and kinins), which activates growth. The Hartley hypothesis [14](1999)builds on the premise that insect-induced galls accumulate large quantities of phenolic compounds, subsequently showing role of auxins in gall development. Phenolic compounds promote cell division and gall growth by interacting with existing plant hormones (IAA)and/or IAA-oxidase, which are usually abundant in meristematic tissues. Galls offer the best opportunity to reconstruct steps in a modified, but geometrical biological structure that arises solely through the trigger messages received from an alien organism viz. the insect.

Therefore, the understanding of the variation in gall morphology is very important. The present study comparatively describes need of development of specialized tissues in leaf galls on *Quercus leucotrichophora* induced by cecidomyiid.

MATERIALS AND METHODS

The study area (Uttranchal, Central Himalaya) was located between 79°23' and 79°42'E, and 29°20' and 29°30' N. The altitude ranges between 1300 and 2600 meter above sealevel. Samples of *Quercus leucotrichophora* (gall and normal counterparts) were collected from within a 3 km radius of forest from youth hostel, Mussoorie (Uttranchal). Samples of different developmental stages of galls and normal leaves were fixed in FAA (37 % formaldehyde: acetic acid: 50 % ethanol, 1:1:18 v/v) for anatomical studies.

Scanning electron microscopic studies

The mature leaf gall was used for scanning electron microscopy. The gall was cut by the attached part and washed with distilled water. Then the specimen was washed with sodium phosphate buffer (0.1 M, pH 6.8), dehydrated in ethanol water series (30%, 50%,70% and 90%- 5 min. each). The specimen was critical point dried under a CO2 atmosphere for 20 min. Mounting was done coated with 90 Å thick gold-palladium coating in a polar on SC 7640 sputter coater (VG Microtech, East Sussex, TN22, England) for 30 min. Coated samples were viewed with Leo-4351 (Leo Electron Microscopy Ltd., Cambridge, UK).

Transmission electron microscopic studies

Transmission Electron Microscopy was performed in galls fixed in gluteraldehyde(2.5%) and paraformaldehyde (4%) buffered with sodium phosphate buffer (0.1 M, pH 6.8).Then fixation was done in Karnovsky's solution overnight at 4º C. Then these were washed in fresh buffer 2-3 times, followed by half an hour change with distilled water. Specimens were post fixed for 2 hours in Osmium teteraoxide (1%) in the same buffer for 2 hours at same temperature. After several washings in buffer the specimens were dehydrated in graded acetone series (30%, 50%, 70%, 80% and 90%) at 4º C and finally in dry acetone for one hour at room temperature. Clearing of specimen was done with toluene at room temperature for 1-2 hours.

Infiltration with toluene and resin series at room temperature in vacuum was done. Resin contained Araldite CY 212- 20%, DDSA-18%, MNA-2%, DNP 30-0.8%.Tolueneand Resin series (3:1, 2:2, 1:3, 0:4) was applied for 2-4

hours. Embedding and polymerization at 50º to 60º C for 12 and 24 hours in oven was done. Then blocks were ready to be cut. Ultra-thin sections of 60-90 nm thickness were cut using an ultra cut-E., Ultra microtome and the sections were stained in alcoholic uranyl acetate (10 min.) and lead citrate (10 min.) before examining the grids in a transmission electron microscope (Morgagne 268 D TEM, Fei Company, The Netherlands) opened at 60-80 KV [15](David *et al*., 1973).

Histochemical localization of various metabolites and enzyme activities was carried out. Fresh hand cut sections of gall and normal counterparts of leaf were used for the localization of various metabolites.

RESULTS AND DISCUSSION

Normal leaves of *Q. leucotrichophora* were dorsiventral with stomata on the lower side, leathery, dull green, white woolly beneath, ovate-lanceolate, serrate etc. Only few bunches of trichomes were present on lower epidermis. One to many leaf galls were found on abaxial side and sometimes on adaxial surface. The galls were variable in size (≈ 2-10 mmdiameter) and were mostly two chambered/bilocular (Fig.1 C & D). Scanning electron microscopic studies showed the dense covering of trichomes on the surface of gall (Fig. 1A&B). These pouch galls were hard, slightly compressed, elongated, mango like and persistent generally with single larva lying inside the gall cavity (Fig. 1 C&D). Mesophyll of the gall was differentiated into parenchymatous, chlorenchymatous and sclerenchymatous compact layers. Sclerenchyma was conspicuous near outer and inner side of gall around the nutritive tissue (Fig.2 A). From the compressed side of the gall two tissue projections were formed and grew towards each other to form septa. On galling, larva develops inside the chambers; it can move inside and dispersed through ostiole after wards. The larval chambers were superficial and formed by tissue projections (septa) leading to bilocular condition of the gall. The apical and basal fusion of the septa was incomplete (Fig. 2 B). The nutritive zone is present just lining the gall chambers. It consisted of isodiametric parenchymatous cells whose contents were very dense (Fig.2 A). Several vascular bundles were observed in the wall of the gall, since several vascular traces entered into the gall. Overall the gall showed complexity at anatomical level. The investigated cecidomyiid gall remained persistent for a considerable length of time until the gall maker's progeny emerged from the ostiole. The sclerenchymatous cells of mechanical tissue showed lignin deposition in response to various types of injury or attack(Fig 2 C). Electron microscopic studies of a mature gall revealed that most of these sclereids showed pits and they were non-living (Fig. 2 E). The cells of nutritive zone and some other cells also showed nuclei in them. These cells may or may not be living. The cells of nutritive zone showed deposition of some granular substances (Fig. 1 E & F). Parenchymatous tissues showed localization of secondary metabolites like tannin (Fig. 2 D).

Trichomes on the galls can play the role of protecting the inducing insect against parasitoids and predators [16]. Higher quantities of phenolic compounds in galls when compared to non-infested leaves are highlighted in this work. Parenchymatic and epidermal tissues in foliar galls are preferable sites of high tannin concentrations [17]. Such compounds are involved with the defense of gall-inducer insects against microorganisms and herbivores [18]. Thus the development of sclerenchymatous tissue and deposition of phenolics and lignin are premisely strengthen the enemy hypothesis. The gall maker's stimulus supported in the differentiation of parenchyma into sclerenchyma instead of a totally 'distinguished' tissue. Sclerenchyma around the larval chamber constitutes a mechanical defense to the galling insect. Development and arrangement of sclerified cells in galls is related to chemical or mechanical conditions present during cecidogenesis and may be advantageous to the gall inducing insect. Thus the viability of the progeny is influenced by the ability of gall to protect and give shelter to its inhabitants from abiotic stress, pathogens, parasitism and predation[16]. This supports the enemy hypothesis. Larval chambers bearing one galling larva were lined by nutritive tissue. Cells of nutritive tissue show characteristic high physiological activity suitable to the gall inducer's(larva) nutrition [19]. Inducing insect stimulates the nutritive tissue and modifies it into parenchyma [20]. Where, protein, lipids, carbohydrates etc. were accumulated more than the other tissues of the gall in the present study. Nitrogenous compounds may be related to the maintenance of inducing insects. Several studies implying the role of auxins and cytokinins in zoocecidia are available [21]. The nutrition hypothesis mainly reveals the importance of nutritive tissue. *Q. leucotrichophora* galls accumulate relatively high carbohydrate contents, which are potential nutrients for the inducer. This is in accordance with the hypothesis of Price [7]*etal*., 1987. This is not surprising if it takes into account the involvement of carbohydrates as precursors of nutrients (starch, protein, lipids) and also of structural (e.g. lignin) and soluble(e.g. phenols) gall secondary metabolites.

The present studies reveal that in *Quercus leucotrichophora*-cecidomyiid gall development there was synchronization of nutritive hypothesis with that of enemy hypothesis. Some tissues were totally made up to nourish

the insect while some were specialized to protect the insects' progeny. With the help of this combination insect feed aswell as remain protected from enemies like herbivores and microbes. These two in responsegive the insect a shelter where it controls the growth of particular plant tissues in a way to complete its life cycle. So we can say that any one hypothesis of adaptive significance is not sufficient to explain the development process of gall.

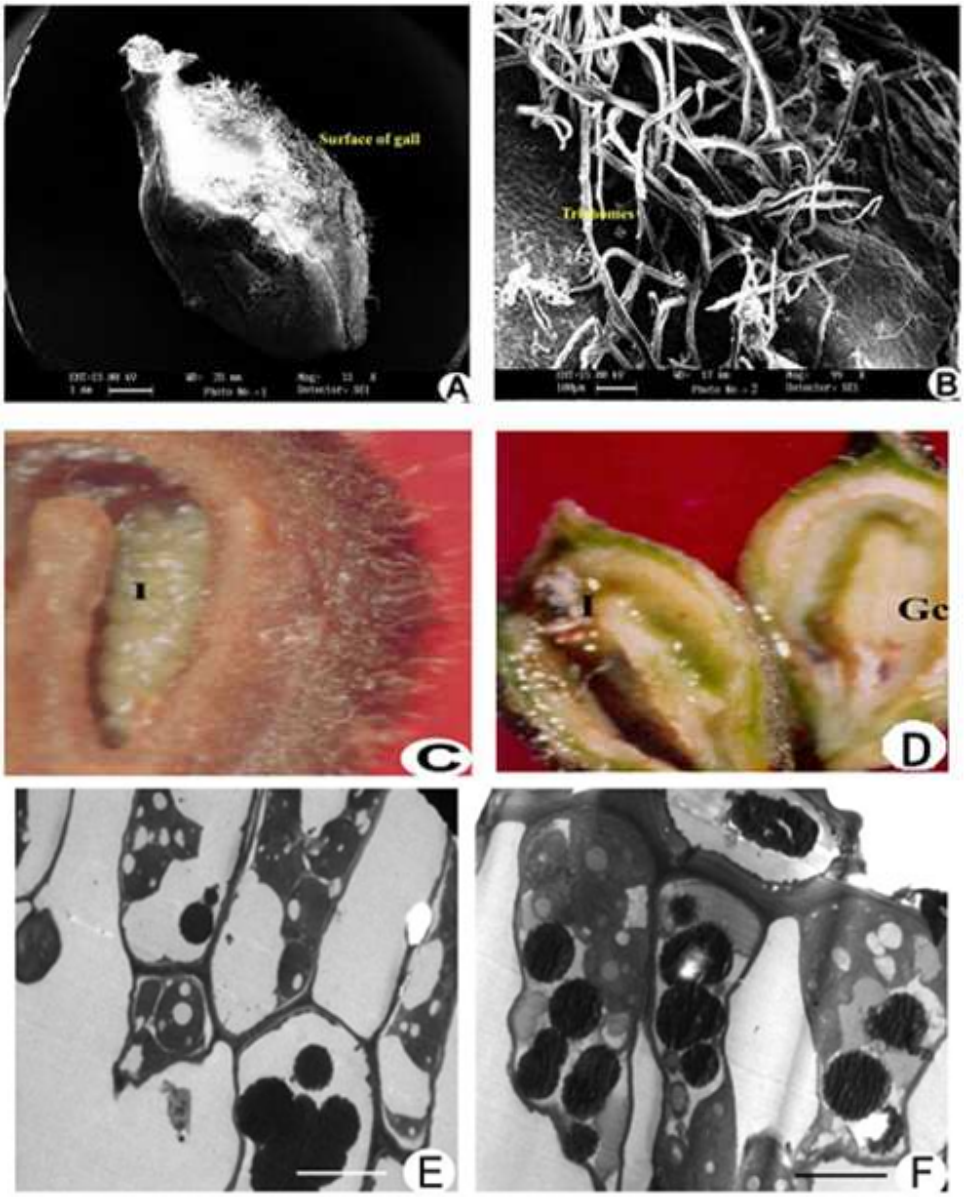

Figure 1: A & B- Close up view of cecidomyiid gall using SEM to show trichomes on outer surface Bar= 1mm and 0.10 mm; C- One part of split open gall showing larva lying inside X20; D- Split open gall showing arrangement of septa and insect lodged in the gall cavity X 16; E & F- Cells of parenchymatous and nutritive zone showing nucleoli and deposition of granular substances. Abbr: I- Insect; Gc- Gall Cavity

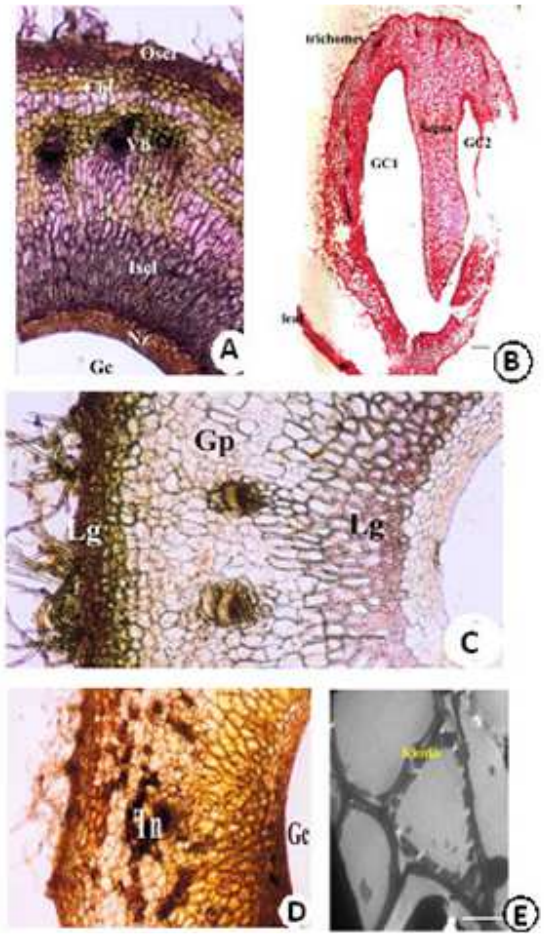

Figure 2: Anatomical studies of foliar galls of *Q. leucotrichophora*; A- Section through mature gall showing well developed tissues; B- L.S. of cecidomyiid gall showing gall chambers, incomplete septa and trichomes (reconstructed); C- C. S. of cecodomyiid leaf gall showing lignin deposition in cell walls of outer and inner sclerenchymatous layer cells; DC. S. of cecidomyiid leaf gall showing localization of tannin in the parenchymatous gall cortex as patches; E- Cells of sclerenchymatous regions of mature cecidomyiid gall showingpits on their cell walls, observed under Transmission Electron Microscope(Bar= 0.10 mm).Abbr. Gc- Gall cavity; Scl- Sclerenchyma; Vb- Vascular bundle; Lg- Lignin; Tn- Tannin; Gp- Gall parenchyma.

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