Histopathological changes in leaf gall of Ficus species

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Abstract

Entomogenous galls are pathological structures which have originated from neoformed tissues as a result of mechanical and/or chemical stimuli. The present investigation reveals that both the processes namely hypertrophy and hyperplasia are common in the development of galls. In *Ficus religiosa* & *Ficus roxburghii* leaf galls, the first effect of cecidozoa attack is the hypertrophy of epidermal cells adjacent to the site of insect attack. Leaf galls show hypertrophy and hyperplasia as evidenced by investigations. The seat of cell proliferation is the mesophyll parenchyma of leaf. The mesophyll cells of the abaxial side of the developing galls are hypertrophied and are in a state of rapid proliferation. Subsequently, the hyperplasia and hypertrophy spread in all the parenchyma cells of the infected area of the leaf.

Keywords: Leaf gall, Ficus religiosa, Ficus roxburghii, Hypertrophy, Hyperplasia, Mesophyll Cells

Introduction

*Ficus religiosa*leaf galls induced by -*Pipaldiplosispipaldiplosis* belongs to the family cecidomyiidae of order Diptera are widely distributed in India. *Ficus* plants are found to be galled with various degrees of infection. The galls occur commonly during October to April.

The normal leaf transverse section shows adaxial and abaxial epidermis. The adaxial epidermis is multilayered. Adaxial epidermal cells are larger as compared to the cells of abaxial epidermis and are coated with thick cuticle. Stomata are observed on abaxial side of the leaf. There is a single layer of palisade cells adjacent to the adaxial epidermis. The palisade cells are cylindrical and closely packed together. Below palisade, lies spongy parenchyma. Some crystals are seen in abaxial epidermis, the common forms of crystals consist of Silica, Calcium carbonate and Calcium oxalate. Calcium carbonate occurs in the form of a crystalline mass, often pear-shaped in appearance. This crystalline mass is called **Cystolith**, which remains enclosed in a large cell. It looks like a bunch of grapes suspended from a stalk .The mesophyll cells are filled with abundant chloroplast. The cells of this region are irregular in shape and are arranged loosely with intercellular spaces.

Methodology

Ficus religiosa and *Ficus roxburghii* leaf galls and their normal counter parts were collected and bagged in polythene envelopes containing cotton swabs soaked in formic acid (40%). Samples of different developmental stages of galls and normal counterparts were fixed in FAA (37% formaldehyde : acetic acid 50% Ethanol, 1:1:18 v/v) (Johansen, 1940) for anatomical studies.

The materials were thoroughly washed with tap water to remove traces of the fixative. Subsequent steps of dehydration, cleaning and embedding were done following tertiary butyl alcohol method (Johansen, 1940). Leaf galls were dehydrated through ethanol series, embedded in histowax and sectioned at 10-15 mm with rotary microtome. Sections were stained with 1% safranin and 0.5% light green. The safranin-light green combination gave best results. D.P.X. mountant was used for mounting. Slides were observed under Nikon Alphaphot trinolcular microscope model SMZ-10 and photomicrograph.

External gall morphology – Galls are hypophyllous larval, localized or extensively ovide fusiform, solid, hard woody indehiscent brown swelling of the midrib and basal part of the other side veins, often continues to form a branched mass, with irregular and narrow, longitudinal larval cavities, extending nearly the whole length of the gall, become more or less deeply fused in irregular patches size of 5-8 mm thick. Galls are simple, sessile, solid, globose and unilocular. Young galls are green in colour and change brownish green with maturity. In case of heavy infection up to 10-13 galls per leaf were observed. Generally galls are isolated, however in rare cases 3-5 galls fuse to form composite structure. The individual galls are 0.5-2.0 cm in diameter (Plate-1; Fig. A&D).

Structure of normal leaf – The transverse section of the normal leaf on upper & lower side of mesophyll shows adaxial and abaxial epidermis. The adaxial epidermis is multilayered. The cells of the adaxial and abaxial epidermis are rectangular. Adaxial epidermal cells are larger as compared to the cells of abaxial epidermis and are coated with thick cuticle. Stomata are observed on abaxial side of the leaf.Mesophyll cells are arranged in definite layers. There is a single layer of palisade cells adjacent to the adaxial epidermis. The palisade cells are cylindrical and closely packed together. Below palisade, lies spongy parenchyma. The cells of this region are irregular in shape and are arranged loosely with intercellular spaces. Some crystals are seen in abaxial epidermis, the common forms of

crystals consist of Silica, Calcium carbonate and Calcium oxalate. Calcium carbonate occurs in the form of a crystalline mass, often pear-shaped in appearance. This crystalline mass is called **Cystolith**, which remains enclosed in a large cell. It looks like a bunch of grapes suspended from a stalk (Plate-2; Fig. A&B).The mesophyll cells are filled with abundant chloroplast and the vascular elements are embedded in this tissue (Plate-3; Fig.A).

Gall anatomy – The epidermis of the gall is covered with a layer of cuticle. No stomata are observed on the gall epidermis. The gall is remarkable for total inhibition of differentiation of the normal tissues of mesophyll. The main bulk of the gall tissue is composed of large sized, thin walled and closely packed secondary xylem. Cecidozoan occurs near primary xylem. The secondary xylem cells are larger in size as compared to the normal. Several tannin filled cells are observed in the secondary xylem. Cells of the mechanical zone are slightly sclerenchymatous in nature (Plate-2; Fig.C-D). Gall chamber lies in the central region of the gall and contains only one cecidozoan. Few inner layers surrounding the gall chamber constitute the nutritive zone. The mesophyll is greatly hypertrophied and the swelling is more on abaxial side than on the adaxial side. Cork formation starts in outer region of mesophyll tissue and after some time lenticels appear. The larval cavity in the mature gall is narrow and lined by the collapsed cells of the earlier nutritive tissue (Plate-3; Fig. B).

In young galls the cells surrounding the larval cavity are characterized by dense cytoplasm and enlarged nuclei. This tissue may be considered as the nutritive zone. However, cells adjacent to the cecidozoa disintegrate in old galls (Plate-4; Fig. A-C).

Gall development –The insect usually attacks veins of young leaves surrounding the growing tips of the plant. The gall formation is initiated by the attack of the insect on the abaxial side may be made continuously on young leaves.Eggs are generally laid on the abaxial surface of leaf, less often on the adaxial surface. Few days after oviposition,

hypertrophy is observed in the cells of area of attack which is followed by hyperplasia. The hypertrophy and hyperplasia turn leaf slightly thicker in this region, so that a small protuberance is formed on veins of leaf. Further growth of the gall takes place as a result of meristematic activity around the cecidozoa. Later on the growth of the gall is horizontal and vertical. The peripheral cells of the gall divide and redivide. This results in a gall of globular shape. Four to six layers of sclerenchyma cells constitute the mechanical zone which surrounds the secondary xylem central region of the gall. The secondary tissues are formed in vascular region due to secondary growth in gall. Outer mesophyll cells are converted in sclerenchyma tissues. Later on mesophyll cells below epidermis forms cork and lenticels. These secondary tissues enlarge during gall development. Small amount of parenchyma cells surrounding the cecidozoa constitute the nutritive zone. An ostiole is formed through which the insect emerges out.The process of gall development is shown in sequence in (Plate -5; Fig. A-C).

Discussion

Mani (1964) has reviewed the work carried out on various aspects of zoocecidia. Cosen (1912) reported that salivary glands are responsible for the secretion of the stimulating substances. Felt (1936) believed that the stimulation for gall formation comes from salivary glands. Reproductive deformities in the galled flowers of *Salvadora persica* are possibly caused by the salivary secretions poured by the cecidozoa while feeding and/or by the drainage of the nutrients sucked by innumerable mites and their young ones (Sokhi and Kapil, 1987).

Several authors have suggested that there is a corelation between the mode of feeding of gall insects and the complexity of their galls (Meyer and Maresquelle, 1983; Rohfritsch, 1992). Although galls are not typical structures of the plant body, the individual components of gall tissues may be found in affected organs or in diverse parts of the plant (Mani, 1964).

So it is concluded that cecidogenous responses are influenced by a specific insect action and directed by the stage of plant cell differentiation at the time of induction.

In leaf gall the mesophyll is not differentiated into palisade and spongy tissues and is represented by simple undifferentiated parenchyma. There is total inhibition of differentiation of normal tissue of mesophyll into palisade and spongy parenchyma. Many workers have reported similar condition in *Piper nigrum* Renjith *et al*. (2007); *Quercus leucotrichophora* Mishra and Patni (2008); *Mangifera indica* Marmit (2010) and *Salvadora persica* Joshi (2011) leaf galls respectively. Gall parenchyma consists of compactly arranged parenchyamtous cells. The parenchyma cells are larger in size as compared to the cells of normal tissue.

In young galls vascular bundles are found scattered in the leaf gall parenchyma but in mature galls, disorganized vascular bundles are found and are almost absent in old galls. The cells of vascular elements are arranged in a most irregular manner and are angular in appearance. After maturation, the gall becomes elongated and ultimately dehisces.

Result

Leaf galls of *Ficus religiosa* are induced by *Pipaldiplosis pipaldiplosis* .Galls are hypophyllous present on the abaxial surface of leaf. The galls are simple, sessile, regular, solid, globose and unilocular. Young galls are green in colour and change yellowish green with maturity. The epidermis of the gall is in continuation with the normal epidermis of the leaf. The mesophyll is not differentiated in to palisade and spongy parenchyma and is represented by simple undifferentiated parenchyma.

Leaf galls of *Ficus roxburghii* are induced by *Pauropsylla sps.* The galls are cone shaped or oval. The insect attacks the growing buds of the shoot. Few days after oviposition, some cells in the region of attack show hypertrophy. The parenchyma cells enlarge during

the gall development. The hypertrophy and hyperplasia turn the leaf slightly thicker in the middle region at maturity, the leaves loose their identity and finally wither.

The epidermis is single layered and becomes irregular. Cell division of mesophyll tissue results in the expansion as well as elongation of the tissue which press the epidermis to form a way outline. Just outside the vascular region a definite ring of cavities is found in the inner side of which can be located a few sieve tube cells with companion cells.

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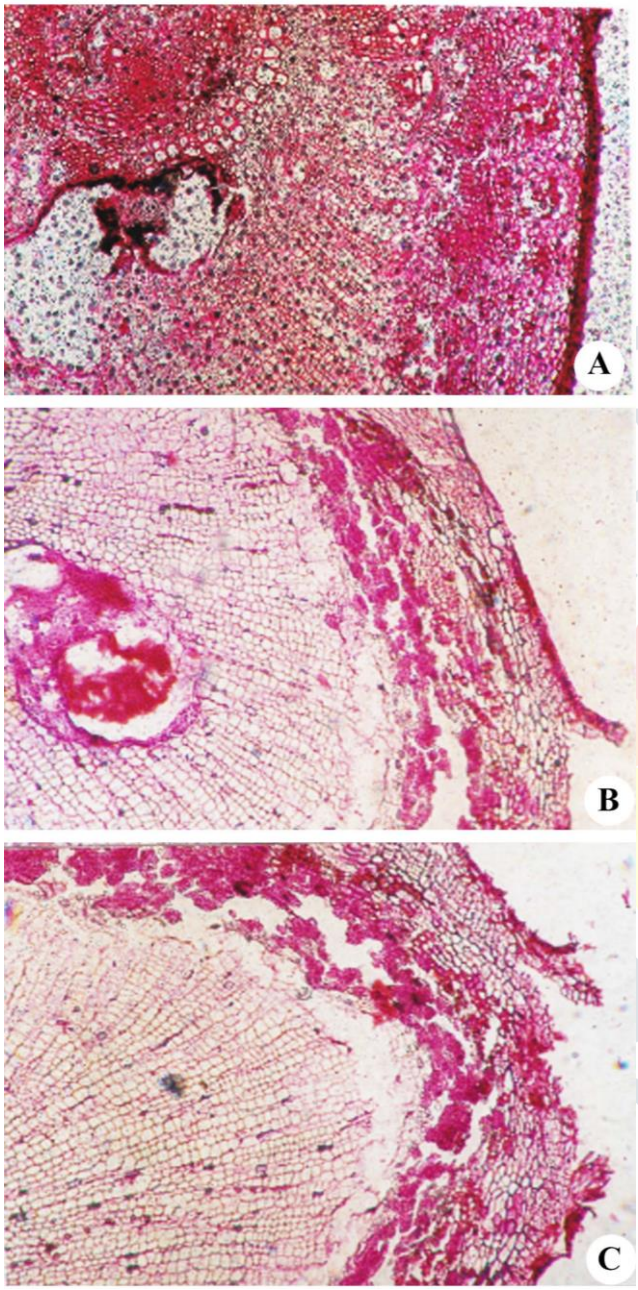


PLATE-1

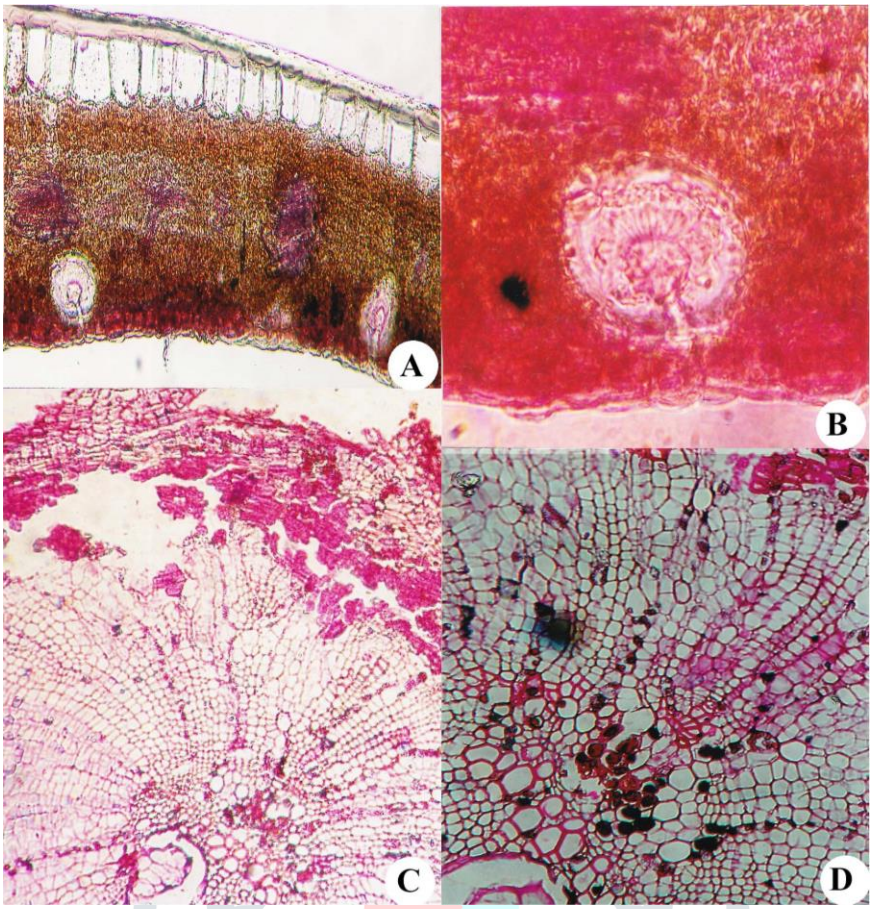
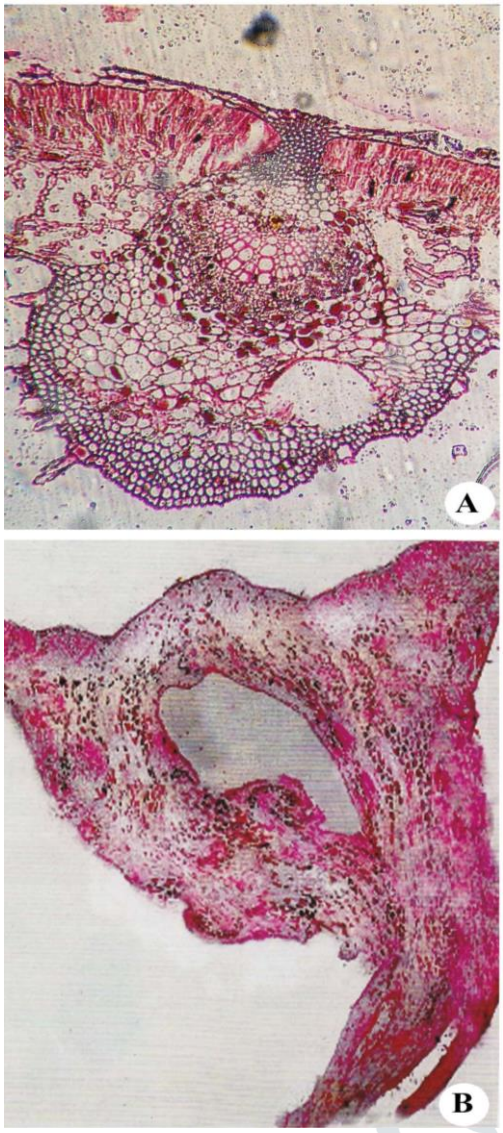


PLATE-2



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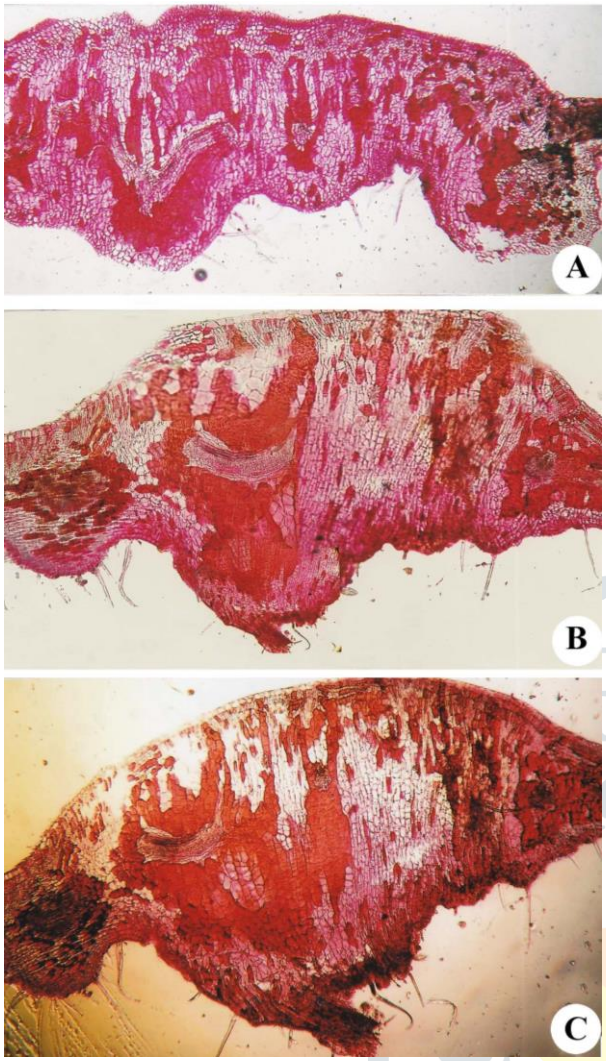
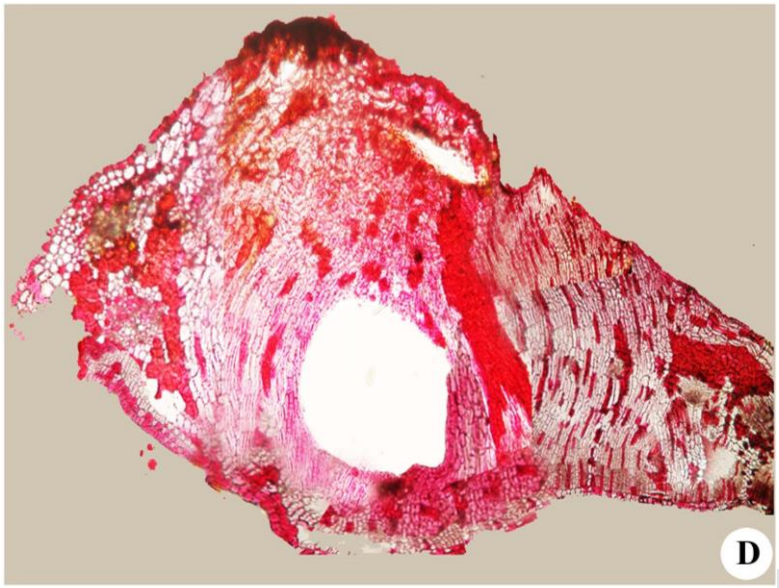


PLATE- 4



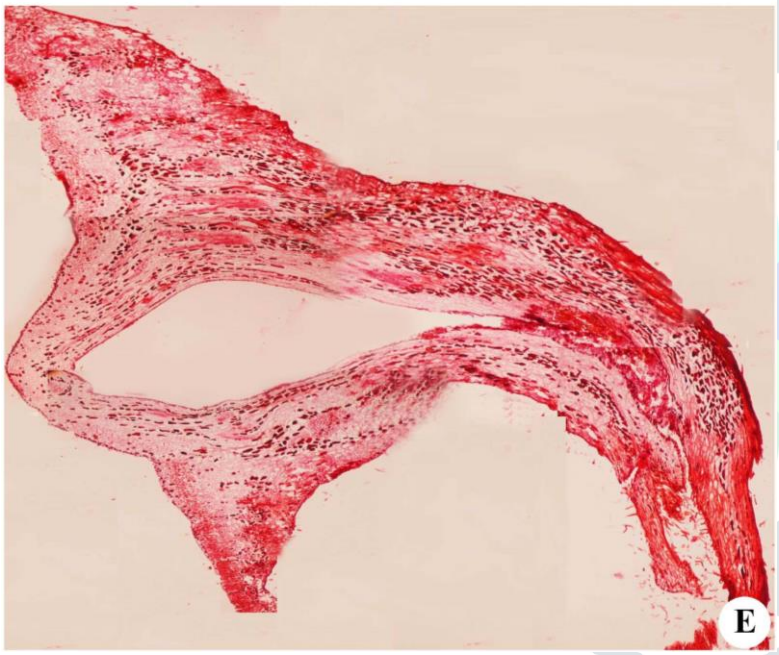


PLATE-5

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