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Morphogical and Histopathological Studies on Leaf Galls of F*icus Mysorensis* Heyne Induced by Unknown *Psyllid*

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Abstract: Plant galls are abnormal growths caused by an inducer that determines their morphology and anatomy. Leaf galls of *Ficus my sorensis* Heyne caused by unknown *Psyllid* are epiphyllous, globose, solitary, sessile and generally persistent pouch galls. The galls may remain separate or may fuse to form a compound gall. In case of heavy infection, entire leaf may be converted into galled mass. The interior of gall contains a cavity, generally circular or oval in shape. The gall cavity opens outside by a wide hypophyllousostiole. The adaxial epidermal cells are larger in size compared to the cells of normal corresponding layer. The abaxial epidermis is well defined in young galls. Stomata are never developed on this side of gall unlike normal leaf. The cells of abaxial epidermis surrounding the gall cavity are hypertrophied and possess dense cytoplasm with prominent nuclei. The attack of insect is on the abaxial side of the leaf. The gall formation is due to bulging on one side of leaf and a corresponding invagination on the other side. The galls are remarkable for total inhibition of differentiation of normal tissue of mesophyll, The mesophyll is represented by a simple undifferentiated parenchyma which is 2-3 times thicker compared to normal. The seat of proliferation is the mesophyll parenchyma of leaf. Several Vascular bundles 1ie scattered in the gall parenchyma.

Keywords: Histopthology, Leaf gall, Gall chamber. Cecidozoa, Hypertrophy. Hyperplasia .*Ficus mysorenesis* Heyne ,*Psyllid*

Introduction:

Galls are abnormal swellings of plant tissue, usually on leaves and stems. They may be caused by Insects, Mites, Viruses, Bacteria, Fungi or Nematodes. Most insect and mite galls are caused by chemicals produced by the egg laying and feeding activities. The chemicals caused the affected plant cell to swell due to hyperplasia & hypertrophy result into a gall. Gall may disfigure stem and foliage, but they do not seriously affect the health of host plant The plant *Ficus mysorensis(*Mysore Fig*)*belongs to family Moraceaeiaa economically important plant.It is used as a source of food,fibre and medicine by local peoples. The family Moraceae is rich in galls, most of which are widely distributed in India. Of the 41 galls known, thirty five are leaf galls, nearly half of which are caused by Psyilidae and one fourth by Itonididae (midges), others caused by Thysanoptera and Hymenoptera. The majority of the leaf galls are pouch galls with some covering galls.

The leaf gall of *Ficus mysorensis* induced by unknown *Psyllid* belongs to family Psyllidaceaeof order Homoptera. Homoptera are important gali Insect all over world. The development and structure of the galls incited by Homoptera are generally correlated with the feeding habit of the insects. The galls induced by Homoptera are relatively complex in structure and predominantly 1eaf galls. Homoptera are confined mostly to Psyllidae and Aphidae with a few in Coccidae. Majority of payllid galls are pit and pouch galls on leaves of Dicotyledons but some leaf margin roll galls and covering galls are also known (Mani, 1964). The leaf gall of *Ficus myorensis*induced by unknown *Psyllid* occurs commonly in Jodhpur and adjoining areas. Generally, the galls mature in months of November and December.

Materials and Methods:

The leaf galls of *Ficus mysorensis* induced byunknown *Psyllid.* appear in the rainy season. Different developmental stages of leaf gall. and normal counterpart of F*icus mysorensis* were collected from the plants and fixed in 70% alcohol. Subsequent dehydration, clearing and embedding was done following the tertiary butyl alcohol method (Johansen, 1940). After dehydration, the samples were infiltrated with paraffin for over one week in an oven at 60 C. The paraffin was replaced every two days and then the samples were embedded in paraffin

until complete solidification. These paraffin blocks were used to cut sections of whole gall at a thickness of 8-12µ using a Weswox microtome. The sections were stained with safranin and fast green. Mounted in DPX Mountant or Canada balsam. These sections were then examined under the microscope to study the histopathology.

External Gall morphology:

Galls are formed singly or in clusters on the adaxial surface of the leaf. They are globose, solitary, sessile and persistent pouch galls.Generally, the galls are isolated and unilocular. Very often many galls fused together and form multichambered structures. The number of galls on a leaf may vary from one to many. As many as 145 galls per leaf have been observed. In case of heavy infection, entire leaf may be converted into a galled mass. The interior of the gall contains a cavity. The cavity is communicated by a large ostiole. The ostiole opens on abaxial surface of the leaf.Size of a single gall is 2.0-6.0mm in diameter and compound galls are 4.0-8.0 mm in diameter.

Structure of Normal leaf:

Leaf is dorsiventral and possesses single layered adaxial and abaxial epidermis. Galls of the adaxial and abaxial epidermis of normal leaf are rectangular, cylindrical and compactly arranged. The cells of the adaxial epidermis are larger in size as compared to the cells of abaxialepidermis. Cystolith (calcium carbonate crystals) occur in the hypodermal region on the upper (adaxdal) side of the normal leaf. Stomata are present only on the lower opidermis. A large subs tomatal chamber is present below the stomatal opening. Hypodernis is present below adaxial as well as abaxial epidermis. The hypodermis on the adaxial side is 2-3 layered while on abaxial side single layered hypodermis is observed (Fig. 1-J).Mesophyll cells are arranged in definite layers of palisade and spongy parenchyma tissues. Adjacent to the adaxial epidermis there is a single layer of palisade cells. The cellls of palisade are cylindrical and closely packed together. Below palisade lies spongy parenchyma. The cells of this region lack regularity in shape and arranged loosely with conspicuous intercellular spaces. The vascular bundles are embedded in mesophyll tissue and surrounded by a thin layer of parenchyma cells.

Gall anatomy:

The epidermis of gall on the adaxial (upper) surface is continuous with the epidermis of the unaffected part. The epidermal cells are rectangular and cylindrical in outline (Fig, 1F,G). Gall of the adaxialepidermis are larger in size as compared to the cells of epidermis of normal leaf. Adaxial epidermis is single layered in thickness. In older galls, the epidermis is cuticularized. Like normal leaf, stomata are not observed on this side. Cystolith are absent in the hypodermal region on the upper (adaxial) side of the gall unlike the normal leaf.

The abaxial epidermis is well defined even in a mature gall. The epidermal cells are thin walled and rectangular in outline. Galls of abaxial epidermis are smaller as compared to the cells of adaxial epidermis. The stomata are absent on the epidermisof the older gall unlike the normal counterpart (Fgs. 1F,G). The cells of the abaxial epidermis surrounding the gall chamber (cavity) are hypertrophied, as compared to galls of normal leaf epidermis. These galls possess dense cytoplasm and prominent nuclei. This layer may be nutritive in function.

The gall is remarkable for inhibition of differentiation of normal tissues of the mesophyll. The palisade or spongy parenchyma are altogether absent and the mesophyll in gall is represented by simple undifferentiated parenchyma. Main bulk of gall tissue is composed of thin walled, closely packed parenchyma cells (Figs. 1F). The parenchyma cells are generally polygonal in shape and are highly hypertrophied. The gall parenchyma cells are larger in size (36.0 x 33.5µ - 47.0 x 42.0 4 µ) as compared to that of the normal parenchyma cells (8.0 x 7.5µ - 22.5 x 13.5 µ). Some of the parenchyma cells possess larger nuclei. The nuclei of nutritive zone and parenchyma cells are of various shapes and size. The nuclei are larger (22.5 - 51.0µ) compared to normal (4.5 6.5µ) (Fig. 1,1). Chlorophyll is present in the parenchyma cells, particularly in young galls. But in older galls, the parenchyma cells are entirely devoid of chlorophyll. Few inner layers surrounding the gall cavity show accumulation of starch in the cells.The parenchyma cells surrounding the opening of the gall are highly proliferated (Fig. 1H). Several tanin filled cells are also observed in gall parenchyma (Fig.1F).

Many vascular bundles are present in the gall parenchyma. The bundles have distinct xylem and phloem. The phloem 1ies towards inner (abaxial) side of the gall and xylem towards outer (adaxial) side like normal leaf. The vascular bundles are largerin size as

compared to normal leaf. The bundles are surrounded by thin walled parenchyma cells (Figs. 1G).

The mature gall is a pouch gall with wide opening on the under (abaxial) side of the leaf. The gall cavity is usually oval or circular and contains only one cecidozoan (Figs. 1C). The number of individuals of the *Psyllid* in each gall is extremely limited. It is never more than one or at the most two nymphs of the*Psyilld* are found in a single gall (Mani, 1964).

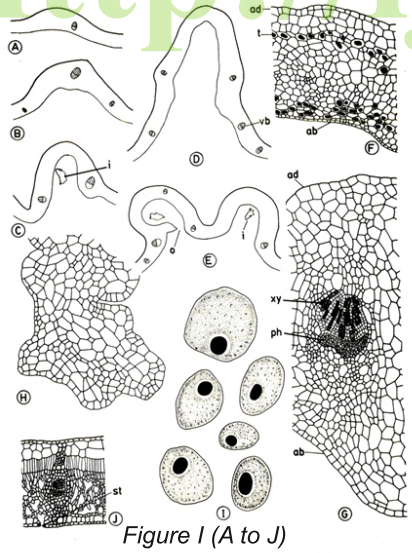

Figure I (A to J)

Abbreviations used for labeling figure 1 (A to J)

ab = abaxial epidermis	**ad = adaxial epidermis**
sg = starch grains	**st = stomata**
t = tanin	**vb = vascular bundle**
xy = xylem	**ph= phloem**
gc = gall chamber	**i = insect; o ostiole**

Gall development:

The insect usually attacks very young leaves surrounding the growing tips of the plants. The attack is generally confined to the abaxial surface of the leaf. New attacks may be made continouslly on young leaves. As a result, a large number of galls appear at their various otage of development on the same leaf. The galls are really the invigilated and swollen parts of the leaf.

Various stages of gall development are shown in Figs. 1A-D. The stimulation of cecidozoa is localized in a small area of the leaf, surrounding the developing insect. Microscopic examination of the affected area of the blade shows a change in the normal histological pattern. In the binning, cell proliferation of the abaxial epidermis and the adjoining mesophyll cells do not greatly occur below the cecidozoa, but it is vigorous around it. This results in the formation of a specific zone of vigorously proliferating cells. The mesophyll cells of the abaxial side of the infected area are greatly hypertrophoid and are in a state of proliferation. The cecidozoa, therefore, is soon lodged inside a developing pouch which is formed by the actively dividing parenchyma cells. Later on, the hyperplasia and hypertrophy spread in all the parenchymatous cells of the infected area of the blade which includes both abaxial and adaxial surfaces. Simultaneously, both inner and outer epidermal cells keep pace with the process of division. As a result, the affected part of the blade arched itself out of the level of the blade (Figs. 1A, B). The bulging is on the adaxial side and corresponding invagination on the abaxial side. In this way typical pouch-like gall formed (Fig. 1D). Hence, this leaf gall is really the invigilated and swollen part of the leaf. The seat of cell proliferation is the mesophyll parenchyma of leaf. Quite often many galls arise very closely together in such a large number that they become fused together, to form a large, multichambered, fleshy, agglomerated mass (Fig. 1E). The mature gall is communicated to outside by a wide ostiole (Fig. 1D).The entire gall is composed of undifferentiated parenchyma of the mesophyll region, which is 2-3 times thicker as compared to the mesophyll of the normal leaf (Figs. 1F, G) .

Discussion:

Plant galls (tumours) are pathologically developed cells, tissues or organs of plants which are formed mostly by hypertrophy and hyperplasia under the influence of gall inducing agents (Mani, 1973). The relative abundance of galls on different parts depends primarily on the plant and the gall maker and it is also influenced by a variety of other environmental factors . The shape and size of gall is determined mainly by the cecidogenic insect and its stimulus

The aim of histopathological studies is to understand the adaptational strategies involved in gall formation. Under the influence of the cecidozoan, the course of morphological events is altered so that a new physiological and morphological environment is available for the gall insects. Gall inducing insects have profound effects on their hosts. These insects live within the plant tissues and induce tumour like growth that provide them with food, shelter and protection from natural enemies (Raman *et al.,* 2005). The host response to feeding or ovipositional stimulus

is sometime unique that alters plant morphogenetic responses (Albert *et al.,* 2013).The stimulus whatever is its nature, oirginates from the point of attack on the host tissue by the cecidozoa and spreads in radial symmetry which results in intensive mitotic activity in the infected area (Mani, 1964). This leads to the formation of a pustule like evaginated pouch like structure. Jayaraman (1979) and Kant and Karnawat (1989) have also made similar observations. There are various opinions regarding the significance of nuclear hypertroply of gall cells. Meyer (1950) believed that it is related to increase in ribose nucleic acid and proteogensis. Of in general, stomata are fewer on the gall epidermis than the normal one and in some cases they may be totally absent (Mani, 1964). In the present investigation, no stomata were observed either on abaxial or adaxial epidermis of the gall.

The present study revealed that both the processes namely hypertrophy and hyperplasia are important in gall development. In F*icus mysoremsis* leaf gall formation is initiated by larva of the insect. The visible effect of the larva is swelling of the affected portion. The gall is really the swollen portion of the leaf blade. Cell proliferation of the mesophysill adds significantly to the swelling of the gall. The cells around gall chamber constitute the nutritive region. These cells were larger in size and rich in cytoplasm because they provided nutrition to cecidozoa.Kant (1967) and Ranwa (1983) made similar observation on stem gall of *Emblica officinalis* induced by *Betousa stylophora* and Vyas (1989) on the stem gall of *Prosopis cineraria* (Linn.) Druce induced by chalcid. The histopathology, physiology and histochemistry of insect and mite induced galls has also been studied by Patni and Arora (2000), Raman *et al.,* (2006) , Konczet *al.,* (2011) , Alvarez et al.(2013),Chaudhary (2015) and Mellah et al.(2016).

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