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Histopathological changes and development of Leaf Gall of *Ficus racemosa* Linn. induced by *Pauropsylla depressa* Crawf

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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 insect. Leaf gall of *Ficus racemosa* Linn. induced by cecidozoan *Pauropsylla depressa* Crawf. were used for histopathological studies with their normal counterpart.The objectives of present investigation was to study the possible alteration in the anatomical characters due to insect attack on *Ficus* leaf and host pathogen interaction The leaf gall of *Ficus racemosa* is an invaginated and swollen part of the leaf.The entire gall is composed of undifferentiated parenchyma of the mesophyll region, which is several times thicker than the mesophyll of the normal leaf.The investigation revealed that hypertrophy and hyperplasia are important in gall development.

Keywords: Histopathological, Gall*, Ficus racemosa, Pauropsylla depressa*

INTRODUCTION

Ficus racemosa Linn. is an economical and medicinally important tree species belongs to family Moraceae. This plant is widely distributed in semi arid regions of India. All parts of the plant are medicinally important.The bark is cooling, acrid, galactagogue and good for gravid uterus,asthma and piles.The roots are useful in hydrophobia and dysentery.The leaves are astringent to the bowels and good for bronchitis,The milky juice is administered in piles and diarrhoea and in combination with sesamum oil in cancer.The insect *Pauropsylla depressa* Crawf. belongs to the family Psyllidae of order Homoptera. The bulk of psyllid galls are pit galls and pouch galls on the leaves of dicotyledons, but some leaf margin roll galls and covering galls are also known.

Galls are abnormal growths that are usually formed as a response by plant to the action of viruses, bacteria, fungi, nematodes, insects or mites. These are unique examples of complex interspecific interaction and mutual adaptation between plants and gall inducing agents. Galls are pathological structures,charactrized by various structural and physiological changes in the host tissues.The host response to feeding or ovipositional stimulus is some time unique that alters plant morphogenetic responses (Albert *et al*.2013). Plant galls shows alteration in the metabolism of affected parts as reflecting in the biochemical analysis of galls and their normal counterparts.They are formed by the interaction of insects on plant tissues and are an example of the unusual transformation and use of plant by insect(Guzicka *et al.*2017).Gall forming insects have the ability to alter the development of plant tissue to cause the formation of tumor-like outgrowths that surround the insect to protect it from the environment and supply it with a source of food.

Histopathology is useful for studies of anatomical alteration of host tissues and development of galls. Histopathological changes in host tissues are associated with the synthesis of various chemical substances including auxin and phenolics which result in hypertrophy and hyperplasia of plant tissues (Gaur *et al*.2021).

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Ficus leaf galls are very common in Rajasthan, particularly around Jaipur. Infection occurs every year from July to November. Leaf gall of *Ficus racemosa* have a specific anatomical and physiological structure (Ushir *et al*.2015).

The present investigation deals with the histopathological studies of leaf gall of *Ficus racemosa* induced by cecidozoan *Pauropsylla depressa* Crawf. (Homoptera) and their normal counterparts.

MATERIALS AND METHODS

Normal leaf and leaf gall material of *Ficus racemosa* Linn. were collected from Jaipur and adjoining areas (Fig. 1 A,B). Fresh, fixed and dried material of normal and gall counterparts were used for experimental studies.

Different developmental stages of leaf gall of *Ficus racemosa* induced by *Pauropsylla depressa* Crawf. and its normal counterparts were collected from the plants and bagged in polythene envelopes containing cotton swabs, soaked in formic acid. Later on, the material was fixed in 70% alcohol. The materials were thoroughly washed with tap water to remove all traces of the fixative. Subsequent dehydration, clearing and embedding was done following the tertiary butyl alcohol method (Johansen, 1940). Microtome sections were cut at a thickness of 8-12 microns using a microtome (Weswox) and sections were stained with safranin and fast green.

DPX Mountant (BDH) and Canada Balsam (Ranbaxy) were used for mounting. These sections were then examined under the microscope to study the histopathology.

Galls were also examined using a Nikon (Japan) stereoscopic zoom microscope model SMZ-10. Free hand cut sections of fresh and preserved material were also studied.

RESULTS AND DISCUSSION

External morphology of the gall

Galls of *Ficus racemosa* Linn. are found singly or in clusters on the adaxial surface of the leaf (Fig.1 B).There may be one to many separate galls lying scattered on a leaf.The galls are simple, regular, globose or obpyriform, sessile, perfoliate and unilocular. These are 5 to 10.5 mm in diameter. Very often, many partially fused galls occur on a leaf. These compound galls are irregular, multilocular and measure 15 to 30 mm in diameter. Sometime compound galls form large, spherical or convex tubercles.The tubercles represent several simple galls which have incompletely fused into a compound mass. Galls are yellowish orange, reddish or reddish brown in colour (Fig.1 B). In young galls a small ostiole is found on the abaxial surface of the leaf while in older galls this passage is closed (Fig.1 C).

Structure of Normal Leaf

Cells of adaxial and abaxial epidermis of the normal leaf are rectangular and cylindrical (Fig.1 D). The cells of the adaxial epidermis are larger than the cells of the abaxial epidermis. Stomata with large sub-stomatal chambers are present on the abaxial side. Mesophyll cells are arranged in definite layers. There are two layers of palisade cells adjacent to the adaxial epidermis. The cells of palisade are cylindrical and closely packed together. Below palisade lies the spongy parenchyma. The cells of this region lack regularity in shape and are arranged loosely with conspicuous intercellular spaces (Fig.1 D). The vascular bundles are embedded in the mesophyll tissue. The bundles are surrounded with thick-walled cells and there are often two strands of fibres, one above and the other below each bundle (Fig.1 E). Cystolith is also observed in palisade region towards the adaxial surface (Fig.1 D).

Gall Anatomy

The epidermis of gall on the adaxial surface is continuous with leaf epidermis of the unaffected part. The epidermal cells are rectangular in outline. It has coating of cuticle like that of normal leaf. The stomata are not observed on this side. In old galls, cork like cells are formed at places on the adaxial surface of the gall (Fig.1,G; Fig. 2B).

The abaxial (inner) epidermis is well defined even in a mature gall. The epidermal cells are thin walled

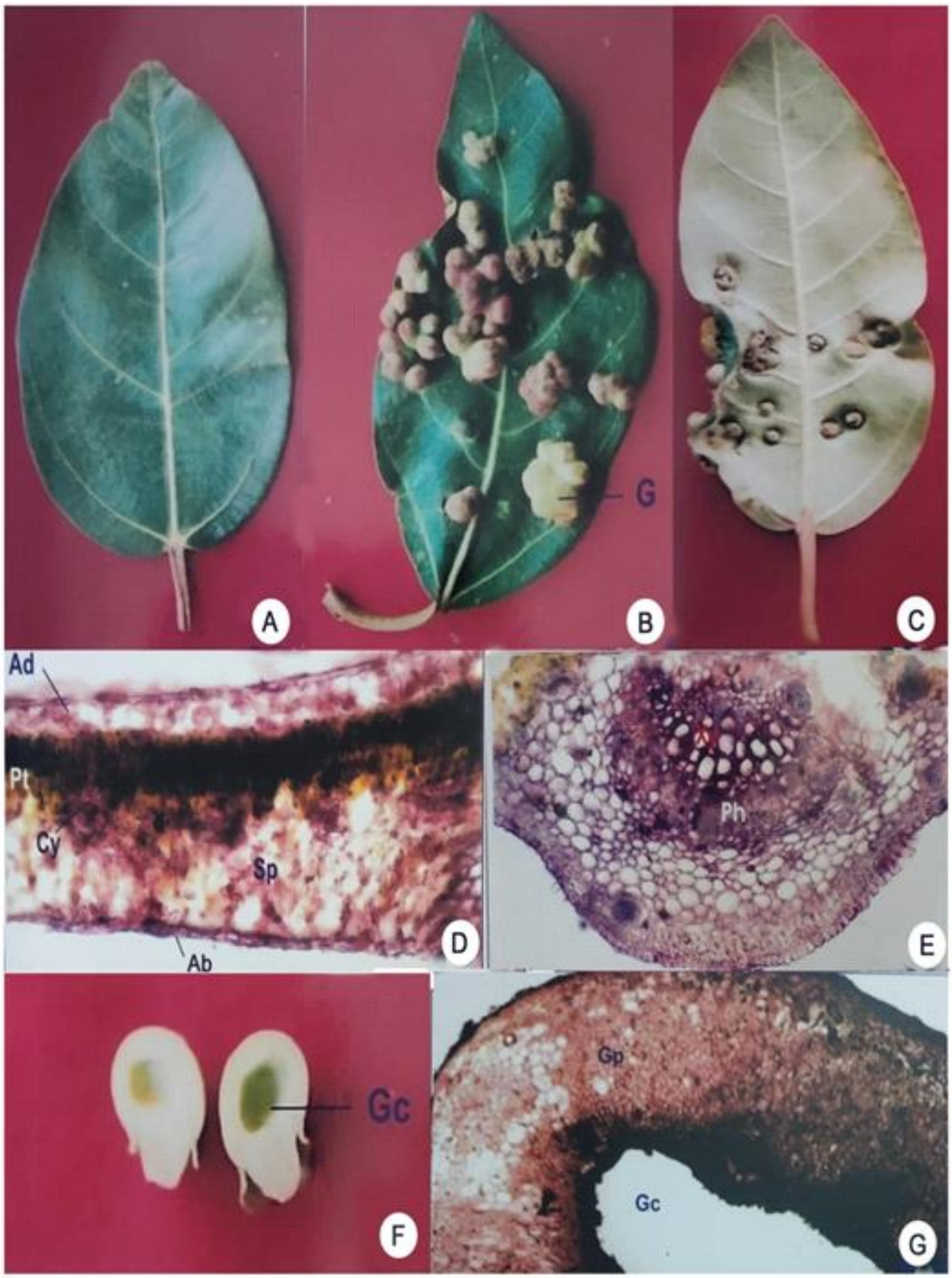

Fig.1: A) Normal leaf of *Ficus racemosa* Linn., (B) Leaf gall of *Ficus racemosa* Linn. showing galls at various stages of development, (C) Abaxial view of *Ficus racemosa* Linn. leaf showing galls, (D) T.S.of normal leaf lamina of *Ficus racemosa* Linn.x300, (E) T.S. of normal leaf of *Ficus racemosa* Linn.showing midrib region x 300, (F) Leaf gall split open, (G) T.S.of leaf gall of *Ficus racemosa* Linn.x150.

Abbreviations :G = Gall , Cy= Cystolith , Sp= Spongy parenchyma, Ph= Phloem ,Gc=Gall cavity ,Gp = Gall parenchyma ,Ab= Abaxial, Ad= Adaxial, Pt=Palisade tissue.

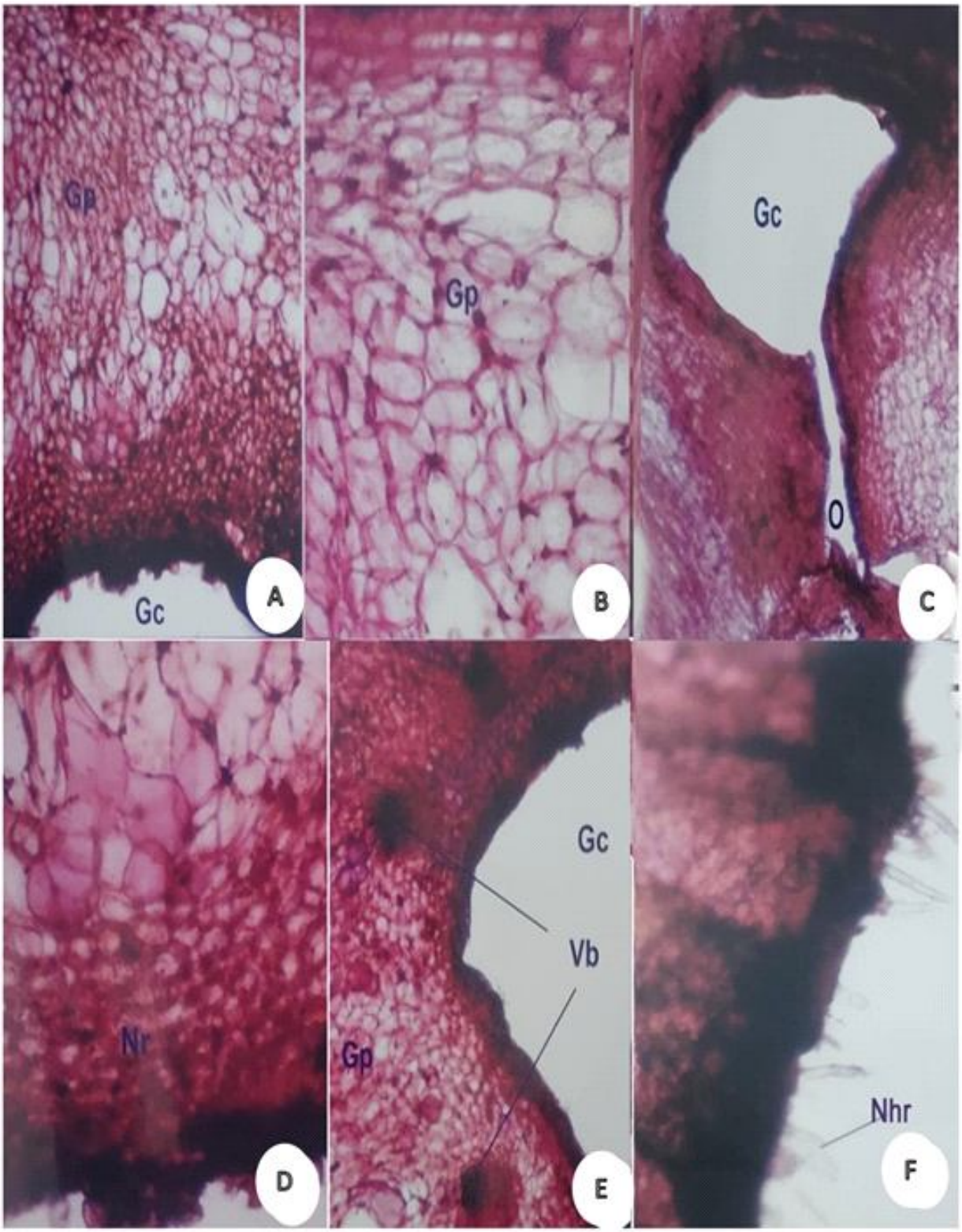

Fig. 2 : (A) T.S.of leaf gall of *Ficus racemosa* Linn.showing gall parenchyma and gall cavity x300, (B) T.S.of leaf gall of *Ficus racemosa* Linn. showing mesophyll represented by undifferentiated gall parenchyma x600, (C) L.S.leaf gall of *Ficus racemosa* Linn.showing gall cavity and ostiole x150, (D) T.S.of leaf gall of *Ficus racemosa*Linn.showing close up view of nutritive region around the gall cavity x600, (E) T.S.of leaf gall of *Ficus racemosa*Linn.showing vascular bundles in gall parenchyma x 300, (MFL.S.leaf gall of *Ficus racemosa*Linn.showing nutritive hairs in ostiolar region x 300.

Abbreviations: Gc = Gall cavity ,Gp = Gall parenchyma, O= Ostiole, Nr=Nutritive region, Vb= Vascular bundle ,Nhr=Nutritive hair

and rectangular in shape, with periclinal walls longer than the anticlinal ones. Stomata are absent on this side of the gall. The leaf gall is remarkable for total inhibition of differentiation of the normal tissues of mesophyll. There is no trace of the palisade or the spongy tissue of the normal leaf. The mesophyll is represented by a simple undifferentiated parenchyma. Bulk of the gall tissue is composed of large sized, thin walled, closely packed parenchyma cells (Fig.1 G; Fig.2A). The cells are generally polygonal or rounded. Few layers of cells adjacent to the inner epidermis are small sized and more closely packed. Chlorophyll is present in these cells, particularly in young galls. However, in mature gall chlorophyll is only sparingly present in a few outer layers of the parenchyma. But in older galls, the parenchyma cells are almost entirely devoid of chlorophyll. Few inner layers surrounding the gall cavity show accumulation of starch in the cells. This region is the nutritive region of the gall (Fig.2 D). The parenchyma cells surrounding the opening of the gall are highly proliferated. In older galls, passage is closed more or less completely due to cell proliferation. Numerous vascular bundles are scattered superficially and deeply in gall parenchyma and are connected with those of the healthy portion of the gall (Fig. 2E). The bundles have distinct xylem and phloem. The phloem lies towards the inner side of the gall and xylem towards adaxial side like normal leaf.

The gall cavity may be oval or circular (Fig. 1F; Fig.2 C) and contains only one cecidozoan. In young galls the cavity is connected to a fistular opening which is found on the underside of the gall (Fig.2 C). Few small thick walled pointed hairs are commonly found near the ostiole (Fig.2 F).

Development of Gall

The insects usually attack very young leaves surrounding the growing tips of the plant. The attack is generally confined to the abaxial surface of the leaf. New attacks may be made continuously on young leaves. As a result, a large number of gall appear at their various stages of development on the same leaf. The galls are really the invaginated and swollen parts of the leaf. The site of cell proliferation is the mesophyll parenchyma of the leaf.

The stimulation of the 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 beginning, cell proliferation of the abaxial epidermis and the adjoining mesophyll cells occur vigorously around the cecidozoan. 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 hypertrophied and are in a stage 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 (Fig. 1 G) 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 arches itself out of the level of the blade. The bulging is on the adaxial side and corresponding invagination on the abaxial side. In this way the typical pouch-like gall is formed. Concurrently, the rapidly dividing abaxial mesophyll tissue projects downward and leaves only a narrow passage. This results in the formation of a bulge on the underside of the leaf. Thus, the gall is developed on both sides of the blade; its major part being epiphyllous (Fig.1B). In older galls, the fistular opening is closed throughout due to rapid cell proliferation around the opening (Fig.1C). Some epidermal cells on the ostiolar end form pointed hairs (Fig.2 F).

Quite often many galls arise very closely together in such a large number that they get fused together to form a large, multi-chambered, fleshy agglomerate mass.Thus the entire gall is composed of undifferentiated parenchyma of the mesophyll region, which is several times thicker than the mesophyll of the normal leaf.

DISCUSSION

Galls are pathologically developed cells, tissues of plants, which are formed mostly by hypertrophy

and hyperplasia under the influence of gall inducing agents.They are found on all parts of a plant from root tip to growing points of shoot, both on vegetative and reproductive organs. 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 (Ranwa, 2017).Insect induced galls arise due to the growth-reaction of plant to the attack of insects. The cecidogenous activity of the insect involves certain complex phenomena which alter the growth and development of host in such a way that the insect becomes partially or completely enclosed within the gall. The different groups of cecidozoa influence the plants in such a way so as to produce galls at different stages in their life history. Most of the cecidozoa cause galls only in their developmental stages but some of them are capable of inducing galls both in larval and adult stages.The feeding of nymph on the sap stimulate the development of galls.(Gawate and Papdiwal, 2011). Many investigators have been attracted towards the plant tumour problem. The main reason for this continued interest is that the plant tumours are in many ways comparable to animal cancer and hence present an alternative experimental system for probing the fundamental processes of tumorigenesis (Mathur, 2002). The physiology and metabolism of gall is different from that of normal tissues of host (Kant, 2000).

The present study revealed that both the processes namely hypertrophy and hyperplasia are important in gall development. The leaf gall of *Ficus racemosa* Linn. is an invaginated and swollen part of the leaf. The site of cell proliferation is the mesophyll parenchyma of the leaf. The mesophyll cells of the abaxial side of the developing galls are greatly hypertrophied and are in a state of rapid proliferation. Subsequently, the hyperplasia and hypertrophy spread in all the parenchymatous cells of the infected area causing the formation of the typical *Ficus* gall.

The insect attack is generally confined to the abaxial surface of the leaf. In leaf galls of *Ficus racemosa* both abaxial and adaxial epidermis take part in the formation of galls. The histological characters of abaxial and adaxial epidermis in gall show fundamental differences. In general, the adaxial epidermal cells are tangentially stretched and cells become narrow. The cells are highly cuticularized in comparison to cells of normal epidermis.

The inner and outer epidermal cells keep pace with the process of division. As a result, the affected part of the blade arches itself out of the level of the blade. The bulging is on the adaxial side and corresponding invagination on the abaxial side. In this way the typical pouch like gall is formed.

The leaf gall is remarkable for total inhibition of differentiation of the normal tissues of mesophyll.The mesophyll is represented by a simple undifferentiated parenchyma.Many workers have reported similar findings in various leaf galls (Ranjith *et al.*2007; Mishra and Patni, 2008; Meena and Meena,2020). Numerous vascular bundles are scattered in gall parenchyma and connected with those of the healthy portions of the gall. The gall cavity may be oval or circular and contains only one cecidozoan. The nutritive region surrounds the gall chamber. The cells of this region are thin walled, large and possess dense cytoplasm. In young galls the gall cavity is connected to a fistular opening which is found on the underside of the gall. In older galls the fistular opening is closed throughout due to rapid cell proliferation around the opening. Some epidermal cells on the ostiolar end form pointed hairs.Histopathology, physiology and histochemistry of insect and mite induced galls has also been studied by Patni and Arora (2000), Choudhary (2015), Mellah *et al.(*2016) and Mishra *et al.* (2020).

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