#### J. Phytol. Res. 1 (1), 1988

# ANATOMICAL STUDIES ON ZIZYPHUS MAURITIANA LAMK. LEAF GALLS

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Development and histopathology of leaf galls on Zizyphus mauritiana incited by unknown Itonididae (Diptera) have been studied. Galls show total inhibition of differentiation of the normal tissue of mesophyll. The mesophyll is represented by simple undifferentiated parenchyma. Calcium oxalate crystals are observed in many cells of the gall parenchyma. In older gall 3-4 layers of hypodermal cells adjacent to both adaxial and abaxial epidermis become thick walled. Gall cavity contains one or two cecidozoa. Gall development is initiated by attack of cecidozoa on the abaxial surface of the leaf. Gall is really a swollen part of the leaf, developed by hypertrophy and hyperplasia in the affected area of the blade. As a result, the affected part swells out of the level of leaf.

Keywords : Zizyphus mauritiana; Leaf gall; Histopathology.

#### Introduction

Studies on origin, development and anatomy of 'Insect' induced plant galls on certain plant species were made by number of workers (Mani, 1948, 1959, 1964; Kant and Arya, 1971; Raman, 1981; Raman and Ananthakrishanan, 1983; Agrwal, 1983; Lalonde and Shorthouse, 1984; Sokhi and Kapil, 1985). Stem galls on Zizyphus mauritiana Lamk, induced by Eriophyes cernuus and leaf galls caused by unknown Itonididae (Diptera) have been reported by Mani (1973). Anatomical studies of stem gall have been worked out by Kant (1967). Present work is concerned with anatomy and development of galls produced on the leaves of Zizyphus mauritiana Lamk. Leaf galls are common is South India.

#### **Materials and Methods**

Plant material (gall and normal leaf) was collected from Sawai Madhopur (Ranthambore forest, Rajasthan) in the month of September. Leaves with galls of various ages were removed. The material was fixed in acetic acid alcohol (glacial acetic acid and 100% ethanol, 1:3). Dehydration, clearing and embedding were done according to tertiary butyl alcohol method as recommended by Johansen (1940). Microtome sections were cut at the thickness of 7-13 $\mu$  and stained with Johansen's Safaranin-Light green staining combination.

# **Observations**

Structure of normal leaf-Cells of the adaxial epidermis of the normal leaf are large, cylindrical and unequal in size. The cells of the abaxial epidermis are rectangular and smaller in size as compared to the adaxial epidermis. Mesophyll cells are arranged in definite layers and some cells of this zone are having calcium oxalate crystals. Adjacent to the adaxial epidemis, there is a layer of cylindrical palisade cells. The palisade cells are arranged tightly with few apparent intercellular spaces. Below the palisade lies spongy parenchyma. Cells of spongy parenchyma are irregular in shape with large intercellular spaces. Stomata are present only on the abaxial surface. The vascular bundles are embedded in the mesophyll tissue surrounded by thick walled cells (Figs. 6 and 15).

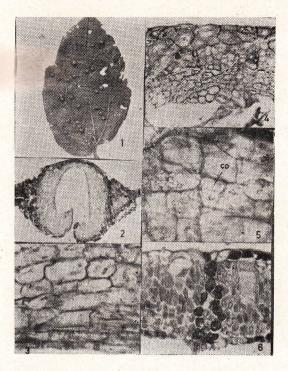
*External morphology*-Galls are sublenticular, free, globose, unilocular, indehiscent, persistant, deep reddish brown to rusty brown, smooth and visible on both sides of the blade. There are 6-20 galls on a leaf. Size of the gall is 2-4 mm in diameter. Mature gall is having wide opening on the underside of the leaf (Fig. 1).

*Gall anatomy*-The epidermis of gall on the adaxial surface is continuous with leaf epidermis of the unaffected part. However, gall epidermal cells ere rectangular, thick walled and small in size (Figs. 4 and 16). Like the normal leaf the stomata are not observed on this side of the gall. The abaxial epidermal cells are thin walled and rectangular in shape. Stomata are absent on this side of the gall unlike the normal counter part.

The Zizyphus leaf gall is remarkable for total inhibition of differentiation of the normal tissue of mesophyll (Figs. 4 and 16). There is no trace of the palisade or the spongy parenchyma of the normal leaf. The mesophyll is represented by a simple undifferentiated parenchyma. The gall consists mainly of thin walled, large sized, closely packed with apparent interce-Ilular spaces parenchyma cells (Figs. 3 and 11). The cells are generally rectangular or polygonal, calcium oxalate crystals are observed in many cells of the gall (Figs. 5 and 12). The frequency of calcium oxalate crystals was more in this part as compared to normal. In older gall, cells of adaxial epidermis and 3-4 layers of hypodermal cells adjacent to both adaxial and abaxial epidermis become thick walled (Figs. 4 and 10). The gall cavity is generally oval and contains one or two cecidozoa. On the under side of the leaf ostiole is present which becomes very wide on maturity (Figs. 2 and 13).

## Gall development

The various stages of gall development are shown In Figs. 7-10. The insect generally attacks the young leaves. New attack may be made continuously on young leaves. As a result,



large number of galls appear at their various stages of development on the same leaf.

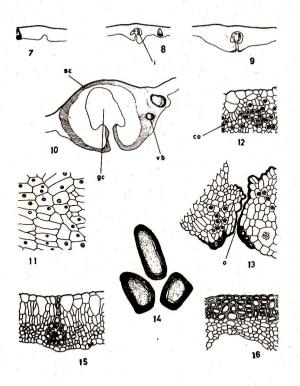
After the attack, some cells in the affected region are stimulated to divide and form new cells. The seat of cell proliferation is the mesophyll parenchyma of the leaf. Cells of this region multiply rapidly to form large number of parenchymatous cells. Cell hypertrophy and hyperplasia is responsible for increase in gall bulk. The cells in palisade region also divide alongwith the spongy parenchyma. Four to six layers of sclerenchyma cell develop around the gall (Figs. 10, 14 and 16). These layers of sclerenchymatous cells constitute the mechanical zone.

Figs. 1-6

Leaf galls of *Zizyphus mauritiana* Lamk. 1 Galls on leaf; 2 median longitudinal section of old gall X65; 3 gall parenchyma X 500; 4 old gall X250; 5 gall parenchyma with calcium oxalate crystals X500; 6 normal leaf (C.S.) X250.

Thus Zizyphus mauritiana leaf gall is really a swollen part of the leaf. Hypertrophy and hyperplasia spread in the affected area of the blade. Simu-Itaneously, both outer and inner epidermal cells, keep pace with the process of division. As a result, the affected part swells out of the level of blade. The parenchyma cells enlarge during the gall development. The gall parenchyma between adaxial and abaxial epidermis is much thicker as compare to mesophyll of the normal leaf.

Parenchyma cells surrounding the cecidozoa constitute the nutritive tissue. The cells of this region are large in size with prominent nuclei and dense cytoplasm (Fig. 3). The mature Dhingra & Singh



gall opens by a wide opening through abaxial side (Figs. 2, 10 and 13).

# Discussion

The present work is undertaken in an attempt to understand the histopathology and gall development. Gall structure primarily depend on the organ on which they are formed, the species of plant and cecidozoa. The present study reveals that hypertrophy and hyperplasia are commn in gall development and leaf galls of *Zizyphus mauritiana* are invaginated and swollen part of the leaf.

One of the interesting feature of the gall epidermis is the structure and

### Figs. 7-16

Leaf galls of *Zizyphus mauritiana* Lamk. *Figs.* 7-10 Comparative stages of gall development (Median longitudinal sections) X32; 11 parenchyma cells X374; 12 calcium oxalate crystalsX233; 13 ostiole X266; 14 sclerenchyma cells X1600; 15 normal leaf ( C. S. ) X 233; 16 old gall X 233

(i=insect; o=ostiole; co=calcium oxalate crystals; gc=gall cavity; sc=sclerenchyma; vb=vascular bundle)

distribution of stomata. In general, the stomata are fewer on gall epidermis than the normal one and in some cases they may be totally absent (Mani, 1964). In present study it has been observed that stomata are devoid on both inner and outer epidermis. Similar conditions have also been reported in other galls (Kant, 1967; Sharma, 1976).

Calcium oxalate crystals are frequently observed in the parenchymatous cells of the gall. Gall parenchyma of *Zizyphus* leaf are loaded with these crystals, while in normal counterpart the calcium oxalate crystals are present in some cells. Presence of calcium oxalate crystals have also been reported in crown gall (Jones, 1947), virus tumors (Burkholder and Nickell, 1984), legume nodule (Spratt, 1919) and 'Insect' induced gall (Hough, 1953; Kant, 1967; Sharma, 1976). Hough (1953) believed that calcium oxalate cryatals in gall tissue are correlated with abnormal metabolism because they are formed in greater amounts in abnormal tissues than in normal.

The mechanical tissue is the common feature of most of the prosoplasmic galls. The tissue generally consists of thick walled cells. The development and arrangement of mechanical tissue differs in different galls and show remarkable complexity. In majority of Diptera galls, the mechanical tissue is radially distributed round the larval chamber (Mani, 1948). During the present investigation 3-4 layers of cells of mechanical tissues around the gall chamber have observed.

The present investigation has thrown open certain interesting aspects of histopathology of gall in plants. The study may prove useful in the experimental part of the problem.

Accepted August, 1988

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