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Research Article

HISTOPATHOLOGICAL STUDIES ON STEM GALL OF *ACACIA LEUCOPHLOEA* WILLD. INDUCED BY INSECT

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ABSTRACT
Galls or cecidia are abnormal plant growths caused by various organisms such as bacteria, nematodes, viruses, insects and mites. The abnormal growth in plants provide interesting object of study because of their pathological significance and economic importance. Stem galls of *Acacia leucophloea* Willd. induced by cecidozoan *Sphadasmus braminus* Pascoe were used for histopathological studies with their normal counterpart. The objectives of the present investigation was to study the possible alternation in the anatomical characters due to insect attack on *Acacia* stem and host pathogen interaction. The investigation revealed that hypertrophy and hyperplasia are important in gall development. Gall is initiated by larva of insect. Larval cavity is surrounded by proliferated cells and affected portion showing swelling. Cortex and medullary rays increased as compared to the normal counterpart.

Keywords*: Histopathology, Gall, Acacia leucophloea, Sphadasmus braminus*

INTRODUCTION
Acacia leucophloea Willd. (white babool) is an economically and medicinally important plant of arid and semi arid regions like Central India, Punjab, Rajasthan, Burma, Malaya etc. In Rajasthan "Rhunj" is the common name for this plant. It is a perennial woody legume of family Fabaceae, sub-family Mimosoidae. Trees are densely branched. Bark is whitish grey. Spines are short, straight, leaves bipinnate, pinnae 5-12 pairs. Leaflets are 10-30 pairs, linear, crowded about 5×1 mm. Flower head is small, pale yellow, terminal, densely tomentose panicle. The leaves are eaten as fodder by goats. The plant has great medicinal value. The bark of *Acacia leucophloea* Willd. is medicinally important. It is bitter, acrid, alexiteric, antihelminthic, antipyretic, useful in leprosy, thirst, vomiting, spermaturia and diarrhoea etc. (Guhabakshi *et al.*, 1999).
Galls are unique examples of complex inter-specific interaction and mutual adaptation between plants and gall inducing agents. These are limited or unlimited neoplasm (Mani, 2000). Galls formed by the interaction of insect on plant tissues are an example of the unusual transformation and use of plant by insect (Guzicka *et al.*, 2017). In recent years, the study of gall has gained considerable significance due to the possibility of understanding the etiology of animal and human cancers. Diverse species of insects which infect plant tissues to spend a part of their life cycle cause gall formation. Gall morphogenesis is the result of interaction between morphogenetic control of the plant body and their insect factor (Miles, 1968).
Histopathology is useful for studies of anatomical alteration of host tissues and development of galls. Histopathological studies when correlated with physiological and biochemical ones could be helpful to determine the fundamental basis for plant tissue alteration that occur during insect parasitism (Choudhary, 2015).
The present investigation deals with the histopathological studies of stem gall of *Acacia leucophloea* induced by cecidozoan *Sphadasmus braminus* Pascoe (Coleoptera).

MATERIALS AND METHODS
The stem galls of *Acacia leucophloea* induced by cecidozoan *Sphadasmus braminus* Pascoe (Coleoptera) appear in the rainy season (July to November).

Different developmental stages of stem gall induced by *Sphadasmus braminus* Pascoe and normal counterpart of *Acacia leucophloea* Willd. and its normal counterparts were collected from the plants around Jaipur 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

Results

External Morphology of the Gall

Galls generally appear on new tender branches in late July or early August. Stem galls are sub globose, oval or fusiform, hard, woody, unilocular, indehiscent, persistent tumescence of tender branches, nearly of the same color as ordinary branches (Figure 1A, B). Generally, only one gall develops on each branch, but sometimes two to three galls may also develop in a series on the same branch. In such cases the youngest gall lies towards the growing tips. Each gall possesses large or fusiform gall cavity (Figure 1C). The larval cavity communicates by a circular hole to the outside of the gall. It is irregularly placed on the side of the gall. Mature gall measures 20-35 mm in length and 10-15 mm in diameter (Figure 1B).

Structure of Normal Stem

The young normal stem has an epidermis, which consists of a single layer of rectangular cells with heavy cuticle. The cortex is 5 to 9 layered and is composed of parenchymatous cells. Cells of this region are rounded or oval without intercellular spaces between them and contain chloroplast in inner layers. The vascular bundles are collateral and surround the pith. The pith cells are parenchymatous and round or polygonal in cross-section (Figure 1D). In older stems the pith cells which are towards the vascular region become thick walled. The pericycle consists of patches of sclerenchyma predominantly (Figure 1D).

In older stem the secondary growth is typically dicotyledonous type. The vessels have a large diameter. The rays in the secondary xylem are uniseriate or multiseriate (Figure 1D).

Gall Anatomy

The epidermal cells of the gall are parenchymatous and rectangular. There is a thick coating of cuticle on the epidermis. In older galls however, the cork develops on the entire gall surface and the epidermis cracks off and peels away. The cork cells are rectangular, parenchymatous and light brown in color. The cortex is parenchymatous, comprising of 10 to 15 layers. Cells are polygonal and are with minute intercellular spaces. Cortical cells are highly proliferated and add significantly to the width of the gall (Figure 1H). The pericycle is composed of sclerenchymatous cells and is not so conspicuous as in the normal stem. The vascular cylinder is reduced in extent (Figure 1H). Secondary vascular tissues are separated from each other by broad rays and parenchymatous cells (Figure 1H). The diameter of vessels is smaller than that of the normal. The phloem is represented by undifferentiated parenchymatous layers. The rays in wood are uniseriate or multiseriate. The medullary rays show prominent cell proliferations and the vascular tissues are widely separated. The cells of medullary rays are thin walled and possess granular cytoplasm. The larval cavity is situated in the middle of the gall and most of the pith is replaced by it (Figure 1E, H). It is surrounded by a nutritive region, which is formed by proliferated medullary tissues (Figure 1F). The cells of the nutritive region are parenchymatous and possess granular and dense cytoplasm with tannins (Figure 1F).

Gall Development

The eggs of the insect are laid near the tips of the young tender branches during the rainy season. The larva on hatching bores into the branch and prepares a larval cavity in the medulla (Figure 1H). Thus, the

gall formation is initiated. In very young galls the entrance hole is minute, circular and lies just below the growing tips. It persists even in mature galls but is loosely plugged by the faecal pellets of the larva (Figure 1I). The visible effect of the larva is swelling of the affected portion, and stunting of the lateral branches. The tip generally continues to grow (Figure 1A) but in extreme cases its growth is arrested.

In very young galls, the pith is present with a small larval chamber. During the process of boring through the medulla, pith cells are crushed and a cavity is formed (Figure 1G). The rest of the pith cells, surrounding the larval chamber undergo cell proliferation. The medullary rays show pronounced cell proliferation, which result in wide dissociation of the vascular tissues. At places near the larval chamber, actively dividing medullary cells form lobes of hypertrophied cells (Figure 1F). The larval cavity is surrounded by proliferated cells which are contributed by pith cells and medullary rays. This region of the gall constitutes the nutritive region. Cells of the nutritive region are characterized by granular and dense cytoplasm. In very young gall the cortex is 7 to 9 layered. It is parenchymatous with few peripheral layers containing chloroplast. Subsequently, as the gall increases in diameter, there is proliferation of cortical cells. Cell proliferation of the cortex adds significantly to the width of the gall. Secondary, growth in galls is similar to the normal stem. The vascular cambium cuts secondary xylem and phloem towards inner and outer sides respectively. The secondary xylem is composed of thick walled elements with narrow vessels. The vascular cylinder is reduced in extent. Secondary growth results in patches of vascular tissues separated by broad rays. In older galls periderm is formed. The phellogen arises around the sub-epidermal region.

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. The Angiosperms, especially the Dicotyledons are remarkable for bearing large number of galls (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 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 present study revealed that both the processes namely hypertrophy and hyperplasia are important in gall development. In *Acacia leucophloea* Willd stem gall formation is initiated by larva of the insect. The visible effect of the larva is swelling of the affected portion and stunting of the lateral branches. The main bulk of the gall develops from cortex and medullary rays. The medullary rays undergo pronounced cell proliferation. Larval cavity is surrounded by proliferated cell which is contributed by medullary ray tissues.

During gall development hypertrophy and hyperplasia are induced in the entire cortex. Cell proliferation of the cortex adds significantly to the width 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.

Secondary, growth in galls is similar to the normal stem. The secondary xylem is composed of thick walled elements with narrow vessels. The vascular cylinder is reduced in extent. Secondary, growth results, in patches of vascular tissues separated by broad rays.

Similar observations were also made by Kant (1967) and Ranwa (1983) 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. Hypertrophy and hyperplasia are important in gall development (Mathur, 2002). The histopathology, physiology and histochemistry of insect and mite induced galls has also been studied by Patni and Arora (2000)**,** Raman *et al*., (2006) and Koncz *et al.,* (2011).

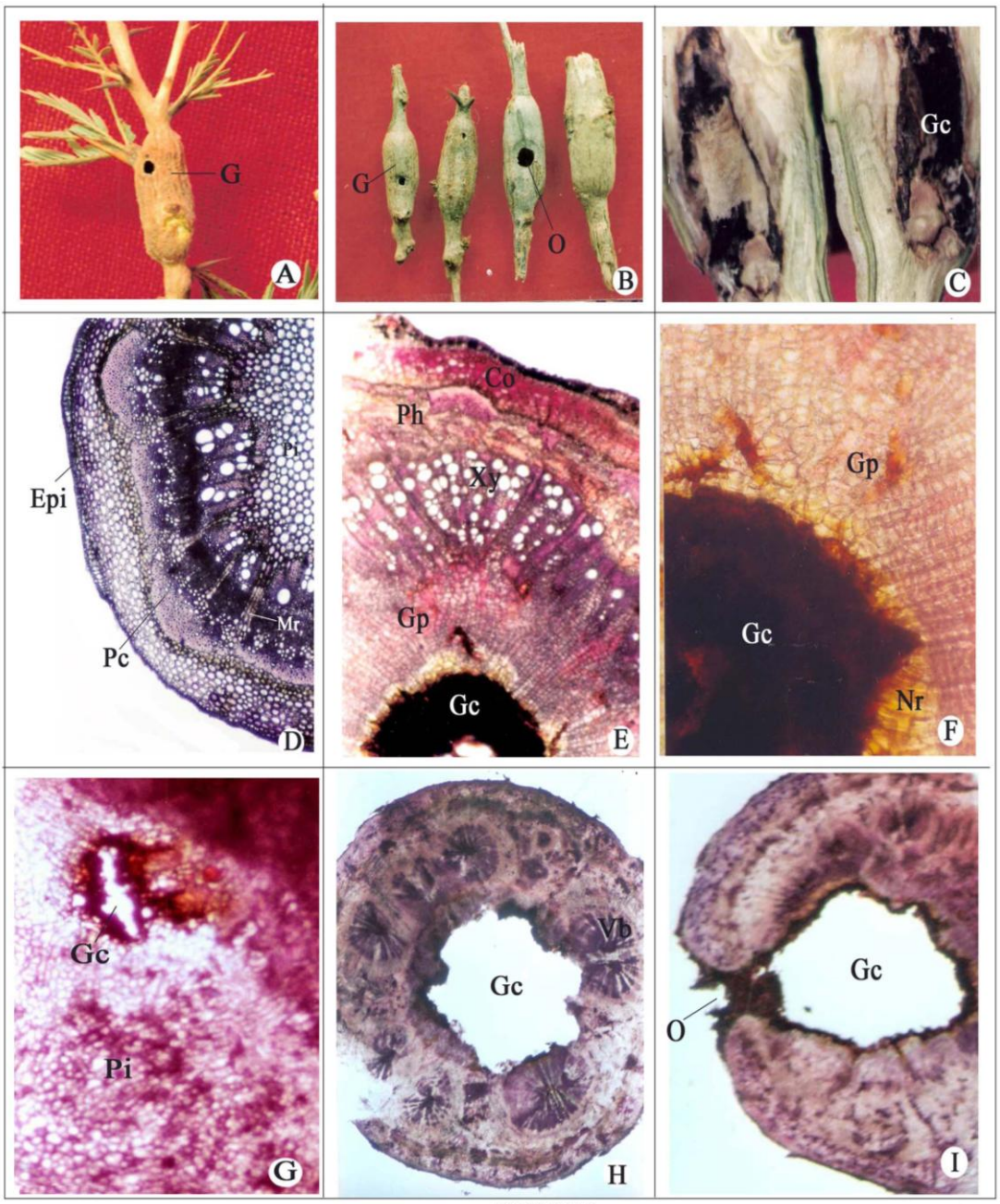

FIGURE-1

A. Stem gall of Acacia leucophloea Willd.
B. Stem gall of Acacia leucophloea showing various stages of gall.
C. Stem gall split open × 30.
D. T.S. of normal stem of Acacia leucophloea Willd × 300.
E. T.S. of stem gall of Acacia leucophloea Willd. induced by Sphadasmus braminus Pascoe showing gall cavity × 300.
F. T.S. of stem gall showing gall parenchyma around the gall cavity × 600.
G. T.S. of stem gall of Acacia leucophloea Willd., showing initiation of gall cavity in pith region. × 120.
H. T.S. OF stem gall showing separate vascular bundles in a ring and gall cavity × 120.
I. T.S. of stem gall showing plugged ostiole in mature gall × 120.

Abbreviations used :
O = Ostiole, G = Gall, Gc = Gall cavity, Epi = Epidermis, Pc = Pericycle, Mr = Medullary rays, Pi= Pith, T.S. = Transverse section, Co = Cortex, Xy =Xylem, Ph= Phloem, Gp = Gall parenchyma, Vb = Vascular bundle, Nr = Nutritive region.

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