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Morphological and Anatomical Studies of the Ovary Galls of *Sesamum indicum* l. Induced by the Gall Midge, *Asphondylia sesami* Felt

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Abstract

The organization of gall on the ovary of *Sesamum indicum* L induced by the gall midge, *Asphondylia sesami* Felt, not only affects biochemical equilibrium of the host but also causes disturbed vegetative growth and reduced seed-setting. The toxicity created by the gall maker induces the re-orientation of vasculature Cecidogenetic stimuli lead to excessive hypertrophy and hyperplasy forming the gall chambers. Each gall chamber is lined by a layer of dense mass of cells called nutritive zone. Vasculature is disturbed during gall formation. One to few larvae are present in a larval chamber. Occurrence of insect galls in the ovary of this taxon is remarkable and it is a new record in the field of cecidology.

Keywords: Anatomy, Morphology, Ovary galls, Plant insect interactions.

INTRODUCTION

In a healthy plant, all the physiological functions are performed in co-ordinated and harmonious manner. Any deviation from this leads to disturbance in metabolism thereby altering the usual mode of ontogeny (Mehalingam, 1999). Plant galls represent such growth modifications (Floate *et al*., 1996). Galls are neoplastic growths resulting from the reactions of a plant species to various kinds of stimuli (Floate *et al.*, 1996, Fernandes and Price, 1988). Plant galls represent an unique and complex interspecific interaction and mutual adaptation between the host plant and the gall maker (Mani, 1964). The nature and origin of the inter-relation, the role of the gall inducing organism, and the reaction of the plant as well as the cytological, histogenetic and ontogenetic processes in gall organization are remarkable morphogenetic problems (Mehalingam, 1999). Insect-induced galls are atypical plant growths which result from a stimulus applied during the feeding activities of specialized insects. The attacked organ loses control over the growth potential of the affected area and the insect redirects tissue proliferation to its own advantage (Rohfritsch and Shorthouse, 1982). During ontogenetic studies on plant galls, we came across the occurrence of galls in a member of Pedaliaceae, *Sesamum indicum* Linn. induced by the gall midge, *Asphondylia sesami* Felt (Mehalingam, 1999). A perusal of the existing literature reveals that there are no earlier reports on the ovary galls in this taxon. Therefore, a detailed study was undertaken to elucidate the morphogenetic aspects of galls in this taxon and the observations made are presented below since it is the first record of ovary gall in Sesamum indicum.

MATERIAL AND METHODS

Normal and galled ovaries of *Sesamum indicum* were collected from Periaperali village, Virudhunagar district. Galls representing sequential stages of development were fixed in FAA. Customary methods of microtechnique were followed. Sections were cut at thickness of 10-12 µm using rotary microtome. Sections were stained in Heidenhain's iron alum- Haematoxylin with dilute Erythrosin in clove oil as a counterstain (Johansen, 1940).

RESULTS

External morphology of normal plant

It is annual, erect, branching herb. The stem is quadrangular, herbaceous and the lower portion is woody, hairy and contains mucilage. The leaves are simple, petiolate, alternate above and opposite below, lanceolate, entire or lobed, often varying on the same plant. Flowers are solitary, axillary and shortly pedicellate. There are two nectary glands, one on either side of the pedicel at its base, and hence, Taxonomists are of the view that the flower is a reduced form of cyme. Calyx has 5 sepals, 5-partite, persistent or deciduous. Corolla has 5 petals, bilabiate or foxglove-like or obliquely campanulate. Stamens 4, free epipetalous, didynamous and dithecous. Ovary superior and two celled, with false septa making it four celled (Fig. 3A). Ovules numerous arranged serially in each cell (Fig. 3B). stigma two lobed. Fruit is oblong, grooved, and beaked at the apex, loculicidally 2-valved, but 4-chambered, seeds numerous, compressed, white or black depending upon the variety.

Gall morphology

Sesamum indicum Linn. (Pedaliaceae) is an important oil-yielding cash-crop plant in India. The organization of gall on the ovary induced by the gall midge, *Asphondylia sesami* Felt, not only affects biochemical equilibrium of the host but also causes disturbed vegetative growth and reduced seed-setting. In this case, the ovules are eaten away by the gall midge, Asphondylia sesami. This is characterized by the transformation of the ovary and finally reduced yield. All abnormalities formed in the ovary ultimately give a reduction of seeds. Cecidogenesis completely arrests seed-setting in the double galls, reniform galls and spherical galls. Consequently, there is 100% reduction of yield of seeds in three types. Ovary gall is one of the most serious diseases of sesame as it arrests the yield causing severe loss to farmers. These ovary galls are formed during February- March.

From the comparative morphological study of healthy plants and plants with galls (Table 1), it is observed that they are found in the ratio of 5:1 in the experimental plot. It is evident from the table that the healthy plants are taller and occupy more breadthwise area than the plants with galls. Relative shoot and root length of healthy plant is more than in plants with galls. The healthy plants are broader than affected plants at their base just above the ground level. The healthy plants have more number of branches. The healthy plants are gregarious in growth and the infected ones are stunted. Average length and breadth of capsule and the number of seeds in healthy plants are more than those of the plants with galls.

The young galls are small, contorted, curved or globose in shape, and measure a length of 0.5 to 1.0 cm. They are mostly semi-circular or reniform in outline. Spherical galls are found on the two adjacently placed nodes (Fig. 1B). Sometimes, galls and normal capsules are intermingled in different nodes (Fig. 1C & I). The upper node bears a reniform gall while the two proximal nodes possess gall in the middle portion of one locule (Fig. 1D). Rarely, all the ovaries are transformed into galls (Fig. 1E). The proximal and distal nodes contain ovary galls while the capsules present in the middle nodes are not involved in gall formation (Fig. 1A). Interestingly, the proximal and distal nodes possess normal capsules. However, the middle nodes bear galls (Fig. 1F). Sometimes, the distal node possesses normal capsules (Fig. 1G) or galled ovaries (Fig. 1H).

Classification of the galled ovary

On the basis of the developmental sequences and shape, ovary galls are classified into the following types.

1. Occurrence of gall in the distal, middle or proximal region of one locule of the ovary

The gall initiation is noticed in the distal (Fig. 2A), middle (Fig. 2B) or proximal (Fig. 2C) regions of the ovary. Due to the appearance of gall in the middle region of the ovary, the distal and proximal regions become bent outwards. Since the gall initiation occurs in the middle region of one locule, the lower and upper regions of the same locule and the entire opposite locule are not disturbed by the gall midge.

Due to galling effect, the length of the capsule ranges from 9 to 20 mm. Within the gall, there is a larval cavity in which the larvae live comfortably by feeding on the ovules. The gall cavity is lined by a layer of dense mass of cells called nutritive tissue. The gall development results in the deterioration of ovules in the infected area. There are normal seeds and aborted seeds.

2. Gall formation occurs on one locule completely

In this type, the gall development occupies one locule completely (Fig. 2D). No change occurs in the other locule. The length of the galled capsule is ranging from 8 to 18mm. In the galled locule, aborted seeds occur. The adult insect escapes from the galls through an ostiole.

3. Gall formation occurs on one locule completely and at the lower part of the second locule

In this case, one locule is completely affected. Moreover, the proximal part of the second locule is also affected by the gall-maker (Fig. 2E).There are no normal and aborted seeds in the first locule. The second locule contains normal seeds in the proximal end. The length of the galled ovary is 16mm. A small orifice is found in the galled region.

4. Gall formation occurs at the proximal portion of both the locules

The proximal portions of both locules are affected by the gall midge (Fig. 2F). The gall development proceeds from the proximal part of the locule. There are normal seeds in the unaffected part, and aborted seeds in the galled region of each locule. The length of the galled ovary varies from 14 to 19mm.

5. Gall formation occurs at the distal portion of both the locules

The distal portions of both locules are converted into gall (Fig. 2G). Interestingly, the gall induction takes place in the distal region of the ovary. Hence, the proximal portion is unaffected. There are aborted seeds in the galled region of each locule. The length of the galled ovary ranges from 7 to 12mm.

6. Double gall

In this type, both locules are completely converted into gall (Fig. 2H). During the early stages of gall development, the tip of the two locules is not disturbed. The length of the galled ovary measures about 10 to 11mm. The length of the gall is significantly reduced sequel to organization of gall. The gall formation, not only reduces the seed-setting, but also reduces the length of ovary. There are numerous hairs on the external surface of the capsule. An interesting feature observed is that the gall initiation occurs prior to anthesis.

7. Reniform gall

Some of the ovary galls become kidney-shaped. These reniform galls (Fig. 2I) are abundant in occurrence than the other types. The speciality of this gall is that one of the locules is completely converted into kidney-shaped gall. Due to gall formation on one locule, the seed development in its counterpart is completely arrested. The unaffected locule has only remnants of ovules. The length of the reniform gall is ranging from 4 to 9mm.

8. Deformed gall

Since the normal shape of the ovary is completely changed during cecidogenesis, these galls are called as deformed galls (Fig. 2J). The gall initiation occurs at the lower part of the capsule. Sequel to this, the gall becomes curved. Undeveloped seeds occur in the curved region. The number of normal seeds depends upon the curvature of the capsule.

9. Spherical gall

In this case, the entire ovary is modified into a spherical gall (Fig. 2K). Sometimes the tip of the ovary gall is pointed or slightly hooked. However, there are no seeds. Length of the gall varies from 4 to 9mm.There is an ostiole in the middle region through which the adult insect escapes.

Anatomical observations

Anatomy of the normal ovary

A transverse section of the normal young ovary is bilocular and bilobed with two rows of ovules in each locule. A false placenta develops between two rows of ovules of a locule, thereby giving a false tetralocular condition (Fig. 3A). Ovules are anatropous and are arranged in a linear row (Fig. 3B). Ovary wall consists of 10-15 layers of rectangular or polygonal cells. Inner cells are compactly arranged (Fig. 3A & B). Ovary wall is traversed by several bundles forming vasculature. Multicellular epidermal hairs are prominent.

Anatomy of the mature galled ovary

Outer layer of mature gall is uniseriate. This is followed by several layers of hypertrophied, polygonal parenchyma cells. Vasculature is disturbed during gall formation (Fig. 3C).One to few larvae are present in a larval chamber (Fig. 3D-H). A few smaller and densely packed cells form the nutritive zone around the larval chamber (Fig. 3I). Cells bordering the exit hole begin to re-arrange thereby leaving a narrow orifice (Fig. 3J).

Sequential ontogeny of the ovary gall

The female adult insect of *Asphondylia sesami* is attracted by nectary and colour and odour of the corolla. The insect tries to search for suitable oviposition sites. Eggs are laid in the surface of ovary wall or into the ovary wall, placenta and ovules (Fig. 3K-O). After the eggs are oviposited, the mother insect flies away. The wound caused during oviposition is healed and so a scar is not visible. These sites provide optimum conditions for incubation. These eggs usually hatch 5-10 days after oviposition. As soon as the first instar larva hatches even before leaving the egg chorion, the host tissue is attacked with its mouth parts. The feeding behavior and secretion of saliva alter the biochemical equilibrium around the larva. The larva is enclosed in the larval chamber (Fig. 3D-H). The toxicity created by the gall maker induces hypertrophy, hyperplasy and re-orientation of vasculature (Fig. 3C). Smaller and densely packed layers of cells organize a nutritive zone around the larval chamber (Fig. 3I). Size of the gall increases until attaining mature stage. Subsequent to second instar stage and third instar stage, larva metamorphoses into a pupa. Finally, adult insect stage is attained. It tunnels the host tissue forming an ostiole and escapes from the gall (Fig. 3J).

Sometimes, eggs are laid in the sepal and corolla (Fig. 3L & M).These eggs don't hatch into larvae and therefore, galls are not initiated. This is presumably due to lack of morphogenetic potentialities of these organs to organize into galls.

DISCUSSION

The availability of young ovaries of *S. indicum* is a major limiting factor and an important prerequisite for the gall maker *Asphondylia sesami* to develop and breed by organizing galls. This conclusion is in agreement with that of Varadarasan and Ananthakrishnan (1981). The formation of ovary gall in *Sesamum indicum* leads to disturbed vegetative growth and reduced seed setting. Etymologically the name 'flower gall' in Sesamum indicum, as it was quoted by Mani (1973), becomes a misnomer since it is the ovary that is transformed into a gall. Hence, the term 'ovary gall' is found to be more appropriate and therefore, the apt term ovary gall is used in the present study. Based on the exomorphological and anatomical features, the ovary galls are classified into nine types. Such a classification has not been made anywhere, even in the works of Mani (1964, 1973), although a comprehensive study on gall morphology of various plants has been made. It will be apt to quote from Mani (1973).

Sesamum indicum Linn. Gall no 251 *Asphondylia sesami* Felt (Diptera) flower gall, irregularly shaped, solid fleshy contorted swellings of flowers with 4 to 5 larvae of the gall midge, often very heavily parasitized by *Eurytoma dentipectus* Gahan, Distribution: South India and Uganda. Except for the above mentioned few lines, nothing has been reported on the developmental and anatomical aspects especially on the ontogeny as for as the ovary galls in *S. indicum* concerned. Anatomical observation made on the ovary galls of *S. indicum* for the first time, report the presence of eggs in the ovary wall, placenta and ovules. Larvae are seen in the gall chamber. Similar observations were made in other taxa by Norris (1979) and Mathur Rajamani (1984) The presence of larva and continuous feeding in the ovary wall stimulate the cells of the ovary wall to divide continuously (hyperplasy) and enlarge in their size (hypertrophy) resulting in the formation of gall. The initial gall development involves the stimulation of cell division leading to altered morphogenesis .The varied shapes of ovary galls in the same plant suggests the involvement of more than one hormone or substances causing cecidogenesis as suggested by Krishnan and Franceschi (1988). Similarly Susy Albert *et al.,* 2011 noticed that the hypertrophy has been followed by hyperplasia and brings about elevation of hypodermal and palisade parenchyma which undergoes repeated anticlinal divisions in *Alstonia scholaris*.

The tracheary elements prior to cecidogenesis, they are thrown towards the larval cavity in the nutritive zone. The occurrence of such vascular strands in the gall tissue has been observed in several foliar and stem galls (Jayaraman, 1980). These stands are designated as 'irrigating strands' by Jayaraman (1980) in the sense that they conduct nutrition to the developing larva in the larval chamber.

In order to achieve a functionally efficient organic compromise by differential elaboration of the tissues in gall systems, the functional elaboration in the cells closer to the feeding sites of the gall maker and the elaboration in terms of morphological criteria of the cells away from the nutritive zone appear impressive. Thus, the galling phenomenon appears to be a turn key mechanism in morphogenesis where the plant organ deviates from the normal histogenetic patterns and incidentally subserves the gall maker.

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Tables

Table 1. Morphological variations of normal and galled plants of *Sesamum indicum* Line.

Sl No	Parameters	Normalhealthy plant	Galled plant
1	Plant height (cm)	90.4± 2.23	70.65± 2.63
2	Length of the root (cm)	17.05 ± 0.61	12.8± 0.578
3	Thickness of the stem (cm)	4.15 ± 0.18	3.0 ± 0.085
4	Area covered by the shoot (cm)	68.4± 1.74	41.03 ± 3.29
5	Number of branches	29.7 ± 2.12	18.2 ± 1.9
6	Number of leaves	593 ± 75.93	203.1± 25.66
7	Number of normal capsules	345.4± 37.09	123.9 ± 17.9
8	Length of normal capsules (cm)	2.99 ± 0.912	2.5 ± 0.065
9	Breadth of normal capsules (cm)	0.66 ± 0.02	0.51 ± 0.02
10	Number of galled capsules	Nil	18.2 ± 1.64
11	Number of normal seeds per capsule	62 ± 1.7	10.2 ± 0.21

* Values are mean + S.E

Figures

Fig. 1 A-I – Ovary Galls of *Sesamum indicum*
A. Galls (arrow) and normal capsules intermingled in different nodes X 1
B. Two adjacently placed nodes, each possessing a spherical gall X 25
C. Galls (arrow) and normal capsules intermingled in different nodes X 1
D A reniform gall (arrow) in the upper node: Capsules in the two proximal nodes possess gall in the middle portion of one locule X 2
E: Galls present in all the nodes (Leaves removed for clarity) X 3
F: Distal nodes with normal capsules and proximal nodes with galls (arrow). Exit hole visible in the gall X 1
G. Galls in the distal nodes while proximal nodes contain normal capsules (Leaves removed for clarity) X 1
H. Galls and normal capsules intermingled X 1
I Galls (arrow) in the middle nodes and normal capsules (C) in the proximal and distal nodes. (Leaves removed for clarity)

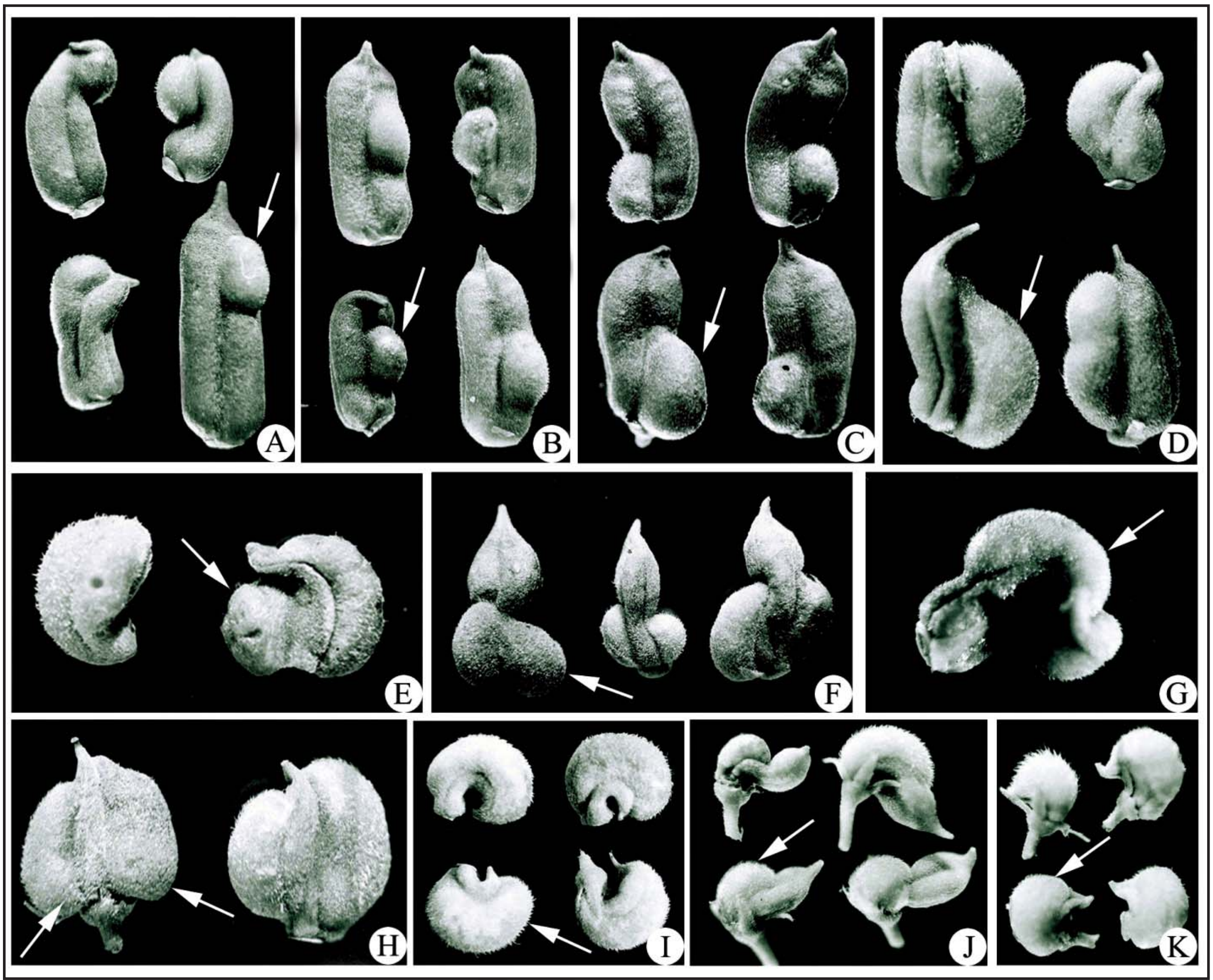

Fig. 2 A-K – Ovary Galls of *Sesamum indicum*
A. Occurrence of gall (Arrow) in the distal region of one locule of the ovary X 2
B. Gall (Arrow) initiation takes place in the middle portion of one locule of the ovary X 2
C. Gall (Arrow) formed in the proximal part of one locule of ovary X 2
D. Gall formation occurs on one locule completely X 2
E. Gall formation occurs on one locule completely and at the lower part of the second locule in the middle row (arrow) X 1
F. Gall (arrow) formation occur at the proximal portion of both the locules X 2
G Galls (arrow) formed in the whole distal part while the proximal part remaining normal X 3
H Double gall X 3
I Reniform galls X 2
J: Deformed galls X 2
K: Spherical gall X 2

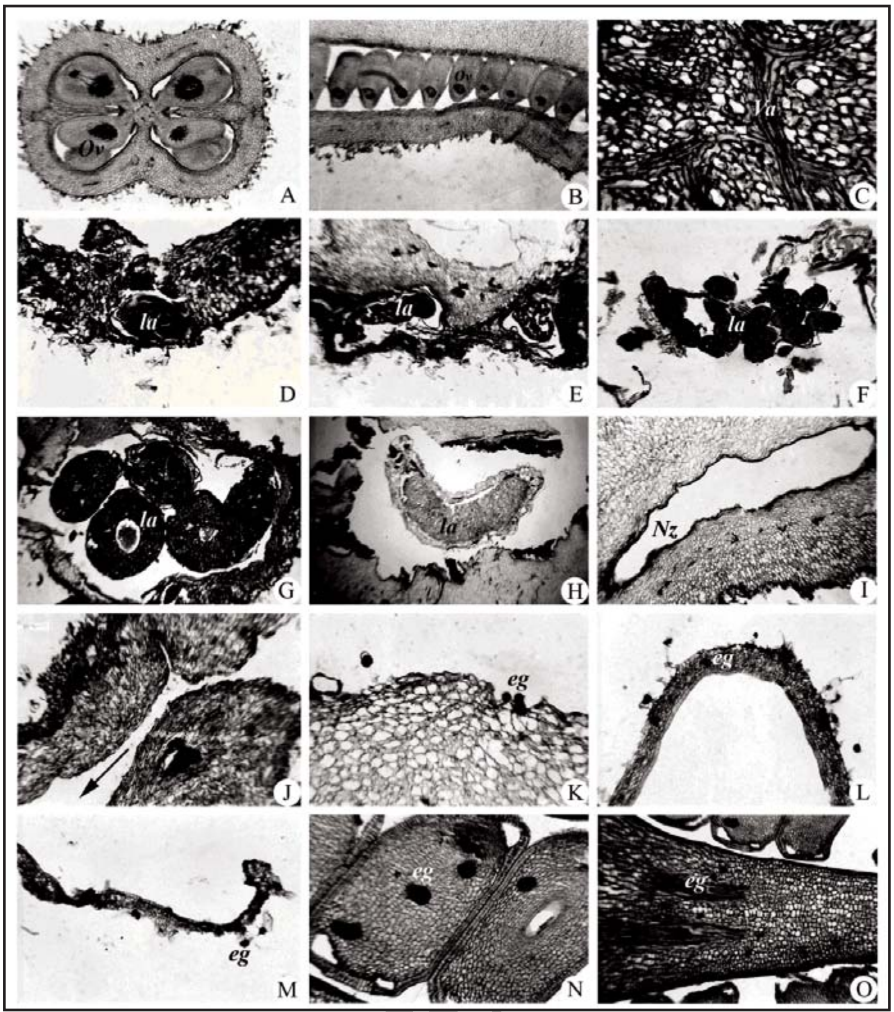

Fig. 3 A-O – Ovary Galls of *Sesamum indicum*
A. T. S. of young normal ovary with ovules (*Ov*) and false placenta x 100
B. L.S. of young normal ovary showing serially arranged ovules (*Ov*), placenta and ovary wall X 120
C. T.S. of gall tissue with hypertrophied cells and inter-connected vasculature X 100
D. T.S. of a part of ovary wall with an embedded larva (*la*) X 100
E. Same with two larvae (*la*) X 100
F. An enlarged view of larval chamber with several larvae (*la*) cut transversely X 120
G. Larval Chamber with 4 larvae (*la*) X 150
H. Same with an obliquely cut larva (*la*) X 120
I. T.S. of gall showing nutritive zone (*Nz*) around the gall chamber
J. T.S. of mature gall showing a tunnel-like ostiole (arrow) X 100
K. T.S. of a part of ovary wall showing eggs (eg) X 100
L. T.S. of corolla tube showing eggs (eg) X 100
M. T.S. of a sepal showing eggs (eg) X 100
N L.S. of an ovule showing eggs (eg). X 200
O. L.S. of placenta and ovule with eggs (eg). X 100

