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On the macro-micro-morphology of organs of host invasion in hemiparasite *Helicanthes elasticus* (Desv.) Danser



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ABSTRACT

Loranthaceae family includes hemiparasitic members which are seen invading a wide range of commercial crops. *Helicanthes elasticus* (Desv.) Danser is very common on mango trees. Though parasitic in nature, this mistletoe is also medicinally important as fetoprotective, against vesicular calculi and kidney infections. This study is an attempt to document macro-microscopical features of parasitic root, fruit and host-mistletoe tissue interaction in the haustorium of *H. elasticus* growing on mango stems. Collection, preservation, sectioning, staining and photomicrography of the root, fruit and hostmistletoe union were done as per standard methodologies of anatomical studies. Though there is resemblance to the normal roots in morphology as well as anatomy, the microscopic finding of large number of branched stone cells in the roots is interesting. The morpho-anatomical features recorded would help in understanding the infection biology of this mistletoe. The eradication during the earlier stages of its establishment from seed or from the root creeping over the surface of the host can help in controlling this parasite infection on commercially important host plants.

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1. Introduction

Parasitic plants are seen distributed around the world in diverse ecosystems. Of the total parasitic flora, around 4000 species are hemiparasitic with self- contained photosynthesis (Tistel et al 2010). Mistletoes are the hemi-parasitic Loranthaceae members which have modified parasitic root capable of invading the aerial parts of other living plants for absorbing water and nutrients required for their survival. These ecological adaptations of mistletoes render them exotic life and have made them highly troublesome to the plants hosting them. Mistletoes are termed as hemiparasite because even being photosynthetically active they

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derive majority share of their carbohydrate from its host (Glatzel 1983). Their peculiar habitat provides them resistance from the drastic biotic and abiotic changes which proves to be deleterious to the growth and survival of many host plants (Marshall and Ehleringer 1990).

The mango mistletoe, *Helicanthes elasticus* (Desv.) Danser is one among the interesting mistletoe species invading almost every mango tree. A total of 46 other plant species have been reported to be hosts for *H. elasticus* (Sunil Kumar, 2011). Traditional medicines make use of the medicinally important mistletoes belonging to both *Loranthus* and *Viscum* genera (Lim et al., 2016). Medicinally *Helicanthes elasticus* is used as a fetoprotective agent and also in the treatment of kidney stones (Kirtikar and Basu, 1935). It is a dichotomously branched shrubby parasite with ovate to elliptic shaped three to five nerved leaves with obtuse tips; flowers are sessile clustered at the nodes; calyx tube ellipsoidal in shape and the limbs are cylindrical; corolla lobes are about 3 cm long; produces sub globose to ovoid berry (Gamble, 1967).

Many species of mistletoes affect commercial crops rendering ill effect on the productivity and life of the host. As far as these ecological adaptations of these species are concerned a keen observa-

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tion on their macro and micro morphology would serve the purpose of understanding the adaptation of these species. There are no previous studies related to the mode of infection of this mistletoe. The present study was taken up to carry out a keen observation on the macro microscopic features of the root which give rise to haustorium affecting the host plant. The fruit which acts as a propogule for new establishment was also undertaken.

2. Material and methods

2.1. Collection, authentication and preservation

Fresh fruiting twig with parasitic root and haustorial associations of *H. elasticus* parasitizing *Mangifera indica* stem were collected from Kasaragod District (12.547104°N/75.243773°E) of Kerala. The plant was authenticated at the Herbarium of King Saud University, Riyadh, Saudi Arabia. Fresh pieces of fruits, parasitic roots and haustorial association were preserved in Formaldehyde acetic acid alcohol (FAA) to carry out microscopic studies.

2.2. Microscopic study

Specimens preserved in FAA were first subjected to dehydration with graded series of tertiary-butyl alcohol as per the schedule (Sass, 1958) and slides prepared. The sections were stained with metachromatic Toluidine blue (O'Brien et al., 1964), safranin and fast green.

To study the cellulosic elements like vessels, tracheids, sclereids, stone cells etc. the sample was boiled in 4%KOH solution and the lignified elements were isolated by Schultz and Jeffery's maceration procedure (Johansen, 1940).

2.3. Photomicrography and description

Zeiss AxioLab trinocular microscope and Zeiss Stemi stereo microscope were used to capture the photomicrographs using different magnifications. For studying the intercellular components like crystals, starch grains and lignified walls polarized light and for histological study bright field light microscope were used. Descriptive anatomical terms provided by Metcalfe, 1964 and Fahn, 1987 were used.

3. Results

3.1. Macroscopy

H. elasticus is commonly found infecting young branches as well as large trunks of girth up to a foot or sometimes more than a foot. After the seeds germinates the roots are formed which moves deep into the host tissue. The mistletoes get established in the crown region of the host where it can harvest maximum sunlight (Fig. 1.1 and 1.2). Root-like structures found growing along the surface of the host plant are attached at intervals by peg-like haustorial penetrations which help in the attachment using the hold fast. Root which spreads on the surface of the stem of the host are cylindrical to somewhat flat, rarely branched, very long, gradually tapering, 4–12 mm in diameter, surface faintly longitudinally striated and dotted at places corresponding to lenticels, pale grayishbrown in color. At places roots penetrate through the host stem forming some protuberance on small branches (Fig. 1.3).

The fruit developed after strong establishment on the host is a sessile sub-globose to ovoid berry greenish to pink dotted in colour and measuring about five millimeters in length. The albumen is white in colour with five toothed apex, embryo is club shaped with minute knobs at the radicle end (Fig. 1.4).



1.4 Helicanthes elasticus Fruit RT - root; STK - stalk of the fruit

Fig. 1. Helicanthes elasticus with host Mangifera indica.

3.2. Microscopy

3.2.1. Parasitic root

TS of root is circular in outline. Parenchymatous cortex embedded with branched tricho sclereids and a narrow band of phloem encircled by lignified pericyclic cells are observed (Fig. 2). Detailed TS of parasitic root shows outermost few layers of irregular cork consisting of sinuously walled parenchyma gradually replaced by 10–15 layers of the thin-walled rectangular, tangentially elongated cells of the cork. Cortex is made up of 20-25 rows of parenchymatous cells embedded with branched sclereids; pericyclic region is characterized by the presence of isolated or groups of branched stone cells which often penetrate deeply into phloem and medullary ray (Fig. 2.1). Phloem is composed of sieve tubes, companion cells and phloem parenchyma in addition to uni to biseriate medullary rays. Ray cells extend and become narrower and elongated, few being scleriedal in nature. Starch grains are embedded throughout the parenchymatous cells of the phloem and cortex; cambium is not distinct, xylem shows isolated or occasionally groups of 2-3 vessels, rarely arranged in radial rows, fibers are plenty, thin-walled forming the major area of the xylem, parenchyma are non-pitted. Xylem rays almost run parallel and ends in isodiametric parenchymatous pith embedded with a few isolated often horse shoe shaped stone cells (Fig. 2.2).

3.2.2. Maceration

The macerated haustorial root shows the presence of thinwalled stone cells, sclerified medullary ray cells, parenchyma, branched stone cells and sclereid, cork in surface view, fragment



CT - cortex; ICK - inner cork; OCK - outer cork; PEP - peeling epidermis; PER - pericycle; PH - phloem; PI - pith; SG - starch grain; STC - stone cells; TSC - trichosclereid; VE - vessel; XY - xylem; XYP - xylem parenchyma; XYF - xylem fibre; XYR - xylem ray.

Fig. 2. TS of Helicanthes elasticus root.

of sclereidal fibre, group of interwoven branched sclereids, pitted vessels, fibre with forked tip, sclereids of different shapes, vessels in groups and isolated, different types of fibres, thick-walled fibre, fibre attached with parenchyma and vessel, group of pitted vessels, bundle of fibre attached with xylem elements, fragment of pitted vessel, fragment of honey-comb like vessel, medullar cells crossing parenchyma, phloem elements, pitted vessels, tracheids (Fig. 3A-V).

3.2.3. Host-mistletoe union

The internal anatomical association of host and mistletoe were observed by taking transverse and longitudinal section of host through haustorial association (Figs. 4 & 5). In transverse (Fig. 4.1) and longitudinal (Fig. 5) sections, the haustorial tissues were found to contain tracheids but no sieve tubes (Fig. 4.2). The cells of the host is seen to be mechanically compressed which can be contributed to enzymatic dissolution of host tissue helping in the penetration of intrusive organs. The cells of the haustorial intrusions are composed of elongated parenchyma cells (Fig. 4.3). Thin ellipsoidal disc formed by the deeper penetration and lateral flattening of the plugs can be seen below the cambial region adjacent to the centrally located host xylem (Fig. 5.1). Dark stained exudates are visible at the host parasite interface which helps in firm adhesion and deeper penetration (Fig. 5.2). As seen in Fig. 5.3 there is a union between the tracheary elements of the host and the haustorium which in turn will be helpful for the unimpeded flow of nutrients and water from the host to the parasite.



A - thin-walled stone cells; B - sclerified medullary ray cells; C - parenchyma; D - branched stone cells and sclereids; E - cork in surface view; F - fragment of sclereidal fibre; G - group of interwoven branched sclereids; H - pitted vessels; I - fibre with forked tip; J - sclereids of different shapes; K - vessels in groups and isolated; L - different types of fibres; M - thick-walled fibre; N - fibre attached with parenchyma and vessel; O - group of pitted vessels; P - bundle of fibre attached with xylem elements; Q - fragment of pitted vessel; R - fragment of hone-comb like vessel; S - medullary ray cells crossing parenchyma; T - phloem elements; U - pitted vessels; V - tracheids.

Fig. 3. Characters of macerated haustorial root.

3.2.4. Fruit

TS of the fruit is circular in outline and shows epicarp, mesocarp and endocarp. A single layered outermost epicarp made up of thin walled cells filled up of mucilage. The outer mesocarp is narrow made up of closely arranged parenchyma cells followed by the inner mesocarp made up of loosely arranged cells. Slimy mucilage is seen all over the mesocarp region. Numerous stone cells and trichosclereids are present in the outer mesocar region only. Following the mesocarp endocarp is present which is composed of 3–5 layers of narrow thick walled closely arranged parenchyma. Testa is 2–3 layered followed by multilayered endosperm enclosing a single cotyledon. The cotyledon has a distinct epidermis enclosing cotyledonary cells with abundant storage (Fig. 6).

4. Discussion

Mistletoes occur in patches or abundantly on different types of host trees or shrubs altering their distributional pattern in forest (Carlo and Aukema 2005, Shaw et al 2005). They are characterized by their growth habit forming compressed thickets in the host crowns except for several root parasitic species. The thickets are generally composed of semi succulent mistletoe stems and leaves. As the endophytic system of the parasitic root grows within host tissues, fusiform swellings enlarge as it age. The infestation of this mistletoe results in the formation of a ball of branches of the host and it forms haustorium to obtain minerals and nutrients from the host (Press and Graves 1995). The swollen holdfast produced also helps in its firm attachment to the host. Glatzel (1983), Stewart



1 mm

4.1. LS of the host showing TS of haustorium.



4.2. Tracheids in the haustorial tissue.
 4.3. Cavity in the haustorial tissue.
 CK - cork; HT - haustorial tissue; PH - phloem; TSC - tricosclereids; XY - xylem.

Fig. 4. TS of haustorial root of Helicanthes elasticus with LS of stem of host Mangifera indica.

and Press (1990) have taken up several studies dealing with the complicated relationship between mistletoes and their hosts in the past.

There are various types of haustorium inclusive of creeping, coupling and knotting recorded among the parasitic plants world over. *H. elasticus* comes under the category where the mistletoe plant creeps and couples around its host plant. The mango mistletoe has been reported to prefer mango tree as host owing to its rough surface which helps in easy attachment and retaining of the mistletoe seeds till its germination (Kumar et al, 2015).

As the mistletoe takes up a large share of water and nutrients it leads to the reduction in growth and reproduction of host and makes it more susceptible to diseases (Hawksworth 1983; Knutson 1984). There are many hypotheses put forward explaining the transmission of water and food reserves from host tissues to the parasitic tissues. One suggests that there are direct links formed between xylary elements of mistletoe and their hosts (Strong and Bannister, 2002). While Glatzel (1983), Stewart and Press (1990) hypothesized that the symplast translocation occurs through the parenchyma cells before entering the xylem with the absence of any type of direct connections. The current study has demonstrated that this parasitic mistletoes form diverse anatomical links with the conductive tissues of the host for absorption.

According to Calvin and Wilson (2006) four types of haustorial tissues can be encountered in these parasitic mistletoes. In the current study it was seen that the haustorium grows deep into the xylem region of the host plant confirming it to be one among the

four as described. As the intrusive organs reaches deep down into the connective tissue of the host the host and the parasite xylem get connected the walls of the cells lining the interface becomes very thick. The anatomical features observed in the current study were in accordance with the haustorium of other Loranthaceous members. The pericycle showed the presence of solitary and clustered crystals and isolated fibres in addition to tanniniferous cells in parenchyma which was observed by Metcalfe and Chalk (1957) in earlier studies of other Loranthaceae members.

Mistletoes, being a hemiparasite even if established from an epiphytic seedling not only achieves a major share of resources through the xylem continuum formed between the host and the parasite but also being positioned at much favourable light conditions are able to photosynthesize more when compared to the understory floras (Těšitel 2016). The ecological adaptation of this species thriving for the higher canopy levels for establishment is noteworthy. *H. elasticus* species is seen parasitizing the mango tress in the crown regions so has to harvest comparatively more amount of sunlight.

The special branched stone cells present in the haustorial infection sites were also encountered in the fruit anatomy. In addition to the mechanical support to the organs these stone cells helps the tender parasite to invade into the rough and thick tissue of the mango tree and helps in reducing the rate of wilting and deter herbivory (Lopez and Barclay 2017). The presence of a well- developed endosperm in the current study supports the fact of photosynthetic endosperms in hemiparasitic mistletoes which is





5.2 Intimate union of tracheids of haustoria with vessels of the host.



5.1 TS of host showing haustorial tissue.

5.3 Establishment of haustoria in the pith region of the host.

CK - cork; CT - cortex; HRT - haustorial root tip; HT - haustorial tissue; PH - phloem; PI - pith; XY- xylem.



infrequent in the case of angiosperms (Nickrent and García 2009). This endosperm provides the seedlings immense energy required for the penetrating the host bark. The establishment of the mistle-toe becomes quiet easier with a free pre attachment stage with the endosperm providing the nutrient supply. The establishment chances of mistletoes are increased by the seed dispersal carried out by birds and in few cases by the stickiness of the seeds.

On reaching the host the mistletoe seeds germinate to produce small radicle followed by a hold fast and an ultimate penetration plug (Hunt et al 1996, Knutson 1984). The radical slowly grows and penetrates into cortex of host tissue followed by the development of root-like endophytic absorptive system (Alosi and Calvin 1985). Many lateral strands grow deep into the cortical region and sinkers are developed in the sapwood region. The transverse sections taken during the present study showed the sinker even deeper into the pith region.

The physiology and morphology of the host plant is altered by the parasite by altering the growth hormone production for its own benefit following which it establishes itself, matures and produces seeds which proceed to infect new hosts (Pennings and Callaway, 2002). The extensive growth of the parasite results in the reduction in growth, wood quality and the life span of the host. In several cases the infection site leads to fungal infections which are further harmful for the host tree. Over a period of time reduction in the diameter of the affected stems is noticed. The long tubular attractive flowers serve well in attracting the birds and small animals which in turn helps in the propagation of the parasite to newer branches or hosts.

Formerly the seed dispersal, establishment and the host parasitic interactions were only focused (Gill and Hawksworth 1961). The previous studies carried out by Press and Phoenix (2005) has credited this mistletoe to be keystone species owing to its ecological roles. Several root parasites including those of mistletoe litter is found to possess high proportion of elements phosphorus and potassium (March and Watson 2007). Kumar et al., 2016 has provided a quality standard for this hemiparasite.

The parasitic plant body can be easily detached from the host during its juvenile stage before the establishment of haustorium. The host tissue subjected to the parasitic penetration also forms tumors. The cutting away of infected branches well low below the site of infection can help in prevention of secondary infection. A variety of horticulture crops are also affected with this mistletoe as they serve as suitable host. Since the level of adherence of the mistletoe seed helps in its germination and final establishment the differences in the physical or chemical nature of the bark will help to reduce the rate of establishment of this hemi parasite. This can be used as a tool by horticulturists to evade this destructive mistletoe.

There is a wide range of habitat scoring different ecosystems worldwide which still remains under investigated. From the observations it is clear that mistletoes should not be considered only as destructive pests, but also as an interesting group of plants due to



6.2 Otter region emarged COT - cotyledon; EPC - epicarp; ENC - endocarp; ENS - endosperm; IMC - mesocarp; OMC - outer mesocarp; STC - stone cell; TE - testa.

Fig. 6. Macro-microscopy of fruit of Helicanthes elasticus.

their interesting biology influencing several forest life forms which can help to assess overall health of an ecosystem with high sensitivity.

5. Conclusion

Macroscopic and microscopic study of the root, haustorial association and fruits of the mistletoe has been recorded in the current study. Early control of the mistletoe will be an effective methodology for controlling the further complications leading to loss in productivity, damage to timber strength, etc in cash crops. Further research into the molecular phylogenetics and physiological interactions together with its significant ecological contributions will be an added knowledge database for these interesting groups of parasitic mistletoes.

Declaration of Competing Interest

Declared none by all the authors.

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