Summary of the thesis

HISTOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON MANGO MALFORMATION

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Mango malformation, a serious malady, is characterised by two distinct stages; vegetative and floral. Typical symptoms of the vegetative malformation are loss of apical dominance and swelling of terminal and axillary buds. Such buds eventually form shootlets bearing small scaly leaves with a bunch-like appearance, the so called bunchy top stage. Vegetative malformation is more pronounced on young seedlings but also appears on mature trees. Development of vegetative malformed shoots on most of the branches of a tree lead to considerably reduced flowering or no flowering at times.

Floral or blooson malformation appears with the emergence of inflorescence. The malformed panicles bear much enlarged numerous flowers crowded around the hypertrophied inflorescence axis. These flowers usually do not open and fail to produce fruits.

1.0 Morphological Study :

The survey of various mango orchards in and around Baroda reveals the occurrence of the malformation. The percentage occurrence of the disease ranges from 10-50% in different orchards.

Eight varieties of *Mangifera indica* viz., Rajapuri, Kesar, Ladva, Limdi, Langra, Alphonso, Dadamio and a local variety have been selected for morphological studies. While cultivars; Rajapuri, Kesar, Langra, Dadamio and Amrapali are observed for histological studies. Morphology of the diseased panicles reveals the increase in number and size of sepals, petals and all the essential organs in both staminate and bisexual flowers compared to those of healthy panicles. A shift from bisexuality to staminate flowers is generally noticed in malformed panicles of all the eight varieties except in var. Kesar. The calyx and corolla of flowers from malformed panicles exhibit quincuncial aestivation contrary to the imbricate among normal flowers. Onset of young panicles starts from terminal buds during mid-December and the elongated stout-buds with large leafy bud scales containing black resin deposits developed into malformed panicles.

Vegetative phase of malformation is predominated by development of many stunted dwarf axillary shoots from axillary buds surrounding the base of the terminal bud.

These dwarf shoots often form a bunch. The leaves on these shoots are reduced in size and appear scale-like. Small lesions with dark blackish resin secretions are often seen on the adaxial side of leaf bases and bud scales. Mango mites are frequently noticed associated with closed and open axillary as well as terminal buds of the vegetative malformed shoots.

The mites identified from the malformed buds and shoots are *Aceria mangifera* sayed, *Cheletogenes* sp., *Cunaxoids* sp., *Tydeus* sp., and Tenuipalpid mites. All these mites are noticed carrying microconidia of the pathogen, *F. moniliforme* on the surface of their bodies.

1.1.0 Histological Study:

Histological and histochemical studies of terminal buds, floral and vegetative malformed parts have been carried out to emphasize the disease development and structural and functional interrelationship of the host-pathogen interaction. These studies have been carried out comparing between healthy and malformed tissues.

1 1.1 Terminal buds: Shoot apical buds of var. Rajapuri collected at monthly intervals have been studied for one year (January-December, 1995). The reproductive apices are predominant than the vegetative ones in winter season. During summer and monsoon the shoots invariably bear vegetative apices. Reproductive apices have elongated axis bearing axillary floral bud meristem. Hyphae of the pathogen are found in between the two overlapping young bract primordia close to the apex and also attached to the tunica layer of young bud meristem.

In early summer, tuft of mycelia or hyphal aggregates are common in the vicinity of the meristem. During mid-summer the apices are relatively free of pathogen and show typical characters of dormancy with reduced length and number of leaf primordia. During monsoon, insect remains are predominant near the vegetative apex and in latter months phenolic compounds accumulate in some of the parenchyma cells below corpus zone. By November tunica layer of meristematic zone is severely infected and degraded by the pathogen. The infected zone is marked by heavy accumulation of phenolic contents.

The gum-resin ducts lumen diameter, frequency and percentage area in the shoots of terminal buds show positive correlation with infection. During winter the lumen diameter is maximum when the temperature is lowest (12.8°C in January) and the infection is found to be more. In summer the decline in the infection is reflected in the reduced lumen diameter, frequency and percentage area of the gum-resin ducts.

4.1.2 Floral malformation: The peduncle of malformed panicles shows ridges with a thick cuticle. Intra and intercellular hyphae are rare in the ground tissue, phenolic contents are often noticed in the outer cortex. Gum-resin ducts have reduced size with no secretion. The peduncle exhibits secondary growth with a prominent ring of xylem with relatively higher vessel frequency. Resin embedded young buds (affected) revealed the association of mycelial fragments, tuft of micro and macroconidia of *F. moniliforme* with the floral organs.

The mature affected buds from var. Rajapuri revealed close structural association between the essential organs and the pathogen. Fusarial hyphal mat is consistently noticed in the basal groove of the anther lobe. The pollen grains are usually round and exhibit thin cell walls while in variety Langra, they are oval-elliptical. Severely infected anthers of all varieties studied show loss of cell identity in tapetal layer followed by phenolic accumulation. Pollen in such locules are attached with fungal hyphae and noticed with thin wavy cell walls enclosing plasmolysed cytoplasm, while normal pollen exhibited dense cytoplasm. Lesions characterised by accumulation of dark contents are noticed along the length of the style. This leads to the formation of canal along the style into the ovular chamber. Fusarial mat is often noticed along this canal and the inner walls of the ovary wall.

The ovary wall also has lesions across it from outer to the inner surface. The ovule is invaded by the pathogen either via micropylar end or the wall layers. These infection sites are characterised by the accumulation of phenolic contents either in intercellular spaces or the cell lumen.

Open flowers from malformed panicles show similar mode of distribution of fungus in the essential organs as described in mature bud. Moreover open flowers from malformed as well as normal panicles usually possess dehisced anther locules lodged with a few pollen grains. Irregularly shaped aborted ovule filled with dark contents and associated fusarial mat characterised the severely infected flowers.

1.1.3 Vegetative malformation :

Most of the cortical cells are filled with phenolic contents. The primary duct lumen is often noticed with gum-resin accumulation. Vessels appear in radial multiples with relatively less lumen diameter in malformed shoots but their frequency is found to be more. Xylem fibres are longer compared to those in normal shoots. The xylem cylinder of healthy shoots is mainly composed of primary xylem.

The dorsiventral leaves on vegetative malformed shoots are covered with a thick cuticle covering the midrib and the lamina regions. A continuous band of sclerenchyamatous fibres encircle the vascular bundles in the mid-rib. The lumen diameter of the gum-resin duct in the mid-rib is less compared to that of healthy ones. The palisade parenchyma of lamina are filled with chloroplasts and stains blue with Toluidine blue while in normal leaves the cells stain green.

1.2 Histochemical Study :

Histochemical study of apical meristems, floral and vegetative malformed tissues in comparison with respective normal tissues has been carried out.

In shoot apical meristems, starch deposits are confined to the pith cells. These deposits gradually increase from September and attain a maximum accumulation in December. However, starch deposition totally declined during summer and monsoon.

The accumulation of protein bodies are found to be more in the vegetative apices of late winter and early summer i.e. March and April. The increase in accumulation of these deposits runs parallel with increase in intensity of infection in both these months.

Resin secretion in the pith ducts lumen also reveal that it is proteinaceous and positive for catechin type of phenolic contents in the apices of both the months. In all the seasons, except in October and November, the apical meristem along with the subtending leaf primordia have relatively more lipid contents.

In malformed panicles the cortical cells of peduncle accumulate phenolic derivatives of catechin type. Gum-resin secretion does not show such type of phenolic contents. However, hydrolysable tannins are encountered in the cortical cells and secretory cells of resin ducts in the peduncle of healthy panicles. Starch reserves are not found in any of the tissues of diseased panicles. Nevertheless epidermal cells, pith parenchyma and secretory cells of resin ducts show more protein bodies. Moreover, cortical, paratracheal and phloem parenchyma along with ray cells accumulate lipid deposits. The infection site i.e., the cells near the basal groove of the anther lobe accumulate more starch and protein deposits but no lipid material. However, the pollen grains are rich in starch, proteins and lipid reserves while the ovary tissues are devoid of these reserve material.

The infection sites of peduncle i.e., the epidermal cells and inner cortical cells show intense positive reaction for the succinic dehydrogenase (SDH) and peroxidase enzymes. Perivascular fibres also localise the SDH but gave faint reaction for the oxidase. Paratracheal parenchyma, phloem and pith parenchyma show both the enzymes precipitation.

The cortical cells and vascular tissues of pedicel exhibit dense reaction product of SDH while peroxidase is noticed only in few inner cortical cells. The infection sites in the anther i.e., the basal groove where the filament is attached and the anther wall in between two anther lobes show intense peroxidase activity while SDH activity is reduced and confined to the epidermal cells. In ovary, the stylar transmitting tissue as well as ovular cells show dense oxidase precipitation but are devoid of SDH activity.

In healthy panicles the peduncle show relatively more starch grains in the epidermis, cortex and pith duct epithelial cells. Starch is commonly found in the young

buds, pedicel, sepals and other organs of young and mature buds. However, proteins and lipids are restricted only to the cortical cells of peduncle.

The secretory cells of gland and cytoplasm of pollen grains exhibit starch, protein and lipid material. Cortical cells and vascular tissues of peduncle and pedicel show scanty reaction product of SDH and intense reaction product of peroxidase. In flowers except for epidermal cells of the basal groove of anther lobe, dehydrogenase activity is absent in stylar transmitting and ovular tissues. However, few anther cell walls and the inner integument cells of ovule do localise the peroxidase.

The shoots of vegetatively malformed twigs are free from starch deposits in the cortical cells, vascular tissue and pith cells. Vascular parenchyma and ray cells are also noticed with accumulation of protein and lipid reserves.

In vegetatively malformed shoots, SDH and peroxidase activity is more in cortical cells. The leaves from vegetatively malformed shoots have starch, protein bodies and lipid material in ground tissue of mid-rib. However, in the lamina region, palisade and spongy parenchyma have deposits of starch and protein bodies. SDH activity is restricted to few secretory cells of gum-ducts in midrib and palisade cells of the lamina. While peroxidase activity is distributed in epidermis, vascular tissues and ground parenchyma in the midrib.

In the healthy shoots, cortical cells have more starch granules and protein bodies. Although starch and protein deposits are seen in parenchyma around gum-resin ducts, lipid reserves are scanty. Phloem parenchyma, ray cells and pith parenchyma accumulate more starch deposits while protein and lipid metabolites are absent. The activity of peroxidase and SDH in the cortical and vascular tissue of healthy shoots is either scanty or absent.

In the healthy leaves starch deposition is found to be more in ground tissue and vascular cells of midrib and spongy parenchyma of the lamina. The peroxidase activity is less in the ground tissue of midrib and confined to only upper epidermal cells in the lamina.

1.3 Ultrastructural Study:

Scanning electron microscopy study of sepals, anthers and ovary from mature flowers of malformed panicles has been carried out. Anthers of malformed flowers do not show distinct grooves on their surface. Hyphal filaments of *Fusarium* are often noticed in contact with the surface of the anthers. The basal portion of the anther lobe where the filament is attached, is often noticed with a tuft of coiled hyphal filaments of the pathogen. In few cases the pathogen is also noticed in the groove between the two anther lobes. The anther locule contains a few oval-elliptical and some disorganised pollen grains. The ovary shows few hyphal filaments attached to the outer surface of the ovule. Ultrathin sections of young buds, mature anthers and ovary have been observed under TEM.

TEM study reveals that tapetal and microspore mother cells are densely cytoplasmic with small vacuoles. The cytoplasm in both the cells is often plasmolysed. Tapetal cells are characterised by the darkly stained cytoplasm containing smooth vesiculated ER. The cytoplasm also exhibit intracellular electron dense material and membranous contents. Lipid globules and electron dense bodies of different sizes are frequently found in the cytoplasm of microspore mother cells.

In mature anther also fragments of hyphae are noticed close to the basal epidermis of the anther lobe. The epidermal cells are covered with thick serrated cuticle and cytoplasm show dark electron dense contents. The inner parenchyma cells close to the basal epidermal cells and the parenchyma around the connective show distinct intracellular hyphae. The tracheary elements of the connective exhibit electron dense granular contents.

The stylar transmitting cells of the ovary possess large lipid bodies, numerous small vacuoles and electron dense contents. The cell walls are irregularly thick enclosing dark contents. Intercellular spaces are distinct among the stylar transmitting tissue. The inner layer of ovary wall shows disrupted cells with thickened walls and electron dense contents. Conidia-like structures are found closely attached to the surface of the ovule.

The cells on the surface of the ovule are irregular with electron dense cytoplasm enclosing irregularly shaped vacuoles.

The host structural, histochemical changes following malformation in vegetative and floral tissues are compared with the healthy tissues and discussed in the light of disease development. On analysing the foregoing results the following conclusions are drawn:

1. Floral malformation is prevalent in all the mango varieties growing in and around Baroda.
2. Malformed panicles bear more number of staminate flowers.
3. Mites other than *Aceria mangifera* sayed also act as vectors in causing malformation.
4 Pathogen (*Fusarium moniliforme*) entry into the host appears at the very early stages of bud development i.e., at meristem level. The entry of the pathogen might also be mediated through mites.
5. The pathogen mainly colonizes in the essential organs (anthers and ovary) of the flowers and makes them abortive or non-functional in the malformed panicles. These observations are further supported by ultrastructural studies.
6. The enhanced SDH activity is reflected in increased accumulation of metabolites (starch and proteins) in diseased tissues which infers high respiratory activity in such tissues. While more peroxidase activity in diseased tissues might indicate a defensive role.
7. The diseased tissues invariably accumulate more phenolic contents (the phytoalexins)