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HOST PATHOGEN INTERACTION STUDY IN MALFORMED AFFECTED TISSUES OF *MANGIFERA INDICA* L.

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ABSTRACT

The malformation disease of mango by *Fusarium moniliforme* var. subglutinan is one of the major causes of huge economical loss every year. Early diagnosis of the pathogens can help cultivators to protect the mango orchid and fruits from successive and secondary infection. The molecular detection is one of the better choices among available techniques for diagnosis. But the farmers or individual cultivars from tropical and subtropical countries like India, Pakistan, Malaysia, Vietnam, Philippines, have lots of limitation to use molecular method. Another problem with this pathogen is lack of specific biomarker. Thus the present study was designed to develop a morphological and histopathological methodology to detect *Fusarium moniliforme* var. *subglutinans* and its host-pathogen interaction between fungus and hot tissues. Fresh and preserved (Formaldehyde: Acetic Acid: Alcohol (FAA) solution) tissues were used to check the efficacy of the method. The inter and intracellular distribution of fungal hyphae, in various tissue like cortex, phloem and parenchymatous pith cell, was clearly revealed by this method. The characteristics which were observed like detection of the mycelium threads at the juncture of the shoot tip, axillary buds, petels and sepals axis of the malformed or infected tissues. The Resin accumulation in the parenchymatous and other cells of vegetative tissue was also found as characteristics of this pathogen infection. This study is being also important in terms of identification and growth behavior of the pathogen.

Keywords: *Mangifera Indica* L., Malformation, *Fusarium Moniliforme*, Var *Subglutinans*, Microscopy, Microtomy

1. INTRODUCTION

Mango is the most popular fruit among millions of people in India and abroad and its known as 'King of Fruits'. Mango is the second highest fruit crop on the basis of production and it's comes second after banana (Kumar *et al*., 2009). For mango, India ranks first in area and production in the world. India's contribution to the world's mango production is highest 16.34 million tons of total mango production of world 38.6 million tons [~2010 data http://en.wikipedia.org/wiki/Mango; (source-Food and Agricultural Organization of United Nations: Economic and Social Department: The Statistical Division)]. The productivity (6.3 mt ha^{-1}) of mango is low in spite of highest area coverage and production (Kumar *et al*., 2011a). This is because of the several abiotic and biotic factors affected the mango production out of these malformation is one of the most important problems and main reason lower the production in India at primary level of mango formation

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and growth The characteristic symptoms in seedlings are loss of apical dominance and swelling of vegetative buds in the leaf axil or at the tip with small shoot lets bearing small scaly leaves. The symptoms of floral malformation are characterized by deformation of panicles, suppression of apical dominance, shortening of primary and secondary axis, giving the panicle a characteristic clustered appearance, thickened rachis of panicles and greatly enlarged flowers with large disc and the preponderance of staminate flowers bearing scanty pollen grains (Ploetz and Freeman, 2009; Youssef *et al*., 2007; Ploetz *et al*., 2002). The rachis of malformed panicle is more branched, thicker and heavier than the normal ones. Several workers isolated and prove through pathogenicity and molecular means and explain the role of *F. moniliforme* var. *subglutinans* as causal agent of mango malformation (Newman *et al*., 2012; Kumar *et al*., 2011b; 2012). Kumar *et al*. (2011b) performed mass isolated and recover 82% of *F. moniliforme* var. *subglutinans* colonies from the malformed affected tissue. These pathogen leads to the malformed, shortening and bunchy top appearance of the vegetative tissues and compactness of the floral parts of the mango. The objective of the present study to revealed the host-pathogen interaction in vegetative and floral malformed tissue by the uses of conventional histopathological techniques.

2. MATERIALS AND METHODS

2.1. Sample Collection

Sample of mango malformed (vegetative and floral) and healthy tissues (control) were collected from mango variety Amrapali (highly susceptible for malformation) from the orchards of Central Institute for subtropical horticulture, Lucknow, India. Samples were dipped in Formaldehyde: Acetic Acid: Absolute Alcohol (FAA) (5:5:90 mL) for the storage and further use (Gupta and Pandey, 2012; Gupta *et al*., 2012; Haggag *et al*., 2011). The samples were left in the above solution till they were processed further. The FAA solution was prepared based on the type of material 25, 50 and 70% ethanol used for soft tissue, moderate tissue or hard tissue respectively.

2.2. Dehydration and Embedding of Sample

The dehydration of samples were processed using series of chemicals (alcohol 30, 50, 70, 80 and 95%; absolute alcohol, alcohol 75% + xylene 25%; alcohol 50% + xylene 50%; alcohol 25% + xylene 75% and pure xylene for 30 min each respectively) using automatic tissue processor (Yorko, New Delhi) and embedded in melted paraffin wax (54 to 56°C) for at least 4-8 h in order to completely replace the xylene with paraffin wax (Gupta and Pandey, 2012; Gupta *et al*., 2012; Pandey *et al*., 2012).

2.3. Sectioning, Staining and Mounting of Sample

About (10 μm thick) sectioning of healthy and malformed samples was done using Microtome (Microm, Germny). Sections were stained in 0.1% aqueous toluidine blue O and were mounted in DPX-mountant after bringing them to xylene through alchohol-xylene series as describe by (Gupta and Pandey, 2012). The samples were examined for anatomical by using Leica LEITZ DMRBE Microscope (Germany) at different zooming 5, 10, 50 and 100x (Pandey *et al*., 2012).

3. RESULTS

Histopathological observations were recorded after carrying out the conventional microtomy of healthy and malformed apical shoot and flower bud to find out damage caused by pathogen as well as presence of fungal mycelium in the malformed apical tissues and flower buds. Longitudinal section of healthy apical portion (**Fig. 1A and C**) of the mango large healthy flower clearly differentiated in parts showing the premature bracts (ps) vascular bundle system (v) cortex (c) and oil ducts containing resins (o) at 5× and 10×. The Longitudinal Section (L.S.) of healthy flower showed ovate to ovate oblong sepals and also high pubescent. There are four to five petals that are oblong to ovoid to lanceolate and also thinly pubescent. The floral discs are four to five lobed, fleshy and large and locate above the base of petals. There are five large, fleshy nectarines that form a five lobed receptacle. Histopathology of longitudinal section of malformed flower showed the sepals, petals, floral discs which are locate above the base of petals and the nectarines were found in damage (necrotic) due to infection hypertrophy was observed in the malformed flowers. Malformed flower (**Fig. 1B and D**) compact, shrink and malformed single flower showing petals (p) shepals (s) damaged male female part (o) at 5 and 10x. It was clearly seen the damaged floral part with mycilium (m) of *Fusarium* penetration of hyphae in tissue of floral part also observed (t) (**Fig. 2A and B**). The cortex and rachis of panicle may develop hyperplasic cells. The number of cells per unit area of cortex, xylem vessels and pith was one and half times less in malformed panicles than in healthy ones.

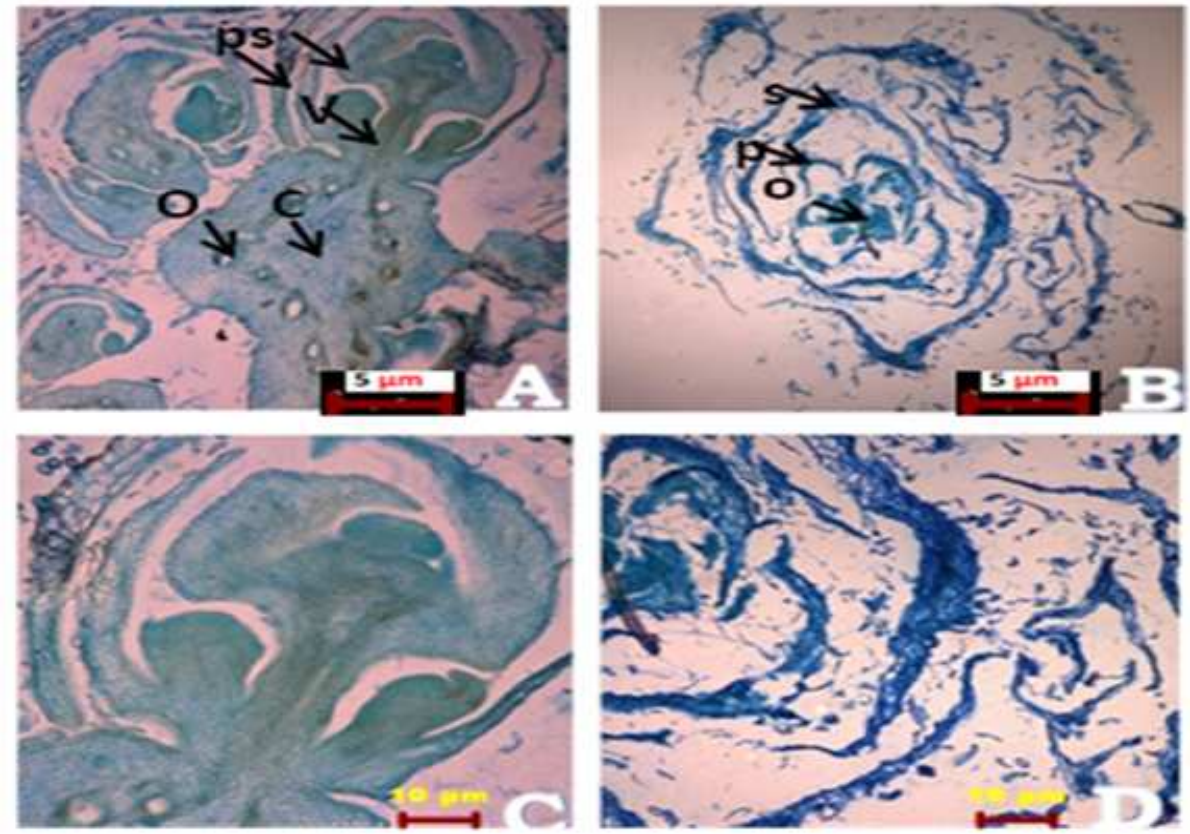
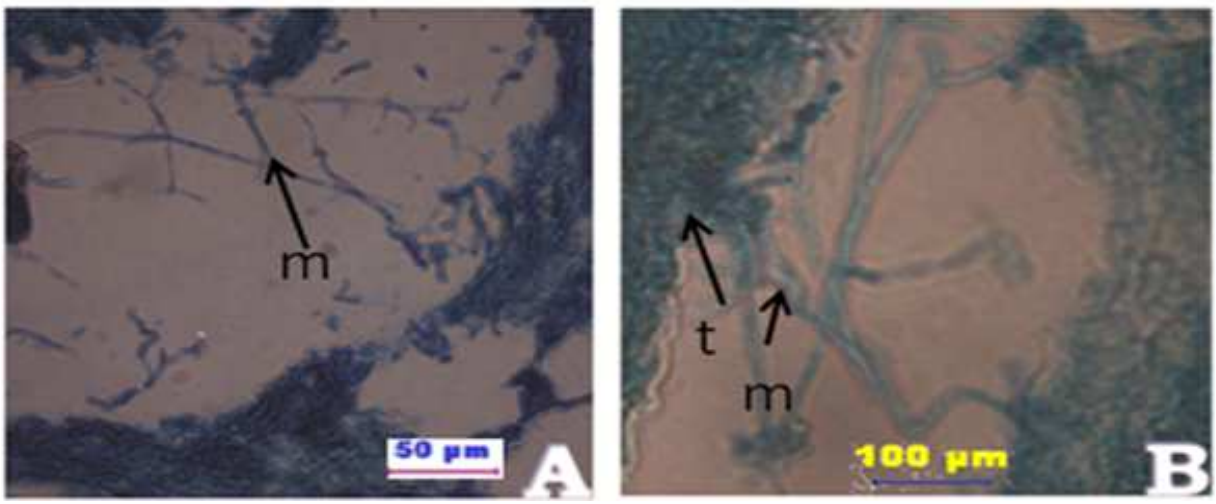


Fig. 1. Logitudunal section of healthy apical part of mango vegetative tissue and malformed floral part of the mango infected by *Fusarium moniliforme* var. *subglutinans.* (A) and (C) Large healthy apical tissue clearly differentiated in parts showing the premature parts (ps) vascular bundle system (v) cortex (c) and oil ducts with resins (o) at 5 and 10×. (B) and (D) Compact shrink and malformed single flower showing stunted petals (p) sepals (s) damaged male female part (o) at 5 and 10x



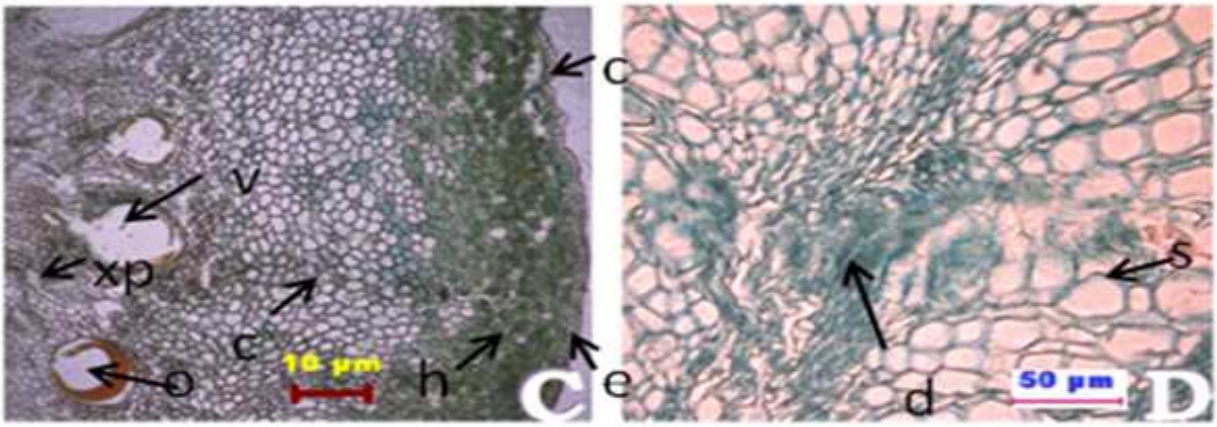


Fig. 2. Logitudunal Section (L.S.) of malformed floral part of the mango infected with *Fusarium moniliforme* var. *subglutinans* (A and B) and Transverse section (TS) of healthy vegetative tissue (C) and malformed tissue (D). Fig. A showing the damaged floral part with mycilium (m) of *Fusarium* at 50×. In figure B mycelium (m) of *Fusarium* were clearly observed and penetration of hyphae in tissue of floral part also observed (t). T. S. of healthy vegetative tissue Fig. C showed out part Cuticle (c), Epidermis (e), Hypodermis (h), Cortex (c), oil duct in cortex and vacuoles (o and v) and vascular system xylem and phloem (xp). Malformed vegetative tissues T. S. showed the hypertrophied or swell (s) cell of cortex and also damaging of vascular system (d) in figure D at 50x

The observations of transverse section of healthy vegetative apical shoot showed that all the cell of cortex as well as vascular system is intact. All the cells are compact without an intracellular space in comparison to malformed tissues, where the cells are loosening due to hypertrophy of cells. At some portion accumulation of cytoplasm was also observed and almost all the cells were deformed. Damage of cortex cells as well as vascular bundle has been observed in the studies. Transverse section of healthy vegetative tissue showed outer part cuticle (c), epidermis (e), hypodermis (h), cortex (c), oil duct (o) in cortex and vacuoles (v) and vascular system xylem and phloem (xp) in **Fig. 2C**. Transverse section of malformed tissues showed the hypertrophy cortex cell (s), damaged vascular system (d), no mycelium were observed in any of the apical vegetative malformed samples (**Fig. 2D**). The thickness of rachis and smaller number of cells per field in the rachis of malformed panicles was due to enlarged cell size.

4. DISCCUSSION

The ability to observe the growth of fungal structures in host tissues under the microscope is an important tool in the study of plant pathogenesis. Over the years many staining techniques that highlight fungal structures in plant tissues have been reported. Inflorescence and vegetative malformation of mango, occurs in many mango-growing countries worldwide and is one of the most important diseases of this crop (Ploetz *et al*., 2002). Haggag *et al*. (2011) reported that vegetative and floral malformation appeared where mycelium of *Fusarium* species was present in the tissue at high concentrations which support our findings. In transverse section of malformed tissues showed necrosis of cortex cells and damage of vascular system caused by pathogen. Gupta *et al*. (2012) reported the similar results in case of guava wilt caused by *Fusarium*. However, gum like substances and tylosis that formed in the infected tissues might bring about dysfunction of the phloem and xylem elements, might interfere seriously with the transportation stream and produce a hypertrophy of the cell at several place the region invaded by pathogen. These results corroborated with findings of other workers in palm and mango crops (Pandey *et al*., 2012).

Resins found in the plant cells may be due to the defense system of the plants activate and increases the synthesis of during the pathogen attack. Microscopic examination of sections taken from infected tissues indicates the ability of pathogenic fungi to invade the different tissues causing changes in cells structure and caused plasmolysis and discoloration of parenchymatous cells (Gupta *et al*., 2012). This alteration of cellular structure was probably due to metabolites, toxins of pathogen. Environmental condition responsible for the production of resins in the plant cell and it may fluctuate time to time, but the

increases amount of resins were observed in the malformed affected cells the result was supported by (Nailwal *et al*., 2006). They showed that the primarily infection via root, completely colonized the seedling root systems and became systemic, spreading to apical plant tissues (apical buds). Apart from competition for nutrients, the fungus may release secondary metabolites, which could create further hormonal imbalance and inhibit the normal growth of the meristematic tissue of the buds. The second infection for long distance dispersal of the pathogen is hypothesized to be via infected nursery stock or by the mango bud mite (Nailwal *et al*., 2006; Haggag *et al*., 2010).

5. CONCLUSION

The etiology of floral malformation in mango has always been controversial. So it is important to understand the exact host-pathogen interaction. *F. moniliforme* var. *subglutinans* causes extensive damage of flower bud of mango was observed because of hypertrophy of cells including sepals, petals, floral discs this may be leads to the abortion of flower.

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