Eur J Plant Pathol (2010) 127:391–398
DOI 10.1007/s10658-010-9605-1

Cytology of infection of *Fusarium mangiferae* Britz in different malformed reproductive parts of mango

Zafar Iqbal · Sohail Hameed · Muhammad Akbar Anjum · Altaf Ahmad Dasti · Ahmad Saleem

Accepted: 15 March 2010 / Published online: 11 April 2010

Abstract Aetiology of mango malformation (MM) has intrigued the scientists since its inception. The objective of the study was to glean an insight into association of the fungus, *Fusarium mangiferae*, with different malformed regions, viz. panicle-shoot juncture, apical buds, primary and secondary peduncles, in five exotic mango cultivars. Tissue assays revealed an infection of 88.5, 84.75 and 82.5% in cvs Zill, Sensation and Tommy Atkins, respectively. Least infection of 69.75% was found in cv. Keitt. No exotic cultivar was found to be free of fungal infection. Apical buds proved to be the potential infection site of the fungus amongst the four malformed regions, hosting maximum within-tissue infection of 86.2%. Determination of *F. mangiferae* at proximal and distant sites of the malformed panicles exhibited maximum recovery of 82.0% at 0 cm and only 3% at >30 cm distance beneath the panicle. In the case of non-malformed panicles, an infection of 14.0% was recorded at 0 cm distance while no detection could be made from non-malformed branches. Moreover, examination of ultra-thin bud sections under Transmission Electron Microscopy (TEM) revealed inter- and intra-cellular ramification of fungal hyphae, indicating fungal ingress in malformed bud tissues of local cv. Malda. The present studies explored the sites hosting the causal fungus in mango and provide convincing evidence that *F. mangiferae* is responsible for turning healthy tissues into the malformed condition. These findings suggest that inoculum specific management strategies are needed in future to curb malformation disease in mango orchards.

Keywords Cytology · Exotic cultivars · *Mangifera indica* · Mango malformation · Transmission electron microscopy

Z. Iqbal
University College of Agriculture, University of Sargodha, Sargodha, Pakistan

S. Hameed
National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad, Pakistan

M. A. Anjum (✉)
University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan
e-mail: akbaranjum@bzu.edu.pk

A. A. Dasti
Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan

A. Saleem
Punjab Agricultural Research Board, Lahore, Pakistan

Introduction

Mango malformation (MM) disease is the major constraint to mango production in the Indo-Pakistan subcontinent and other mango growing areas of the world, causing yield losses ranging from 60 to 90%

(Ginai 1965; Ploetz 1999; Freeman et al. 2004). The disease affects vegetative shoots and floral panicles resulting in phyllody and hypertrophy (Singh and Dhillon 1990a; Ploetz 1994). Various aetiologies, including viral (Das et al. 1989), nutritional (Prashad et al. 1965; Singh et al. 1991) and hormonal (Singh and Dhillon 1987, 1988, 1990b), have previously been claimed in the literature. A novel experiment by Freeman et al. (1999) unequivocally proved the causal relationship between *Fusarium subglutinans* (Wollenweb. & Reinking) and MM, fulfilling Koch's postulates for both forms of the disease. Taxonomy of this fungus has been revised recently. Three species, viz. *F. mangiferae* Britz, *F. sterilihyphosum* Britz, Wingfield & Marasas and an undescribed *Fusarium* species, have been found associated with MM in South Africa. *F. sterilihyphosum* has been reported to be present in South Africa and Brazil. The *F. subglutinans* strains are conspecific with newly characterized species *F. mangiferae* causing MM (Britz et al. 2002). Efforts to examine the association of fungi with malformed parts have been reported previously (Ploetz and Gregory 1993) but previous attempts to determine the fungus within symptomatic tissues or at sites behind the panicles are based on study of one or two cultivars, or a few trees or tissues only (Crookes and Rijkenberg 1985a; Darvas 1987). Diversity of tissues from different symptomatic regions has not been studied specifically. Scanning electron microscopic studies in India (Usha et al. 1997) confirmed the infection in malformed mango buds by *F. subglutinans* but the illustrations depicted only superficial features. No systematic ultrastructural study has been conducted yet.

The aim of the present study was to detect fungal infections in malformed regions of different exotic cultivars and study their ultrastructure to confirm the infection site. We present conclusive evidence that mango buds are the sites for latent infection of the *F. mangiferae*. The present work will prove useful in tracking movement of the pathogen and to adopt suitable cultural and chemical means to eradicate inoculum.

Materials and methods

Sampling

Studies to ascertain the association of *F. mangiferae* with malformed regions were conducted during the flowering cycle (March–April) in the Punjab province of Pakistan. Five exotic cultivars, viz. Keitt, Maya, Sensation, Tommy Atkins and Zill, growing at three mango growing districts (Lodhran, Multan and Faisalabad) were selected to obtain the samples. The sampled trees showed high disease severities with typical MM symptoms like bud swelling, short thick rachis, peduncles thickening and compact/deformed panicles (Singh and Dhillon 1990a; Kumar and Beniwal 1992). Ten samples each of four symptomatic regions, viz. panicle shoot-juncture, apical bud and primary and secondary peduncles, were cut from non-contiguous branches of every cultivar. Each sample was excised into 10 tissues/subsamples. In this way 500 tissues of each symptomatic region representing 100 tissues of every cultivar were trimmed to investigate the deep seated infection.

To determine the fungus at different proximal and distant sites of the panicles, sixteen widely separated malformed and non-malformed mango trees of cv. Sensation growing at the Horticultural Research Institute, Faisalabad, Pakistan, were selected. Three branches were clipped from one tree followed by excision of 15 tissues from each branch. Forty five tissue samples, 5 mm long, were removed for each target distance, viz. 0, 1–5, 5–10, 10–20, 20–25, 25–30 and<30 cm, with a total of 360 tissues each for malformed and non-malformed branches.

For Transmission Electron Microscopy (TEM), mango trees of uniform age (10 years old), growing under similar soil and climatic conditions were selected. Young emerging healthy and fully swollen malformed apical buds were obtained from healthy and malformed branches of cv. Malda tagged in the preceding flowering cycle of the year in 2002. Healthy buds excised from non-malformed branches served as the control. In all the cases, the samples were maintained on ice from field collection to laboratory assay as described by Iqbal et al. (2003).

Identification of the fungus

Tissues sterilized for 2 min in 1% NaOCl solution were rinsed twice in sterilized deionized water, dried on sterile blotting papers, and placed into 9 cm Pyrex glass Petri plates containing Potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich.) amended with 100 mg streptomycine sulfate to avoid bacterial contamination (Pathak 1987; Ploetz and Gregory

1993; Akhtar 2000). The plates were incubated at 25°C with a photoperiod of 12 h and examined after 6–7 days of incubation. The colonizing fungus was identified based on characteristics specific for the species (Britz et al. 2002). The culture was purified on Carnation leaf agar (CLA) medium [5 g of 5 mm sterile Carnation (*Dianthus caryophyllus* L.) leaves; 15 g agar, 1000 ml distilled water] specific for *Fusarium spp.* (Nelson et al. 1983; Iqbal et al. 2005a) and the identification was verified on the basis of micro and macroconidia using light microscope (Olympus BX 50, Japan). The culture was lyophilized and additional copies were stored at 8°C for immediate use.

Ultrastructural assay

Culture proliferation

The sterilized 5 mm long bud pieces excised from healthy and malformed buds were immersed in liquid Potato dextrose medium in 1.5 ml Eppendorf tubes. The tubes were incubated at 25°C with 12 h alternating cycles of light and darkness for 96 h. The proliferating fungal growth was picked out aseptically and purified on PDA. Agar blocks with conidiating hyphae were cut into small pieces. Healthy bud pieces yielded no growth of the fungus.

Processing for TEM

Culture blocks and healthy and malformed bud pieces trimmed to 5 mm for ultrastructure observations were fixed in 5% glutaraldehyde for 16–18 h. Post fixation was done with 0.2% osmium tetroxide followed by staining with 5% aqueous uranyl acetate for 16–18 h. The material was then dehydrated in an ethanol series (30, 50, 70 and 90%) with 30 min per change and exchanged with 100% acetone (2× 15 min) prior to infiltration for 16–18 h in 1:1 acetone/spur resin, then in spur resin alone (Roberts 2002). The specimens were embedded in low viscosity epoxy resin (Spurr 1969). Ultra-thin sections 120–200 ηm thick were cut on Ultramicrotome RMC-7000 and ribbons of sections lifted on copper grids were double stained with uranyl acetate and lead citrate as described previously (Anjum 2001) and examined under TEM (JEOL JEM-1010, Tokyo, Japan) operating at 80 kV.

Experimental results

Identification of the fungus

The examination of malformed tissues of five exotic cultivars revealed the dominance of fungus *F. mangiferae*. The colonies appearing from a single conidium exhibited an initially orange colour on PDA, turning thereafter to varying colours on the reverse and obverse sides of Petri plates. Colonies showed purple or rosy-buff colours on the reverse of glass Petri plates after 12–14 days. The upper surface gradually manifested a purple or mixed pigmentation. The colony growth on CLA was granular white and sometimes cottony with a pinkish tinge. These distinctive cultural characteristics were retained after repeated sub-culturing. The sporulation started quickly after two days in the aerial mycelium, followed by development of subdeveloped macroconidia two days later. The macroconidia were 3 septate (Fig. 1a), slender, falcate, borne on monophialides and fairly abundant and typical on CLA. The size of macroconidia conformed to the reported range for the species, approximately, 3.5–5 to 45–60 µm (Gerlach and Nirenberg 1982; Britz et al. 2002). The microconidia were abundant, fusoid, oval and obovoid, 0 to 1 septate, apiculate on both ends and produced on polyphialides in false heads (Fig. 1b & c). Chlamydospores or sclerotia-like fungal fragments were absent.

Infection of *F. mangiferae* among exotic cultivars differed significantly. Tissue assay exhibited maximum infection of 88.5% in cv. Zill, colonizing 354 tissues out of 400, while Sensation and Tommy Atkins followed with 84.75% (339 of 400) and 82.5% (330 of 400), respectively (Table 1) . Least infection of 68.25% (273 of 400) was shown by Keitt which is still high. None of the exotic cultivar was found free of infection. Apical buds proved to be the potential infection site hosting significant infection of 86.2% amongst the four malformed regions (Table 1). Panicle-shoot juncture tissues and primary and secondary peduncles varied with tissue infection of 81.6, 79.4 and 74.8%, respectively. Zill exhibited maximum infection of 93.0, 90.0, 89.0 and 82.0% in all the four malformed regions, viz. apical buds, panicle shoot-juncture, primary and secondary peduncles, respectively, while Keitt showed least infection of 76.0, 71.0, 66.0 and 60.0% in the same order.

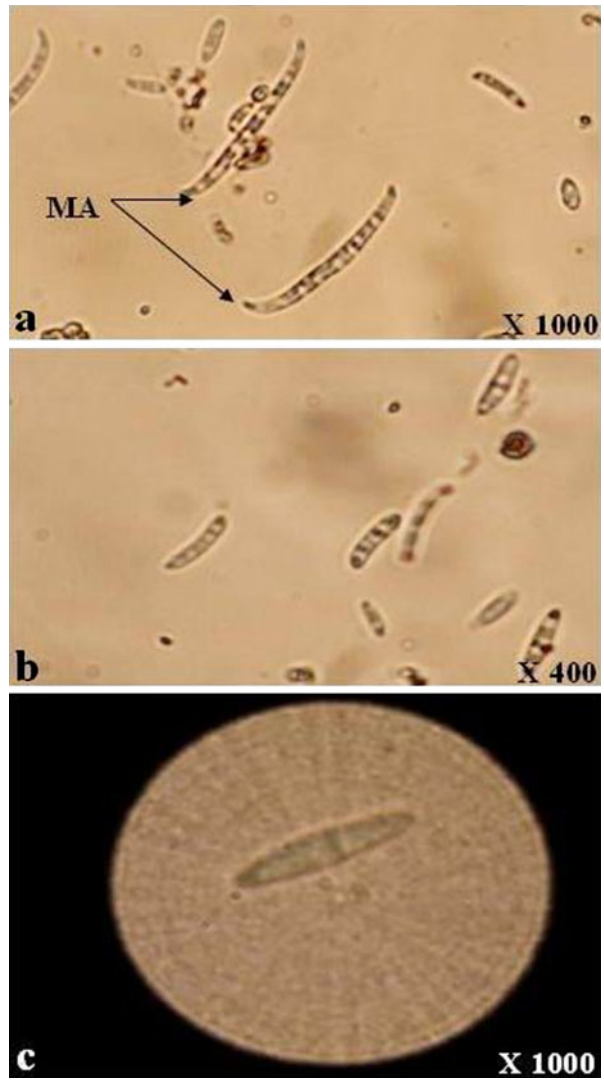

Fig. 1 Macro and microconidia of fungus *F. mangiferae*: **a** Macroconidia (MA); **b** & **c** Microconidia

Determination of *F. mangiferae* at proximal and distant sites of the malformed panicles revealed maximum recovery of 82.0% at 0 cm and only 3% at <30 cm distance beneath the panicle (Table 2). The fungus was frequently isolated from panicle shoot-juncture tissues but there was a gradual decrease in recovery of the fungus from panicles to the main stem in malformed organs. In case of non-malformed panicles, an infection of 14.0% was recorded at 0 cm distance while no isolations could be made from shoots/branches that supported non-malformed panicles. On an average, 34.76% of the tissues in malformed and only 1.75% in non-malformed branches were found infected; this difference was significant at $P<0.003$.

Interaction of *F. mangiferae* with malformed buds

Culture proliferation

The *in vitro* grown fungal culture obtained from malformed buds of cv. Malda revealed longitudinal and crosswise arranged sections of hyphae when observed under TEM (Fig. 2a & b). The longitudinal sections of hyphae were septate. Oval to fusiform, single and bicelled microconidia (Fig. 2a & c), a

Table 1 Recovery of *F. mangiferae* from different malformed regions of exotic mango cultivars

Cultivar	District	% infection in malformed regions				
		Panicle-shoot juncture	Apical buds	Primary peduncles	Secondary peduncles	Mean infection
Keitt	Lodhran	71.0 k	76.0 i	66.0 l	60.0 m	68.25 e
Maya	Multan	80.0 gh	87.0 bcd	72.0 jk	75.0 ij	78.50 d
Sensation	Faisalabad	86.0 cd	90.0 ab	86.0 cd	77.0 hi	84.75 b
Tommy Atkins	Multan	81.0 fg	85.0 c-e	84.0 d-f	80.0 gh	82.50 c
Zill	Multan	90.0 ab	93.0 a	89.0 bc	82.0 e-g	88.50 a
Mean		81.6 b	86.2 a	79.4 c	74.8 d	80.50

Means followed by the same letter(s) do not differ significantly (p=0.05) by LSD test

characteristic feature of *F. mangiferae*, were also observed with similar morphology as under the light microscope but varied in magnification and degree of resolution.

Cytology of infection of mango buds by F. mangiferae

On examination of ultra-thin bud sections, no fungus was detected in healthy tissues, cells or intercellular spaces. Well organized and clear cell orientation was observed in healthy tissues of cv. Malda (Fig. 3a & b). No such distinction could be made in tissues of malformed buds, wherein cell walls were mostly thick and closely packed. The hyphae displayed the ability to penetrate bud tissue and invasion of the fungus was inter- and intra-cellular. Intensity of colonization in the cells resulted in ramification and frequent non-osmicated, transverse hyphal sections were observed (Fig. 3c). The non-osmicated sections appeared slightly blurred. Intercellular ingression of the fungus was also confirmed by presence of well defined, osmicated cross sections lying in tissue of malformed bud (Fig. 3d).

Table 2 Determination of *F. mangiferae* at proximal and distant sites of the panicles of mango cv. Sensation

Distance behind panicle (cm)	% Infection	
	Malformed	Non-malformed
0	82.0	14.00
1–5	50.14	0.00
5–10	44.0	0.00
10–15	37.0	0.00
15–20	31.0	0.00
20–25	19.0	0.00
25–30	12.0	0.00
>30	3.0	0.00
Mean	34.76±24.92	1.75±4.95

T-test: Values are percentages (± SD) of healthy and malformed tissue pieces from which *F. mangiferae* was isolated

Discussion

The fungus causing MM has remained a focus of study due to the complexity of the disease. A high percentage of tissue infection and frequency of *F. mangiferae* in malformed tissues of all the exotic cultivars in the present study confirms the possible role of the fungus in causing MM. Zill proved to be the most susceptible amongst the exotic cultivars showing 88.5% infection. Recovery of *F. mangiferae* as a causal agent from malformed tissues of different cultivars grown in diverse agro-climatic zones of the world has already been proven (Britz et al. 2002; Freeman et al. 2004). Apical buds proved to be the potential infection site giving an infection of 86.2% in 500 malformed tissues of five exotic cultivars. Other symptomatic regions like panicle-shoot juncture and primary and secondary peduncles also showed almost equally high infection levels. Mean infection of *F. mangiferae* in five exotic cultivars and four symptomatic regions was 80.5% (Table 1). Scanty information is available on quantitative distribution of *F. mangiferae* in malformed and healthy mango trees. Crookes and Rijkenberg (1985a) reported frequent isolations of *F. subglutinans* from malformed tissue and infrequent iso-

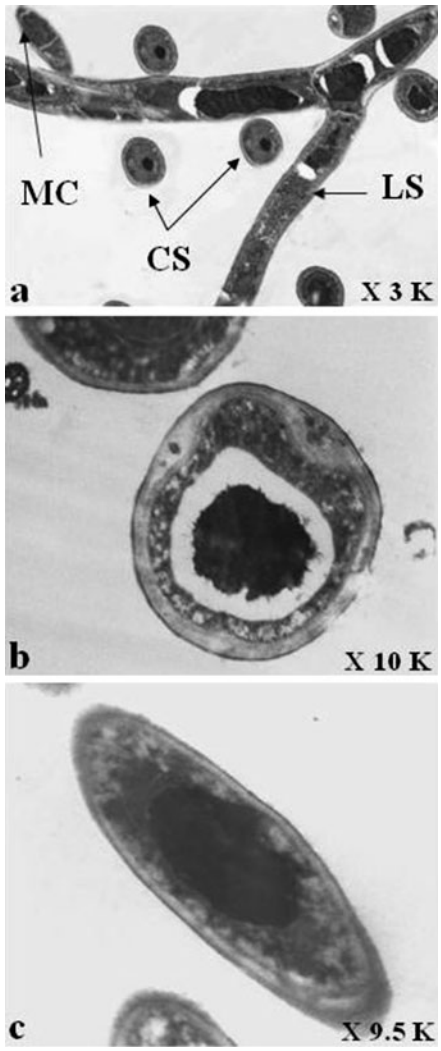

Fig. 2 Culture proliferation: **a** Cross sections (*CS*) and longitudinal sections (*LS*) of fungal hyphae yielded from malformed buds of mango cv. Malda. Bi-celled microconidium (*MC*) is also visible; **b** Magnified image of cross section of hypha; **c** Single celled, fusiform, microconidium of fungus *F. mangiferae* observed under TEM

lations from supporting branches, but did not present quantitative data. Darvas (1987) corroborated the findings of Crookes and Rijkenberg (1985b) but his conclusions were inferred from data from a single tree. Ploetz (1994) made direct comparisons at 0, 1–10, 11–30 and >31 cm distances behind the panicles in six branches. The fungus was isolated more frequently from malformed than from non-malformed tissues (68.3 vs. 11.7% of the sampled tissues, respectively). In the present study, 720 tissues were assayed from 48 scaffold branches of 16 widely separated trees. Recovery of *F. mangiferae* was examined at 8 different distances either proximate or distant to the panicles at narrow spacings to provide a true disease estimation. At 0 cm distance, an infection of 82.0 and 14.0% was recorded in malformed and non-malformed tissues, respectively. These data suggest that massive colonization by the fungus is a prerequisite to development of symptoms of malformation in mango panicles. The fungus was less frequently isolated from branch tissues that supported malformed panicles and was never isolated from branches having non-malformed panicles. This means that systemic colonization by *F. mangiferae* with increasing distance down the stem is quite rare. Data corroborating this conclusion is supported by the findings of Ploetz (1994) who observed only 1.3% infection at >31 cm distance from the panicles. In our study, infection level was 3.0% at distance above 30 cm. This is the reason that standard management strategies rely on removal of malformed tissues at three basipetal nodes or 0.5 m distance from the panicle (Manicom 1989; Iqbal et al. 2005b).

The colonizing fungus in apical buds and other malformed regions was identified as *F. mangiferae*. Complementary confirmations through electron microscopy were essential to strengthen the light microscopic studies. The ultrastructure of vegetative and reproductive fragments of the fungus provided a good comparison with their physical features at ordinary magnifications (Figs. 1b, 2a, b & c and 3c & d). Morphology of hyphal sections and microconidium was identical with light microscopic observations of *F. mangiferae*. Fine structure of conidial surfaces and conidial morphology were observed by means of TEM only. Conidial surface ornamentation and matrix could not be reliably distinguished with light microscopy unlike as observed with TEM, and this may be significant in taxonomic studies. Examination of ultra-thin bud sections revealed successful inter- and intra-cellular invasion of the fungus. No such infection was observed in healthy tissues or cells. When hyphae of the pathogen multiplied extensively, their ramification was marked with ultrastructural changes characterized by drastic tissue alterations. Organelles were no longer discernible in such altered fungal cells. This extensive cell colonization affecting the

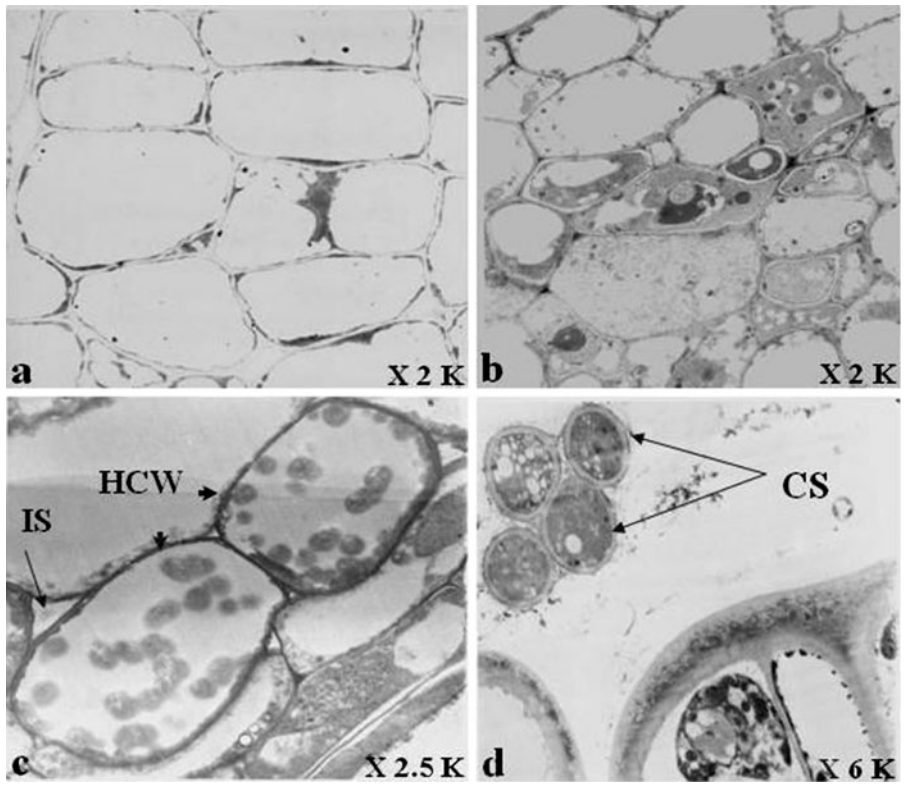

Fig. 3 Healthy and infected bud tissue of mango cv. Malda: **a** & **b** Healthy cells free of fungal infection; **c** Infected cells showing intracellular ramified growth of fungal hyphae. Host cell wall (*HCW*) is indicated by arrow heads and intercellular space (*IS*) by arrow; **d** Distinct osmicated (treated with osmium tetroxide) cross sections of hyphae lying within tissue

host defence response coincided with disturbance in cell metabolism and expression of external symptoms such as swelling and hypertrophy of bud cells thereafter (Kumar and Beniwal 1992). In all of the examined malformed buds, a positive correlation was established between bud damage and the presence of *F. mangiferae* because the fungus was readily isolated from tissue sections collected in and around the symptomatic areas. Fungal colonization was also found associated with some structural changes including marked thickening of the host cell walls in the invaded cells. This might occur due to host response to fungal ingress. No systematic study on ultrastructure of mango buds or *F. mangiferae* has been reported so far. Usha et al. (1997) found mycelia of *F. subglutinans* in malformed mango buds while no such growth was observed in healthy buds. Their study illustrated only superficial mycelia with the help of a electron microscope but inter- and intra-cellular fungal ingression and related ultrastructural detail were not be elucidated.

Plant-pathogen interactions are mediated by a complex network of cytological, histopathological and molecular events that ultimately turn resistance to susceptibility (Lamb et al. 1989; Kumar and Beniwal 1992). A novel finding of the present study concerns the interaction of *F. mangiferae* with apical buds. Emerging tender buds, containing low amounts of the defensive chemical mangiferin, but high carbohydrate content may serve as the preferential site of infection for the fungus (Chakrabarti and Ghosal 1989; Usha et al. 1996). Wounds, mechanical injuries or mite infection provide avenues for fungal infection. The buds on healthy branches which are proximate to the diseased ones are at risk of infection (Ploetz and Gregory 1993; Iqbal et al. 2005a). From buds, with the growing tissues, infection moves to other regions including the rachis of the emerging panicle and primary, secondary and tertiary peduncles. Symptoms may remain latent after preliminary infection, but massive colonization by macroconidia results in typical misshapen shoots and compact inflorescences. Studies are under way to devise an integrated strategy thet include different pruning practices to curb this malady in mango orchards.

References

Akhtar, K. P. (2000). Fresh potato extract the best source for the growth of *Colletotrichum gloeosporioides* causing anthracnose of mango and *Fusarium subglutinans* isolated from malformed inflorescence of mango. *Pakistan Journal of Phytopathology, 12*, 134–136.

Anjum, M. A. (2001). Cytology of potato callus cells in relation to their frost hardiness. *Biologia Plantarum, 44*, 325–331.

Britz, H., Steenkamp, E. T., Coutinho, T. A., Wingfield, B. D., Marasas, W. F. O., & Wingfield, M. J. (2002). Two new species of *Fusarium* section Liseola associated with mango malformation. *Mycologia, 94*, 722–730.

Chakrabarti, D. K., & Ghosal, S. (1989). The disease cycle of mango malformation induced by *Fusarium moniliforme* var. *subglutinans* and the curative effects of mangiferin-metal chelates. *Journal of Phytopathology, 125*, 238–246.

Crookes, C. A., & Rijkenberg, F. H. J. (1985a). Isolation of fungi associated with blossom malformation of mangoes. *South African Mango Growers' Association Research Report, 5*, 10–14.

Crookes, C. A., & Rijkenberg, F. H. J. (1985b). A literature review of the distribution, symptomatology, cause and control of mango blossom proliferation. *South African Mango Growers' Association Research Report, 5*, 15–24.

Darvas, J. M. (1987). Control of mango blossom malformation with trunk injection. *South African Mango Growers' Association Research Report, 7*, 10–14.

Das, G. C., Rao, D. P., & Lenko, P. C. (1989). Studies on mango malformation in mango clone *Chiratpudi*. *Acta Horticulturae, 231*, 866–872.

Freeman, S., Maimon, M., & Pinkas, Y. (1999). Use of GUS transformants of *Fusarium subglutinans* for determining etiology of mango malformation disease. *Phytopathology, 89*, 456–461.

Freeman, S., Klein-Gueta, D., Korolev, N., & Sztejnberg, A. (2004). Epidemiology and survival of *Fusarium mangiferae*, the causal agent of mango malformation disease. *Acta Horticulturae, 645*, 487–491.

Gerlach, W., & Nirenberg, H. (1982). *The genus Fusarium—a pictorial Atlas*. Berlin: Kommission sverlag Paul Parey.

Ginai, M. A. (1965). Malformation of Mango inflorescence (West Pakistan). *Journal of Agricultural Research, 3*, 248–251.

Iqbal, Z., Saleem, A., & Dasti, A. A. (2003). Comparative distribution of *Fusarium subglutinans* in malformed and non-malformed tissues of mango. *Pakistan Journal of Phytopathology, 15*, 17–20.

Iqbal, Z., Saleem, A., & Dasti, A. A. (2005a). Manifestation of vegetative malformation on mango seedlings by artificial inoculations of fungus *Fusarium mangiferae*. *Pakistan Journal of Phytopathology, 17*, 1–6.

Iqbal, Z., Dasti, A. A. & Saleem, A. (2005b). Combating mango malformation through an integrated management strategy. (Paper presented at 1st International Conference on Mango and Date palm: Culture and Export Production for Global Market. Faisalabad,University of Agriculture).

Kumar, J., & Beniwal, S. P. S. (1992). Mango malformation. In J. Kumar, H. S. Chaube, U. S. Singh, & A. N. Mukhopadhyay (Eds.), *Plant diseases of international importance: Diseases of tropical fruit crops* (Vol. 3, pp. 357–393). New Jersey: Prentice Hall.

Lamb, C. J., Lawton, M. A., Dron, M., & Dixon, R. A. (1989). Signals and transduction mechanisms for activation of plant defense against microbial attack. *Cell, 56*, 215–224.

Manicom, B. Q. (1989). Blossom malformation of mango. *South African Mango Growers' Association Yearbook, 10*, 11–12.

Nelson, P. E., Tousson, T. A., & Marasas, W. F. O. (1983). *Fusarium species: An illustrated manual for identification*. University Park: The Pennsylvania State University Press.

Pathak, V. N. (1987). *Laboratory manual of plant pathology* (2nd ed.). New Delhi: Oxford & IBH.

Ploetz, R. C. (1994). Distribution and prevalence of *Fusarium subglutinans* in mango trees affected by malformation. *Canadian Journal of Botany, 72*, 7–9.

Ploetz, R. C. (1999). Malformation: A unique and important disease of mango, *Mangifera indica* L. In B. A. Summerell (Ed.), St. Paul Minnesota: APS.

Ploetz, R. C., & Gregory, N. (1993). Mango malformation in Florida: distribution of *Fusarium subglutinans* in affected trees, and relationships among strain within and among different orchards. *Acta Horticulture, 341*, 388–394.

Prashad, A., Singh, H., & Shukla, T. M. (1965). Present status of mango malformation disease. *Indian Journal of Horticulture, 22*, 254–265.

Roberts, I. M. (2002). Iso-butanol saturated water: a simple procedure for increasing staining intensity of resin sections for light and electron microscopy. *Journal of Microscopy, 207*, 97–107.

Singh, Z., & Dhillon, B. S. (1987). Occurrence of malformin-like substances in seedlings of mango (*Mangifera indica* L.). *Journal of Phytopathology, 120*, 245–248.

Singh, Z., & Dhillon, B. S. (1988). Hormonal changes associated with vegetative malformation of mango (*Mangifera indica* L.). *Journal of Phytopathology, 125*, 193–197.

Singh, Z., & Dhillon, B. S. (1990a). Comparative developmental morphology of healthy and malformed floral organs of mango (*Mangifera indica* L.). *Tropical Agriculture, 67*, 143–148.

Singh, Z., & Dhillon, B. S. (1990b). *In* vivo role of indole-3-acetic acid, gibberellic acid, zeatin, abscisic acid and ethylene in floral malformation of *Mangifera indica* L. *Journal of Phytopathology, 128*, 235–245.

Singh, Z., Dhillon, B. S., & Arora, C. L. (1991). Nutrient levels in malformed and healthy tissues of mango (*Mangifera indica* L.). *Plant and Soil, 133*, 9–15.

Spurr, A. J. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research, 26*, 31–43.

Usha, K., Sharma, H. C., Goswami, A. M., & Singh, B. (1996). Photosynthetic efficiency and translocation pattern in relation to floral malformation in mango. Paper presented at International Conference on Tropical Fruits, Kuala Lumpur.

Usha, K., Goswami, A. M., Sharma, H. C., Singh, B., & Pande, P. C. (1997). Scanning electron microscopic studies on floral malformation in mango. *Scientia Horticulturae, 71*, 127–130.