

Proceedings:

International Conference on
Mango and Date Palm: Culture and Export.
20th to 23rd June, 2005.
Malik *et al.* (Eds), University of Agriculture, Faisalabad.

SELECTIVE GROWTH MEDIA TO STUDY MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF *Fusarium mangiferae*, THE CAUSE OF MANGO MALFORMATION

*Zafar Iqbal¹, Altaf Ahmad Dasti² and Ahmad Saleem¹

¹Plant Protection Institute, Faisalabad-Pakistan.

² Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan-Pakistan

*Corresponding author's e-mail: zafarrohils@yahoo.com

ABSTRACT

Different growth media viz. Potato dextrose agar (PDA), Carnation leaf agar (CLA), Fresh Carnation leaf agar (FCLA), Banana leaf agar (BLA), Mango leaf agar (MLA) and Spezieller Nährstoffarmer Agar (SNA) were tested to study cultural and morphological features of isolates FM-1, FM-2 and FM-4 of fungus *Fusarium mangiferae*, the causal agent of mango malformation. PDA promoted maximum mycelial growth, comparatively less sporulation and varying pigmentation. The tested isolates showed different colony colours on reverse and obverse sides of petridishes with dominant purple tinge. PDA proved to be a good medium for isolation and morphological differentiation of strains/ isolates. CLA and FCLA equally exhibited maximum macroconidial production with least phenotypic variation. Both the media ideally suited for microphotography, conidial morphology and measurements. BLA and MLA also showed abundant macroconidia of the fungus. SNA enhanced production of both micro and macroconidia. For isolates/strains studies, PDA and CLA are suggested to be the best media.

Key words: *Mangifera indica*, isolates, conidia, morphology, pigmentation, micrometry

INTRODUCTION

Mango (*Mangifera indica* L.) occupies a prominent position among the commercial fruits of the world. Global mango production is 26.28 M tonnes from an area of 3.69 M ha. In Pakistan, it is cultivated on an area of 0.1 M ha with annual production of 1.072 M tonnes (FAO, 2004). Several animate and inanimate diseases attack mango. Malformation is the most important symptom causing colossal losses every year. It has been reported from Pakistan, Egypt, South Africa, Brazil, Israel, Central America, Mexico, USA, Sudan, Cuba, Australia, Bangladesh and UAE (Kumar *et al.*, 1993). Two types viz. vegetative and floral have been reported. Vegetative malformation is manifested on seedlings, saplings and even on mature trees whereas floral malformation attacks floral organs on flowering plants. Both symptoms are confirmed to be expression of the same disease (Tripathi, 1954; Schlosser, 1971). Despite strenuous efforts, complete control has not yet been achieved. Different etiologies like viral (Kausar, 1959), acarological (Singh *et al.*, 1961; Singh, 1997) and physiological (Sattar, 1946; Sharma, 1953)

have previously been reported but they lack causative relationships. Recent literature confirms that a fungus *Fusarium mangiferae* is the cause of the disease. Koch's postulates have been completed by artificial inoculations with strains of *F. mangiferae* (Freeman et al., 1999; Saleem, 2004). These results confirmed that vegetative and floral malformation of mango were both caused by *F. mangiferae* and other factors or agents, such as eriophyid mites had secondary roles in the disease development.

Fusarium is a cosmopolitan genus of filamentous fungi, the members of which represent an important group of plant pathogens. Species in this complex are important because of their association with diseases of agronomically important plants and are best noted for their many secondary metabolites which include mycotoxins (Correll et al., 1991; Marasas et al., 1984; Leslie, 1995). Due to complex nature of the malformation pathogen, the fungus and its strains are still an interesting topic for the scientists. Suitable growth medium is a prerequisite to study the fungus under in vitro conditions. Potato dextrose agar medium (PDA) is a universal medium used to study *F. mangiferae* (Ploetz and Gregory, 1993; Iqbal et al., 2003). PDA is a valuable medium principally for gross morphological appearances and colony colorations. Carnation leaf agar has the advantage of promoting sporulation rather than mycelial growth. Conidia and conidiophores are produced in abundance with least phenotypic variation (Nelson et al., 1983). Banana leaf agar and Spezieller Nährstoffarmer agar media are also good media to purify and examine the fungus *F. mangiferae* (Ploetz and Gregory, 1993).

The present studies were imperative to examine the cultural characteristics and morphological features of the fungus *F. mangiferae* empirically on selective media. The major objective of the study was to simplify and standardize the best media to facilitate preliminary processing as some plant source media are not specifically quantified.

MATERIALS AND METHODS

Potato dextrose agar (PDA)

White skinned, baking grade peeled potatoes (200 gm) were cut into small pieces and boiled for 30 to 45 minutes in 500 ml distilled water until the pieces became soft. The distilled water containing starch was poured into a conical flask through a cheese cloth and 20 gm each of agar agar and glucose were added. Volume was made up to 1000 ml.

Carnation leaf agar (CLA)

Carnation (*Dianthus caryophyllus* L.) leaves were harvested from fresh potted carnation plants and washed with distilled water to remove surface dust. Leaf pieces (5 mm²) were excised and sun dried. Sun drying might be substituted by oven drying at 45-55°C for 2 hr (Nelson et al., 1983). Agar agar (15 gm) and carnation leaves (5 gm) were added to 1000 ml distilled water in a Pyrex glass flask.

Fresh Carnation Leaf Agar (FCLA)

Fresh Carnation leaves weighing 20 gm were ground with pestle mortar adding distilled water. Sap was obtained by passing through muslin cloth. After addition of Agar agar (15) gm, volume was made up to 1000 ml.

Banana leaf agar (BLA)

Fresh banana leaves (20gm) were collected and medium was prepared as described under FCLA.

Mango leaf agar (MLA)

Fresh mango leaves (20 gm) were picked from mango plant and medium was prepared as described above.

Spezieller Nährstoffarmer Agar or Synthetic nutrient agar (SNA)

SNA was prepared with the following ingredients: KNO₃ 1.0 gm, KH₂PO₄ 1.0 gm, MgSO₄ 7H₂O 0.5 gm, KCL 0.5 gm, glucose 0.2 gm, sucrose 0.2 gm, agar agar 15 gm and dH₂O 1000 ml (Singh et al., 1991). The flasks were sealed and all the media were autoclaved for 30 min and then for additional 10 min to avoid any resistant contaminants. Streptomycin sulfate was

added @ 1gm/litre of medium to check bacterial contamination when media cooled down to 40°C. Uniform pouring (25 ml/dish) was done into sterilized 9 cm dia. glass Petriplates and allowed to solidify at room temperature.

Isolates

Three isolates of *F. mangiferae* obtained from Bahawalpur, Lodhran and Vehari, identified and maintained at Plant Protection Institute Faisalabad, Pakistan, were selected for the study. The isolates were prefixed as FM-1, FM-2 and FM-4, respectively.

In vitro culture

The single spore subsets of isolates were used for morphological and cultural studies (Saleem, 2004). A few mycelial threads were inoculated on each medium. The plates were incubated at 25°C under cool white light to promote maximum macroconidial production and varying pigmentation. The cultures received alternating cycles of 12 hr of light and darkness. Colony diameter of isolates was observed after 7 and 12 days of incubation on CLA and PDA media while FCLA, BLA, MLA and SNA were used for morphological and microscopic observations.

RESULTS AND DISCUSSION

The isolates showed different type of colorations on PDA medium. The undersurface of colonies of isolates showed distinct purple pigmentation. PDA achieved maximum mycelial growth with comparatively little or meager sporulation due to much carbohydrate content (Table 1). Growth rate of all the isolates was faster on PDA as compared to CLA after 7 and 12 days of incubation. The colony growth on CLA was granular white and sometimes slight cottony with light pinkish tinge on obverse of the Petri dish. On BLA and MLA the growth was almost white and less prominent. On SNA, clear white growth was observed.

Four celled macroconidia were always found. Conidia were slightly sickle shaped to straight with dorsal and ventral surfaces almost parallel. The microconidia were abundant, fusiform, obovoid, produced on polyphialides and none to one septate (Nelson et al., 1983; Britz et al., 2002). Ideal macroconidial production was observed in CLA and FCLA. The size of the macroconidia was in the range of 3.5-5 x 45-60 µm. BLA, MLA and SNA also showed excellent macroconidia. Abundant microconidia were observed in SNA because KCL in SNA promotes microconidia. Poly phialides were observed on all the tested media but chlamyospores were absent.

Despite provision of unequivocal evidence that *F. mangiferae* is the cause of the disease, some citations still confuse the etiology. So malformation fungus remains under focus of study. The latest approach is to study the isolates of worldwide collection and find isolates conspecific to newly characterized species. Cultural and morphological studies require selection of suitable growth media to examine specific phenotypic and morphological features. Most common method for isolation of *Fusarium* from plant material is by culturing colonized material on agar media. Since morphology of the macroconidia is the basis for identification, it is necessary to standardize the methods to make the task of identification easier.

Careful selection of media ensures promising colony growth, distinct micro and macroconidial morphology and varying pigmentation to screen strains of ecological proximity or different national and international origins. Mutations are enhanced when *Fusarium* species are grown on PDA and similar media. The problem is minimized by subculturing a little as possible, using the single spore or hyphal tip techniques and by not subcluturing or storing fungi on media high in carbohydrates (Nelson et al., 1983).

Best reported identification is based exclusively on CLA. In the present study, CLA proved to be the best medium to study the micro and macroconidia of *F. mangiferae*. Abundant macroconidial production with least phenotypic variation was ensured. This is in conformity to the findings of Nelson et al. (1983) and Viljoen et al. (1997). CLA promises excellent sporulation and least mycelial growth. The uniformity of macroconidia avoids complications in identification.

It is low in carbohydrates and contains naturally occurring substances as encountered by *Fusarium* in nature. The fungus can be grown on CLA like a naturally occurring substrate. Constitution of the medium determines and satisfies the host loving nature of the pathogen. So CLA provides host like properties. BLA and MLA also provide natural substances essential for *Fusaria*. Mango is the natural host of *F. mangiferae*. So typical and prominent macroconidia were observed on MLA.

Although every individual aforementioned medium is a good choice against *F. mangiferae*. But it is suggested that for isolate/strain screening, PDA and CLA are the best media (Viljoen et al., 1997; Saleem, 2004). According to the species or the ecological situation, either micro or macroconidia may dominate on a natural substrate. All of these forms must be observed carefully to identify a *Fusarium* species with certainty. Identification of *Fusarium* species requires careful observation and attention. Color differentiation, diversity and colony morphology of isolates can be visualized on PDA and morphology of micro and macroconidia are best ensured by CLA. Microphotography and conidial measurements (micrometry) can also be best done on CLA.

REFERENCES

- Correll, J.C., Gordon, T.R., McCain, A.H., Fox, J.W., Koehler, C.S., Wood, D.L. and Schultz, M.E. 1991. Pitch canker disease in California: Pathogenicity, distribution and canker development on Monterey pine (*Pinus radiata*). *Plant Disease* 75:676-682.
- Food and Agricultural Organization. 2004. www.fao.org
- Freeman, S., Maimon, M. and Pinkas, Y. 1999. Use of GUS transformants of *Fusarium subglutinans* for determining etiology of mango malformation disease. *Phytopathology* 89(6):456-461.
- Iqbal, Z., Saleem, A. and Dasti, A.A. 2003. Comparative distribution of *Fusarium subglutinans* in malformed and non-malformed tissues of mango. *Pak. J. Phytopathol.* 15(1-2):17-20.
- Kausar, A.G. 1959. Malformation in inflorescence of mango. *Punjab fruit J.* 22:19-21.
- Kumar, J., Singh, U.S. and Beniwal, S.P.S. 1993. Mango malformation: One hundred years of research. *Ann. Rev. Phytopath.* 31:217-232.
- Leslie, J.F. 1995. *Gibberella fujikuroi*: Available populations and variable traits. *Canad. J. Bot.* 73(1):282-291.
- Marasas, W.F.O., Nelson, P.E. and Toussoun, T.A. 1984. Toxicogenic *Fusarium* spp.: Identity and Mycotoxicology. The Pennsylvania State University Press, University Park. p. 328.
- Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* species: An illustrated manual for identification. The Pennsylvania State University Press, University Park. p.123.
- Ploetz, R.C. and Gregory, N. 1993. Mango malformation in Florida: Distribution of *Fusarium subglutinans* in affected trees and relationships among strains within and among different orchards. *Acta Hort.* 341:388-394.
- Saleem, A. 2004. Studies on malformation of mango. 3rd Annual report of Agricultural Linkage Programme (ALP) Project, Plant Protection Institute, Faisalabad, Pakistan. p. 85.
- Sattar, A. 1946. Diseases of mango in the Punjab. *Punjab Fruit J.* 10:56-58.
- Schlosser, E. 1971. Mango malformation: Incidence of bunchy top on mango seedling in West Pakistan. *FAO Plant Protec. Bull.* 19(2):41-42.
- Sharma, B.B. 1953. Studies in the diseases of *Mangifera indica*. *Proc. 40th Ind. Sci. Congress III.* p. 70-71.
- Singh, K., Frisvad, J.C., Thrane, U. and Mathur, S.B. 1991. An illustrated manual on identification of some seed-borne *Aspergillus*, *Fusaria*, *Penicillia* & their mycotoxins. Danish Govt. Inst. of Seed Pathology for Developing countries, Denmark. p. 133.
- Singh, L.B., Singh, S.M. and Nirvan, R.S. 1961. Studies on mango malformation I. Review symptoms, extent, intensity and cause. *Hort. Advance* 5:197-217.

- Singh, P.K. 1997. Biology of some mites associated with mango malformation. Thesis, M.Sc. Entomology, N.D.Univ. of Agric. and Tech. p. 50.
- Tripathi, R.D. 1954. Bunchy top and malformation diseases of mango. Ind. J. Hort. 11:122-124.
- Viljoen, A., Marasas, W.F.O., Wingfield, M.J. and Viljoen, C.D. 1997. Characterization of *Fusarium subglutinans* f. sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. Mycol. Res. 101(4):437-445.

TABLES

Table 1: Morphological characteristics of *F. mangiferae* isolates on selective growth media

Medium	Colony color on medium			Fluffy mycelial growth	Macro conidia	Micro conidia	Poly phialides
	3 days	7 days	12 days				
PDA	Orange	Purple	Dark purple	Excellent	Poor	Good	+
CLA	White	Granular white	Granular white with light pink tinge	Poor	Excellent	Excellent	+
FCLA	White	Granular white	Granular white with light pink tinge	Poor	Excellent	Excellent	+
BLA	White	White	White	Poor	Excellent	Good	+
MLA	White	White	White	Poor	Excellent	Good	+
SNA	Clear white	White	White	Poor	Good	Excellent	+

+ = Present