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VARIABILITY AMONG ISOLATES OF FUNGUS *FUSARIUM MANGIFERAE* ASSOCIATED WITH MALFORMATION DISEASE OF MANGO

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Abstract

Twenty isolates of the fungus *Fusarium mangiferae* were isolated and identified from 14 different locations of Pakistan. The isolates FM-1 to FM-20 showed prominent purple or rosy buff pigmentation on the under surface of the Petri dishes on Potato dextrose agar medium. On Carnation leaf agar the colony growth was granular white and sometimes cottony with pinkish tinge. Some differences among cultural and morphological features were detected after 3, 7 and 12 days of inoculation. Maximum colony diameter of 32.16 mm after three days was exhibited by isolate FM-2 on PDA and 32.5 mm by FM-17 on CLA, after seven days, 66.0 mm by FM-20 on PDA and 65.5 mm by FM-18 on CLA. After twelve days, maximum colony diameter of 81.33 and 80.0 mm was shown by FM-20 on PDA and CLA, respectively. Isolates FM-20 proved to be fast and FM-7 slow growing with mean colony size of 57.50 and 42.96 mm on both the media, respectively. The present study elucidates mycological variability among isolates of ecological proximity or different national origins.

Introduction

Mango (*Mangifera indica* L.) is affected by various biotic and abiotic stresses. Malformation is the most important malady causing colossal losses every year. Since its inception (Waite, 1891), the problem defines complete solution. Two expressions of malformation *viz.*, vegetative and floral have been characterized (Schlosser, 1971). Identical morphology and etiology of vegetative and floral isolates identified from mango have been demonstrated (Varma *et al*., 1974; Mitra & Lele, 1981). Almost all the cultivars lack resistance and have succumbed to diverse virulence of *F. mangiferae* isolates. The significance of mangiferin-induced changes in evolving the host-specific strains of the pathogen on mango has been reported. These include the production of more aerial hyphae but, less pigment and an increase in parasitic ability with reduced saprophytic growth (Kumar & Chakraborty, 1995). Terao *et al*., (2001) observed mango trees in Brazil to exhibit floral and vegetative malformations. The obtained isolates from infected shoots and panicles were identified as *F. subglutinans* [*F. mangiferae*]. Both the floral and vegetative malformations were considered to be two different expressions of the same species. Steenkamp *et al*., (2000) studied a set of malformation isolates that were subsequently described by Britz *et al*., (2002) as a new species *F. mangiferae*. Ploetz *et al*., (2002) identified 77 isolates of fungi from mango orchards of Egypt.

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All the isolates conformed to the species *F. mangiferae*. Study supported the conclusion that the isolates of the pathogen from Nile Delta were responsible for the appearance of the disease in El-Faiyum. Saleem (2005) compared isolates of *F. mangiferae* obtained from different locations of Pakistan for their morphological and specific characteristics. The isolates were found similar to the previous descriptions of conidial morphology (Britz *et al*., 2002). The objectives of the study were to glean an insight into the association of isolates of *F. mangiferae* with malformation disease and find out mycological variability among isolates of diverse origins.

Materials and Methods

Sampling: Infected mango shoots along with malformed panicles were collected randomly from different susceptible cultivars showing typical symptoms of malformation after surveying 14 districts of Pakistan. After tagging, samples were immediately brought to the laboratory in an ice box to avoid drying during transit. The parameter of origin was given due consideration to represent almost all the commercial mango producing areas.

Morphological and cultural characteristics: The experiment was arranged *in vitro* in completely randomized design with three replications. Ten tissue pieces, 5 mm long, excised randomly from peduncles and panicle-shoot juncture of each sample were surface disinfested for 10 sec in 70 % ethanol and for 2 min., in 1 % NaOCl solution. The tissues were rinsed twice in sterilized deionized water, dried on sterile blotting papers and placed into glass Petri plates containing PDA (Ploetz & Gregory, 1993; Akhtar, 2000). The plates were incubated at 25º C under cool white fluorescent light with 12 h cycling of light and darkness to promote typical coloration and maximum micro and macroconidial production. Identification was verified on the basis of typical micro and macroconidia (Britz *et al*., 2002). Isolates were single spored on PDA and CLA media. Morphological and cultural features such as colony colour, colony growth, number of septations, presence/ absence of polyphialides and chlamydospores were recorded. The colony diameter of isolates was observed after 3, 7 and 12 days of incubation. After initial culturing, duplicate samples were lyophilized and also maintained at 8º C for future use. Colony diameters of isolates were analyzed by analysis of variance as a three way factorial combination of isolate, media and days. Mean separations were determined at P= 0.05 by LSD test.

Results and Discussion

Prefixing and origin: The single-spored subsets of recovered isolates on PDA and CLA media exhibited varying pigmentation and abundant macroconidial production with least phenotypic variation, respectively. Twenty representative isolates were identified from 14 different locations of Pakistan. The isolates were prefixed from FM-1 to FM-20. Nineteen isolates were selected from mango growing areas of 13 districts of the Punjab province. One isolate FM-12 was from Mardan (N.W.F.P. province). Three isolates viz., FM-5, FM-13, and FM-16 were isolated from vegetatively malformed part, while, 17 categorized under floral type malformation (Table 1).

Morphological and cultural characteristics: All the malformation isolates possessed morphological features of *F. mangiferae* conforming to the attributes of the species (Britz *et al*., 2002). The colonies originating from single-conidium isolates exhibited an initial orange colour on PDA, which later turned to varying colours on reverse and obverse sides of Petri dishes. The colonies were mostly dense and tinged with purple. The under surface of the Petri dishes was orange with a purple mid point at first, which after 14 days led to a prominent purple or rosy buff pigmentation spreading across the whole surface. Top of the cultures mostly showed mixed coloration (Table 1). The colony growth on CLA was granular white and sometimes cottony with pinkish tinge. The distinctive cultural characteristics retained when single-spored isolates were sub-cultured. Aerial mycelia were usually abundant on PDA. None of the isolates developed sclerotia or chlamydospores like fragments. The sporulation started quickly after two days in the aerial mycelium as microconidia cohering always in false heads. Again two days later, sub-developed macroconidia appeared between them. The macroconidia were slender, falcate and fairly abundant and typical on CLA. Three-septate macroconidia were always found. Conidia were slightly sickle shaped to straight with dorsal and ventral surfaces almost parallel. The size of macroconidia was from 3.5-5 x 45-60 µm. The microconidia were abundant, fusiform, oval to elliptical, sometimes spindle shaped, 0 to 1 septate, apiculate on both ends and produced on polyphialides in false heads. Obovoid microconidia specific for *F. mangiferae* were also identified.

Maximum colony diameter of 32.16 mm after three days was exhibited by isolate FM-2 on PDA and 32.5 mm by FM-17 on CLA, after seven days, 66.0 mm by FM-20 on PDA and 65.5 mm by FM-18 on CLA (Table 2). After twelve days, maximum colony diameter of 81.33 and 80.0 mm was shown by FM-20 on PDA and CLA, respectively. Least growth of 16.0 and 43.0 mm was given by FM-7 on CLA medium after 3 and 7 days, respectively. After 12 days the least growth of 45.66 mm was shown by FM-7 on PDA and 58.66 mm by FM-9 on CLA. FM-20 and FM-7 proved to be fast and slow growing with mean colony size of 57.50 and 42.96 mm on both the media, respectively. PDA medium achieved maximum colony growth with poor or scanty sporulation. PDA being rich in carbohydrates promoted mycelial growth of all the isolates radiating swiftly to periphery as this is empirically a mycelia promoting medium (Fig. 1).

Mean values of isolates after comparative study of 3, 7 and 12 days reflected minor differences in growth except for isolates FM-2 and FM-7 (Table 2). Isolate FM-2 showed normal growth of 30.58 and 57.83 mm after 3 and 7 days, respectively but growth rate slowed down (65.91 mm) as compared to other isolates after 12 days. Major deviation was displayed by FM-7 showing decline in colony diameter after two observation periods during the course of study. The growth was reduced to 17.91 and 44.66 mm after 3 and 7 days, respectively, which are the least values amongst all the isolates. FM-20, although at par with FM-19 after 3 days, proved to be the fast growing isolate amongst all showing significant rise of 63.0 and 80.66 mm after 7 and 12 days of inoculation, respectively.

Fifteen isolates *viz.*, FM-1, FM-2, FM-3, FM-4, FM-6, FM-7, FM-8, FM-9, FM-10, FM-11, FM-12, FM-13, FM-15, FM-17 and FM-20, showed almost similar trends having significant growth on PDA although vacillating among themselves. Four isolates, FM-5, FM-14, FM-16 and FM-19 were at par giving least difference on both the media. CLA values for different isolates fluctuated from each other with minor differences. Only one isolate, FM-18 showed comparatively more growth of 58.11 mm on CLA (Table 2).

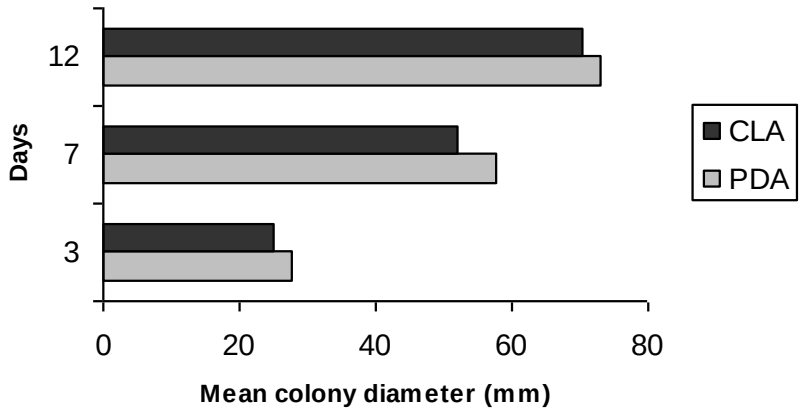

Fig. 1. Effect of PDA and CLA media in promoting colony growth of *F. mangiferae* isolates after 3, 7 and 12 days of inoculation.

Selective media and culture conditions: The species of the genus *Fusarium* are variable because of their genetic make up and variation in the environment in which they grow also causes morphological changes. As the morphology of the macroconidia, is the basis for identification, it is necessary to take all possible steps for standardized procedures to make the task of identification easier. Each conidium represents a single genotype, so the developing colony barring mutations is of the same genotype. It is a clone recognizable by its own particular characteristics. In this manner giving ideal conditions, the clones can be maintained indefinitely in cultures (Nelson *et al*., 1983).

Due to complexity of the disease, malformation fungus remains under focus of study. Careful selection of media ensures promising colony growth, distinct micro and macroconidial morphology and varying pigmentation to screen strains of different origins. *Fusarium* species can be identified with certainty only if the cultures have been grown under optimum conditions for sporulation. The cultures initiated from single conidia in the present studies were provided optimum conditions of temperature and light. The single-spored subsets were studied for various characteristics. The isolates retained the same pigmentation and features after subsequent culturing. This shows the uniformity of growing conditions. PDA proved to be a good medium for isolation and phenotypic characters like colour and morphological differentiation of isolates achieving maximum mycelial growth with scanty sporulation. Pigmentation specific to isolates was ever conspicuous on PDA. Mutations might be enhanced when *Fusarium* species were maintained on PDA. The problem was minimized by sub-culturing a little times as possible, using the single spore or hyphal tip techniques and by not sub-culturing or storing fungi on media with high carbohydrate concentration (Nelson *et al*., 1983).

The most imperative problem is to reduce the phenotypic variation of macroconidia. The uniformity of macroconidia avoids complications in identification. This was best done by purification of isolates on Carnation leaf agar (CLA). CLA proved to be good medium to study the micro and macroconidia of *F. mangiferae*. The characteristic colony growth on CLA was granular white and sometimes slight cottony with pinkish tinge. CLA

ensured abundant production of macroconidia with least phenotypic variation. Being low in carbohydrates it contains naturally occurring substances as encountered by *Fusarium* in nature. The fungus was grown on CLA like a naturally occurring substrate (Nelson *et al*., 1983). According to the species or the ecological situation, either micro or macroconidia could dominate on a natural substrate. The composition of the medium conformed to the host loving nature of the pathogen. So CLA provided host like properties. Cool white light also enhanced macroconidial production. For isolate/ strain screening, PDA and CLA proved to be the excellent media. Conidial measurements were also best done on CLA. The studies are in consistency to the work of Szecsi (1994), Viljoen *et al*., (1997) and Iqbal *et al*., (2005).

Cultural and morphological features: The major morphological characteristics used in the description of *Fusarium* spp. include macro and microconidial morphology and conidiogenesis. The isolates in the present study matched the previous descriptions and displayed the characteristics described for the species (Britz *et al*., 2002). However, obovoid microconidia specific for *F. mangiferae* were confirmed in the present study for the first time in Pakistan after first report by Britz *et al.,* (2002). They have been reported to be absent in *F. subglutinans* having a wide host spectrum in addition to mango. Minimum differences in morphological characteristics and physical features among isolates representing cultivars of different origin were detected. Colonies from single-conidium of most of the isolates formed light orange colour at first and later turned into dominant light to dark purple. Mixed coloration was visible on obverse side of Petri dishes but bearing minor variation. Macroconidia of all the isolates were four celled (3-septate). Similarly sclerotia or chlamydospores like fungal fragments were not found in any isolate. The size of macroconidia conformed to the reported standards for the species ranging from 3.5-5 x 45-60 µm (Gerlach & Nirenberg, 1982).

The isolates proved to be prolific at 25º C. Some differences in cultural appearances including colony growth were found. Isolates FM-20 and FM-7 appeared fast and slow growing, respectively on both selective media (PDA and CLA). FM-20 showed mean colony diameter of 57.50 mm while FM-7 secured 42.96 mm only (Table 2). Rest of the isolates, although, differed in growth but with comparatively less variation.

Similar results consisting of limited morphological differences but variation in culture are reported for other *Fusarium* spp., including isolates of *F. subglutinans* f. sp. *pini* (Viljoen *et al*., 1997). The increase in colony diameter after 3, 7 and 12 days incubation under the same conditions was variable for different isolates. Differences in cultural appearances among isolates from different hosts, as well as within hosts, representing *Fusarium* spp., or formae speciales have been reported (Ploetz, 1990).

Mycological similarities do not ensure relatedness at molecular level. Even closely related or sibling species may be different at genetic level. Future work on DNA sequencing of isolates will be helpful to detect differences in gene regions.

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