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Characterization of Malaysian Isolates of *Fusarium* From Tomato and Pathogenicity Testing*

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Abstract: Differentiation of 22 different isolates of *Fusarium* from tomato fields in Cameron Highlands, based on their cultural and morphological characteristics grouped them into five representative species aggregates; *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *F. chlamydosporum* and *F. lateritium*. This was further confirmed by expression of the DNA polymorphism which showed two main clusters of *F. oxysporum* and *F. solani* at the genetic similarity of 56%. The minor clusters include *F. moniliforme*, *F. chlamydosporum* and *F. lateritium* which are not related to *F. oxysporum* and *F. solani*. Pathogenicity testing proved the pathogenicity of isolate M1 on tomato. M1 was situated in Cluster I (*F. oxysporum*) and thus identified as *F. oxysporum* f. sp. *lycopersici*. There was no direct relationship between clustering in the RAPD dendrogram and pathogenicity testing of the isolates.

Key words: Fusarium isolates, tomato, DNA polymorphism, pathogenicity

INTRODUCTION

Large scale tomato cultivation in Malaysia is confined mainly to the highlands, Cameron Highlands, primarily because of the mild temperature. The production of tomato is being threatened by the wide spread of Fusarium wilt. *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a fungal pathogen commonly associated with wilt of tomato. Identification and characterization of *Fusarium* species and especially of formae speciales is difficult according to morphological features because of characteristics like mycelial pigmentation, shape and size of conidia are unstable and highly dependent on media composition and environmental conditions. Modern approaches applied in *Fusarium* taxonomy include RAPD assay (Crowhurst *et al*., 1991) and isozyme analysis (Saito *et al*., 1980). RAPD markers has been successfully used to study inter and intra-specific variation of twelve *Fusarium* species isolated from cotton-growing areas in Egypt (Abd-Elsalam *et al*., 2003). Fusarium wilt has not been extensively studied in this country. Correct identification of the causal pathogen is needed to enable formulation of effective strategy for disease control. An attempt was made to study the possibility of identifying and differentiating *Fusarium* isolates from tomato fields in Cameron Highlands using the cultural and morphological traits and DNA polymorphism and their association with pathogenicity.

MATERIALS AND METHODS

Isolation of Fusarium

Fusarium spp. were isolated from root and stem samples of tomato collected from fields in Cameron Highlands, Pahang. Stem and root segments were surface sterilized with 10% sodium

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hypochloride solution (Clorox®) for 5 min and rinsed twice with sterilized distilled water. The excised segments were then placed onto sterilized filter paper to dry. After drying, 0.5 cm sections were cut from both ends of the segments and the center segment placed on Potato Dextrose Agar (PDA). The plates were incubated at 28°C for 24-48 h. Fungal colonies growing out of the stem or root segments were transferred to fresh PDA. The transfers were allowed to grow until sporulation had occurred and single spore culture was then prepared.

Characterization of Fusarium Isolates from Tomato

The characterization of *Fusarium* isolates into species aggregates was made on the basis of cultural and morphological characters (Booth, 1977) and DNA polymorphism (Amoah *et al*., 1995, 1996; Saito *et al*., 2003).

Pathogenicity Testing

Pathogenicity of *Fusarium* isolates was tested on two varieties of tomato; Baccarat 322 and Cherry. Individual tomato seed was sown in 10 cm diameter pots and allowed to grow for three weeks. Inoculum consisted of spore suspension obtained from 14 day old culture on PDA and each seedling was inoculated with 50 mL of the spore suspension (5×10^6 spores mL^{-1}). Each treatment consisted of 10 seedlings (replicates). The experiment was arranged in a Completely Randomized Design (CRD). Symptoms development was observed at intervals for six weeks. Watering was done daily and plants were fertilized with NPK Green (15:15:15).

Disease Incidence (DI) was calculated based on the foliar-associated symptoms (Campbell and Madden, 1990). Plants were considered infected when they expressed symptoms of epinasty, yellowing of lower leaves, wilting of leaves, defoliation or marginal necrosis of the remaining leaves. The Area Under Disease Progress Curve (AUDPC) was then assessed using the same data plotted as disease progress curve based on the formula: $AUDPC = \sum_{1}^{n-1} (y_{(l+1)} + y_l /2) (t_{(l+l)}-t_l)$, where n = the number of assessment times; y = disease incidence and t = time (weeks). The epidemic rate of Fusarium wilt expressed as the slopes of the disease progress curve was obtained by the multiple regression analysis using the Sigma Plot Software Program (SPSS Version 9). To confirm the infectivity of the isolate, infected root tissues were randomly plated on PDA and colonies of *Fusarium* regenerated were identified based on the cultural and morphological characteristics.

RESULTS AND DISCUSSION

Isolation of *Fusarium* spp. from Tomato

A total of 22 different isolates of *Fusarium* were obtained from three different locations in Cameron Highlands; seven from MARDI Research Station (M1-M7), six isolates from Tringkap (T8-T13), eight from Ringlet (R14-R21) and one from Sungai Palas (S22). Even though the isolates were associated with tomato roots and stems, it could not be confirmed that these isolates were pathogenic to tomato. This is because most of the wilt-causing *Fusarium* belong to the species *F. oxysporum*. Different host plants are attacked by special forms or races of the fungus. The fungus that attack tomato is designated as *F. oxysporum* f. sp. *lycopersici* (FOL). Therefore identification through cultural and morphological characteristics, DNA polymorphism and pathogenicity testing were attempted to confirm the authenticity of the isolates as causal pathogen of the vascular wilt in tomato.

Table 1a: Cultural and morphological characteristics of *Fusarium oxysporum* on PDA

Cultural characteristics	Morphological characteristics	Isolates identified
Rate of growth:	Conidia:	
growth rapid, greater than 7 cm diameter	microconidia were abundant generally single-celled,	
after 10 to 14 days.	5-12×2.2-3.5 μ oval to kidney-shaped and produced	M1
	only in false heads.	M2
Color of aerial mycelium and colony	macroconidia were abundant, 3-5 septate, 27-60×3-5 μ	M3
from below:	only slightly sickle-shaped, thin walled and a foot-shaped	M4
	basal cell, produced a sporodochialike groups.	M6
color may varies from whitish to pale	Conidiophores:	M7
pink, salmon, vinaceous grey to purple,	unbranched and branched monophialides. The	T8
the undersurface may be colorless or	monophialides bearing microconidia were short compared	T9
light purple pigment diffusing into agar.	to those produced by *F. solani.*	T10
	Chlamydospores:	T11
	one or two-celled, thicked-walled round spores produced	T12
	on older cultures.	T13

Table 1b: Cultural and morphological characteristics of *Fusarium solani* on PDA

Cultural characteristics	Morphological characteristics	Isolates identified
Rate of growth:	Conidia:	
growth rapid, greater than 7 cm diameter	microconidia vary from sparse to abundant	
after 10 to 14 days.	generally single-celled,	
Color of aerial mycelium and colony	8-16×2-4 μ oval to kidney-shaped, larger and have	
from below:	thicker walls, produced only in false heads.	R14
color may varies from whitish to yellow,	macroconidia were abundant, 1-5 septate,	R15
brownish, pink or reddish and the	35-55×4.5-6 μ, thick walled, the apical cell was	R16
undersurface may be colorless or light to	blunt and rounded, basal cell was rounded or was	R18
dark purple pigment diffusing into agar.	distinctly foot-shaped basal cell.	R19
	Conidiophores:	R21
	unbranched and branched monophialides. The	
	monophialides bearing microconidia were long	
	compared to those produced by *Fusarium oxysporum.*	
	Chlamydospores:	
	one or two-celled, thicked-walled round spores	
	produced on older cultures.	

Cultural and Morphological Analysis

Identification of *Fusarium* spp. through cultural and morphological analysis, grouped the isolates into five representative species aggregates, *Fusarium oxysporum* (M1, M2, M3, M4, M6, M7, T8, T9, T10, T11, T12, T13), *F. solani* (R14, R15, R16, R18, R19, R21), *F. moniliforme* (M5 and S22), *F. chlamydosporum* (R17) and *F. lateritium* (R20) (Table 1a, b). However, only pathogenic formae speciales *lycopersici* of *F. oxysporum* can cause severe losses in tomato while little information is available concerning *F. solani* and no disease caused by *F. moniliforme*, *F. chlamydosporum* and *F. lateritium* was related to tomato. *F. moniliforme* and *F. chlamydosporum* attack crops such as asparagus, banana and paddy while *F. lateritium* attack maize and leguminous plants. The most distinguishing characteristics for *F. oxysporum* were the presence of fusoid three to five septate macroconidia (27-60 μ×3-5 μ) with pointed and curved ends and microconidia (5-12 μ×2.2-3.5 μ) borne in false head on short monophialides. Even though the presence or absence of chlamydospores is also a useful character for identification, it was less reliable than other criteria. For *F. solani,* the morphology of the macroconidia (35-5×4.5-6 μ) and the elongate monophialides bearing microconidia (8-16×2-4 μ), helped to distinguish it from *F. oxysporum. F. oxysporum* produced white to pale violet pigmentation, while *F. solani* produced white to cream pigmentation on PDA. The most distinguishing

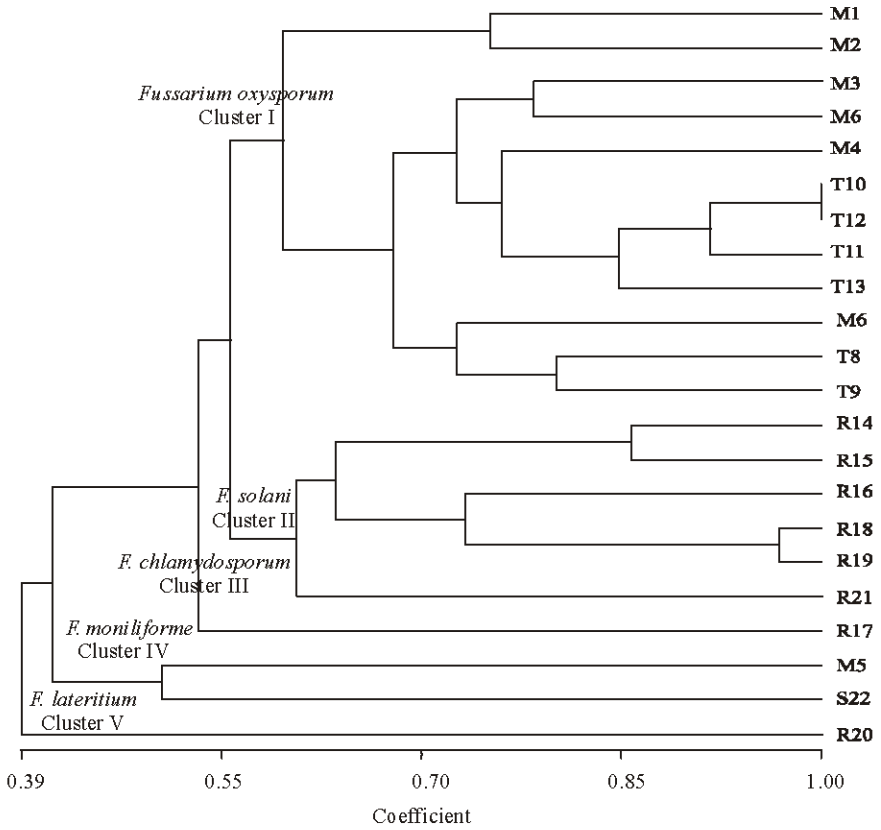

Fig. 1: Dendrogram based on RAPD-PCR profiles generated by UPGMA analysis showing the genetic similarity among *Fusarium* isolates with primers OPC-11, OPC-15 and OPC-18

characteristic for *F. moniliforme* was the microconidia formed in chains on monophialides, whereas *F. chlamydosporum* showed the presence of spindle-shaped microconidia borne on polyphialides and *F. lateritium* in the shape of the macroconidia with special emphasis on the apical cell. However, the colonies of *F. oxysporum* f. sp. *lycopersici* (FOL) appearance could not be distinguished from those of the non-pathogenic *F. oxysporum* just based on colony appearance and morphological characteristics alone. *F. oysporum* was anamorphic and comprises of non-pathogenic strains which asymptomatically colonize plant roots or grow saprophytically, as well as pathogenic strains which colonize the xylem, causing diseases. The plant host specificity of the pathogen denotes a specialized form of the fungus; the formae speciales are not morphologically distinguishable. Therefore an attempt was made to differentiate the pathogenic *F. oxysporum* f. sp. *lycopersici* (FOL) from non-pathogenic isolates based on the DNA polymorphisms.

RAPD-PCR Analysis

OPC-11, OPC-15 and OPC-18 were used to profile twelve isolates of *F. oxysporum*, six isolates of *F. solani*, two isolates of *F. moniliforme*, one isolate of *F. chlamydosporum* and one isolate of *F. lateritium*. Based on the cluster analysis of the genetic similarities using UPGMA clustering, two main clusters were defined (Fig. 1). *F. oxysporum* with 12 isolates (M1, M2, M3, M4, M6, M7, T8,

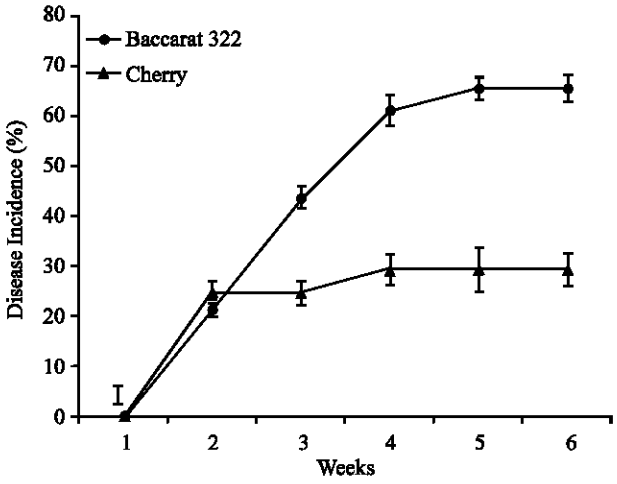

Fig. 2: Disease development on tomato seedlings cultivar Baccarat 322 and Cherry inoculated with *F. oxysporum* (M1 isolate), based on foliar-associated symptoms. Each value mean of 10 replicates. Bars represented the standard deviation

T9, T10, T11, T12, T13) were grouped in Cluster I with genetic similarity values ranged from 60.9 to 91.7%. The six isolates of *F. solani* (R14, R15, R16, R18, R19, R21) were grouped in Cluster II with genetic similarity values between populations of this cluster ranged from 60.9 to 96.8%. Genetic similarities ranged from 56 to 61% between *F. oxysporum* and *F. solani* for inter-specific comparisons as was reported previously (Khalil *et al*., 2003). The polymorphisms observed from the RAPD markers revealed a high degree of genetic diversity within *Fusarium* spp. at the inter-specific level. The genetic similarities ranged from 61 to 97% for intra-specific comparisons. High intra-specific variability was observed in *F. oxysporum* and *F. solani* from tomato. The two isolates of *F. moniliforme* (M5 and S22), one isolate of *F. chlamydosporum* (R17) and one isolate of *F. lateritium* (R20) were distinctly isolated from these two main clusters with genetic similarities ranged from 39 to 50%. UPGMA clustering analysis was able to characterize all 22 isolates of *Fusarium* and grouped them together into their own species, but it could not differentiate FOL from non-pathogenic forms of *F. oxysporum*. RAPD-PCR has been successfully used in *Fusarium* identification, characterization and genetic variability study within and between species. Among these are *F. culmorum* and *F. graminearum* (Schilling *et al*., 1994), *Fusarium* section Liseola (Amoah *et al*., 1996), *F. lateritium* (Jae and Clark, 1998), *F. moniliforme* (Mitter *et al*., 2002), *F. oxysporum* f. sp. *phaseoli* (Alves-Santos *et al*., 2002) and *F. oxysporum* f. sp. *lentis* (Belabid *et al*., 2003). To further confirmed the differentiation between FOL and other forms of *F. oxysporum* in this study, pathogenicity testing was carried out on tomato cultivars Baccarat 322 and Cherry to establish the causal agent and to fulfill Koch's Postulate.

Pathogenicity Testing

Pathogenicity testing confirmed isolate M1 to be pathogenic to tomato cultivars Baccarat 322 and Cherry. Disease incidence for cultivar Baccarat 322 was highest (65.55%) at six weeks after inoculation compared to cultivar Cherry with value of 29.44% (Fig. 2). The initial symptoms appeared as yellowing of the older leaves. The yellowing gradually spread to affect most of the foliage and was normally accompanied by wilting of the plant during the hottest part of the day. The wilting becomes more extensive from day to day until the plant collapsed and died. In cross-sections of the stem near

Table 2: Area Under Disease Progress Curve (AUDPC) and epidemic rate of *F. oxysporum* f. sp. *lycopersici* (M1) on tomato cv. Baccarat 322 and cv. Cherry, six weeks after inoculation

	[2]Area under Disease Progress Curve (AUDPC) according to weeks (unit/square)					
Tomato cultivar	2[1]	3	4	5	6	Epidemic rate (unit/week)
Baccarat 322	10.85	32.77	52.50	63.33	65.55	0.23
Cherry	12.50	24.99	27.22	29.44	29.44	0.06

[1]Weeks, [2]AUDPC values

the base of infected plants, a brown ring is evident in the area of the vascular bundles. Infection starts from the lateral roots, progressing into the stem systematically. In this study, the upward progress of the browning in the vascular tissues for tomato cv Baccarat 322 and cv Cherry were 8.36 and 3.27 cm respectively, six weeks after inoculation, supporting the observations that M1 was pathogenic to tomato and cv Baccarat 322 was more susceptible than cv. Cherry. The disease progress over time based on the Area under Disease Progress Curve (AUDPC) and epidemic rate of the disease was highest for cv Baccarat 322 (r_m = 0.23 unit/week) than cv. Cherry (r_m = 0.06 unit/week) (Table 2). Re-isolation from infected roots and stems on PDA yielded colonies of *F. oxysporum* f. sp. *lycopersici* (M1), thus proved positive for Koch's Postulate.

However, this finding indicate no clear relationship between the RAPD profiles and pathogenicity testing for *Fusarium* spp. and the conclusions are in accord with previous reports (Abdel-Sattar *et al*., 2003; Kiprop *et al*., 2002; Satyaprasad *et al*., 2000; Schilling *et al*., 1994). However, it has been used widely in identification, characterization and genetic variability studies between species as the polymorphism markers revealed a high degree of genetic diversity in *Fusarium* spp. at the inter-specific level. RAPD gives more comprehensive information regarding the genetic variability among the pathogen population as it is based on the entire genome of an organism. Therefore, identification of intra-specific elements for *Fusarium* isolates from tomato in Cameron Highlands, has to be complemented with cultural and morphological characterizations and pathogenicity testing.

REFERENCES

Abd-Elsalam, K.A., F. Schnieder, M.S. Khalil, A.A. Aly and J.A. Verreet, 2003. Genetic variation at the intra-and interspecific level in *Fusarium* spp. associated from Egyptian cottons. J. Plant Dis. Prot., 110: 46-53.

Abdel-Sattar, M.A., M.S. Khalil, I.N. Mohmed, K.A. Abd-Elsalam and J.A. Verreet, 2003. Molecular phylogeny of *Fusarium* species by AFLP fingerprint. African J. Biotechnol., 2: 51-56.

Alves-Santos, F.M., L. Cordeiro-Rodrigues, J.M. Sayagues, R. Martin-Dominguez, P. Garcia-Benavides, M.C. Crespo, J.M. Diaz-Minguez and A.P. Eslava, 2002. Pathogenicity and race characterization of *Fusarium oxysporum* f. sp. *phaseoli* isolates from Spain and Greece. Plant Pathol., 51: 605.

Amoah, B.K., M.V. Macdonald, N. Rezanoor and P. Nicholson, 1996. The use of the random amplified polymorphic DNA technique to identify mating groups in the *Fusarium* section *Liseola*. J. Plant Pathol., 45: 115-125.

Amoah, B.K., N. Rezanoor, P. Nicholson and M.V. Macdonald, 1995. Variation in the *Fusarium* section *Liseola*: Pathogenicity and genetic studies of isolates of *Fusarium moniliforme* Sheldon from different hosts in Ghana. J. Plant Pathol., 44: 563-72.

Belabid, L., M. Baum, Z. Fortas, Z. Bouznad and I. Eujayl, 2003. Pathogenic and genetic characterization of Algerian isolates of *Fusarium oxysporum* f. sp. *lentis* by RAPD and AFLP analysis. African J. Biotechnol., 3: 25-31.

Booth, C., 1977. *Fusarium* Laboratory Guide to the Identification of the Major Species. Commonwealth Mycologia Institute Kew, Surrey, England.

Campbell, C.L. and L.V. Madden, 1990. Introduction to Plant Disease Epidemiology. USA: John Wiley and Sons.

Crowhurst, R.N., B.T. Hawthrone, E.H.A. Rikkerink and M.D. Templeton, 1991. Differentiation of *Fusarium solani* f. sp. *cucurbitae* races 1 and 2 by random amplification of polymorphic DNA. Current Genetics, 20: 391-396.

Jae, W.H. and C.A. Clark, 1998. Analysis of *Fusarium lateritium* using RAPD and rDNA RFLP techniques. Mycol. Res., 102: 1259-1264.

Khalil, M.S., M.A. Abdel-Sattar, I.N. Aly, K.A. Abd-Elsalam and J.A. Verreet, 2003. Genetic affinities of *Fusarium* spp. and their correlation with origin and pathogenicity. African J. Biotechnol., 2: 109-113.

Kiprop, E.K., J.P. Baudoin, A.W. Mwang'ombe, P.M. Kimani, G. Mergeal and A. Maquet, 2002. Characterization of Kenyan isolates of *Fusarium* from Pigeonpea [*Cajanus cajan* (L.) Millsp.] by cultural characteristics, aggressiveness and AFLP analysis. J. Phytopathol., 150: 517-527.

Mitter, N., A.C. Srivastava, S. Renu, A.K. Ahmad, Sarbhoy and D.K. Agarwa, 2002.Characterization of gibberellin producing strains of *Fusarium moniliforme* based on DNA polymorphism. Mycopathologia, 153: 187-193.

Saito, M., M. Ichinoe and O. Tsuruta, 1980. Usefulness of gel electrophoretic comparison of peroxidase for identification of *Fusarium* species. Trans. Mycol. Soc. Japan, 21: 229-235. (Translated from Japanese into English by Michael J. Osgard..)

Satyaprasad, K., G.L. Bateman and E. Ward, 2000. Comparisons of isolates of *Fusarium avenaceum* from white lupin and other crops by pathogenicity tests, DNA analyses and vegetative compatibility tests. J. Plant Pathol., 4: 211-219.

Schilling, A.G., E.M. Moller and H.H. Geiger, 1994. RAPDs of *Fusarium Culmorum* and *Fusarium Graminearum*: Application for Genotyping and Species Identification. In Modern Assays for Plant Pathogenic Fungi: Identification, Detection and Quantification, (Eds.) Schts A, Dewey FM, Oliver R. University Press, Cambridge, pp: 47-56.