Characterization of *Fusarium oxysporum* F. sp. *elaeidis* (Fofse) causal agent of vascular wilt disease of oil palm

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Abstract

Studies were carried out on *Fusarium oxysporum* f. sp. *elaedis* (Fofse) isolates from the Crops Research Institute and Oil Palm Research Institute fields at Akumadan and Kusi, respectively. Isolates P.38, P.50, P.459 and P.568 were obtained from Akumadan and isolate P.93 from Kusi. The growth and cultural characteristics of the isolates were studied on potato dextrose agar (PDA) prepared from dehydrated commercial formulation, PDA prepared from fresh potato tubers and potato sucrose agar (PAS). Cultural characteristics of the isolates on agar plates showed differences with respect to the pigments produced. The sizes of the macroconidia varied for all isolates. Four isolates namely P.38, P.50, P.93 and P.459 were found to be variants of Fofse while isolate P. 568 was found to be either *Fusarium solani* or a mixture of *F. solani* and Fofse.

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Introduction

Cultural and morphological variation is a common phenomenon in *Fusarium* sp. (Armstrong *et al.*, 1940). The originally isolated parent type with abundant conidia and floccose mycelium seldom remained constant in culture (Armstrong *et al.*, 1940). Such cultural variats also showed variation in pathogenicity (Armstrong *et al.*, 1940), and were usually significantly less pathogenic than their parental wild types (Oswald, 1949). It is, therefore, important to study the cultural variations in the *Fusarium* species before conducting pathogenicity tests since only the parent types should be used for such tests. Subramanian (1951) demonstrated that *Fusarium* species in nature occur in a multiplicity of forms. However, these are generally vigorous in growth and uniform within the particular strain. According to him, these forms are not to be confused with the cultural atrocities that one frequently receives for identification.

Fusarium oxysporum, apart from being the most economically important members of the genus *Fusarium* is also one of the most labile and variable species (Booth, 1971). Isolates of *F. oxysporum* from one particular host or geographical area usually vary as much as isolates from different hosts or regions. Although modern studies have shown that it is possible to recognize a particular clone from one area or host on the basis of colony morphology, such an association is not found when isolates are obtained from more hosts or from one host over a wider area (Booth, 1971).

Not much has been done on *F. oxysporum* f. sp. *elaeidis* (Fofse), casual agent of vascular wilt disease of oil palm in Ghana, and it is not known whether variants of the fungus is present in the various oil palm plantations. Information on the variability of Fofse in oil palm growing areas in Ghana will be useful to oil palm researchers and the economy at large, if appropriate control measures are to be achieved in order to sustain the industry in Ghana.

Materials and methods

Diseased specimen were taken from Kusi and Akumadan for isolation purposes. More specimen were taken from Akumadan than Kusi because of the high incidence of vascular wilt at Akumadan. The isolates were labelled after trees from which they were taken. These were as follows: Palm No. 93 - Field K. 16, Kusi; Palm No. 38, 50, 459 and 568

- Field K. 16, Akumadan.

The five isolates P.38, P.50, P.93, P.459 and P.568 isolated from diseased trees in the fields were used in the study. The isolates were maintained on potato dextrose agar slants and sandy silt loam soil in McCartney bottles. These were incubated at room temperature (26 ± 2 °C) for 7 days and immediately transferred into the refrigerator at a temperature of 5 °C. Isolations were made from brown fibres of the petioles of infected trees. The specimen were surface-sterilized with 1 per cent sodium hypochlorite (Naclo) solution and inoculated onto water agar plates. The plates were incubated at room temperature (26 ± 2 °C) for 7 days.

Discs (3 mm diameter) were removed from the growing edge of cultures of the Fofse isolates with a cork borer. These were cultured on PDA prepared from dehydrated commercial formulation, PDA prepared from fresh potato tubers and PSA. Each isolate and medium was replicated three times. The cultures were incubated at room temperature. Characterization of the isolates was made on three media; *viz.* dehydrated PDA, fresh PDA and PSA. The lengths and breaths of the macrononidia were also measured under the high power objective of the microscope with the eyepiece graticule.

Pathogenicity studies were conducted on the five Fofse isolates using six oil palm *tenera* progenies (G.9, G.25, G.30, G.44, G.51 and G.84). Germinated nuts of the six progenies with single developing roots were dipped into potato dextrose broth inocula of the five Fofse isolates (P.38, P.50, P.93, P.459 and P.568) in beakers for 63 h. By this period more than 50 per cent of the spores were germinating. Sterile distilled water served as the control. The beakers containing inocular and germinated nuts were then placed in polyethylene bags. The mouth of each bag was tied with a piece of twine. After 63 h the germinated nuts were transferred into polybags containing 1.5 kg of sterile soil in the screen house. Each treatment was replicated three times. The bags were arranged randomly. Observations were made at 2, 4, 8 and 12 weeks after planting and the following records were taken: height of plants above soil, length of leaves and number of leaves. Reisolations were made from the roots of seedlings inoculated with the five isolates after 3 months to confirm successful infections.

The composition of the culture media used were as follows: Potato dextrose broth: potatoes, 200 g; dextrose, 20 g; distilled water, 1000 ml; Potato dextrose agar: potatoes, 200 g; dextrose, 20 g; agar, 20 g; distilled water, 1000 ml; Potato sucrose agar: potato extract, 500 ml; sucrose, 20 g agar, 20 g; distilled water, 500 ml.

*p*H readings of the culture media were taken using a Phillips PW 9420 *p*H meter. Readings of *p*H were made before autoclaving. All the cultures were incubated at room temperature (26 ± 2 °C)

Results

Structures of F. oxysporum *f. sp.* elaeidis *(Fofse)*

The various isolates were described under two headings: culture characteristics and the nature of spores (Tables 1 and 2).

Measurements of macroconidia of Fofse

The mean sizes of 50 macroconidia of the five isolates is shown in Table 3. The macroconidia of isolate P.38 appeared to be longer (33.2 μ) than the others even though the number of cells in a macroconidium were few (3.5). The macroconidia of isolate P.50, however, appeared to have the shortest length (15.4 μ).

Cell sizes within a macroconidium

The sizes of cells within a macroconidium for the five isolates are shown in Table 4. The tip cells had smaller widths than the centre cells. The lengths of the tip cells of most of the isolates (P.50, P.93 and P.568) were also longer than those of the centre cells. The measurements in Tables 3 and 4 thus give a picture of the structures of the macroconidia. They also show that the isolates are different.

TABLE 1

Culture Characteristics of Five Fofse Isolates

Isolates	*Mycelium*	*Chlamydospore*
P. 38	i. Reddish purple pigment produced on medium. This is intermingled with White mycelium when viewed from the top of Potato sucrose agar (PDA) plates ii. Mycelium looks reddish purple when viewed from bottom of the plates. iii. The edge of the mycelium is white. iv. Mycelium looks fluffy on the surface v. The hyphae are septate	i. Chlamydospores are intercalary and measure approximately 6.4μ
P. 50	i. Culture is whitish on fresh PDA and PSA plates when viewed from the top but arange or pink with concentric rings from a dehydrated PDA plate ii. Mycelium looks fluffy on the surface. Margins of mycelium are diffused. iii. The hyphae are septate	i. Most of the chmamydrospores are intercalary ii. Chlamydospores are large (15.8μ) and abundant (about twelve in a field view)
P. 93	i Culture is white on top and light orange-pink at the top of a dehydrated PDA plate ii. Mycelium looks fluffy on the Fresh PDA and PSA plates iii. The hyphae are septate	i. Chlamydospores are few in number about three in a field view) and are intercalary. ii. Chlamydospores are small in size (5.4μ)
P. 459	i. Culture is white on fresh PDA plate. It is however purple intermingled with white on PSA and dehydrated PDA plates ii. The mycelium is fluffy on all the media iii. The edges of mycelia are diffused iv. The hyphae are septate	i. Chlamydospores are intercalary and measure approximately 7.6μ
P. 568	i. Culture is creamy and flat on dehydrated PDA plate ii. It is white and fluffy on fresh PDA and PSA plates but has greenish tinge at the centre or the periphery when viewed from the bottom of a PSA plate. iii. The hyphae are septate	i. Chlamydospores are intercalary and measure approximately 9.8 μ

Discussion

Measurements of width of the macroconidia of all the isolates fell within the reported range of 3-4.5 μ for *F. oxysporum* described by Messiaen (1959). All the isolates had different measurements in length and width of macroconidia and chlamydospores, as well as cultural characteristics which showed that they were variants. Even though the description of P.568 made it a *F. solani* rather than *F. oxysporum,* the measurement of its macroconidia fell within the range for *F. oxysporum* and not *F. solani.* This made it difficult to decide whether P.568 was *F. solani* or *F. oxysporum* or a mixture of both.

The number of cells in a macroconidium ranged from 3-6. P.93 and P.568 had macroconidia with

TABLE 2

Spores of Five Isolates of Fofse

Isolates	*Mycelium*	*Chlamydospore*
P. 38	i. Micronidia are stocky. The ends do not end at a fine point ii. Microconidia may be one or two-celled iii. Very few about 4.3% of the micronidia are boat shaped iv. There are more micro than macroconidia. The ratio of micro to macronidia in a field view is about 190:1	i Macroconidia are typically boatshaped and ends at a point ii. Macroconidia are three or four-celled
P. 50	i. About 94.9% of the microconidia are stocky. Very few are slightly boatshaped ii. Micro and macroconidia have up to four cells within iii. Single-called microconidia look stocky while double and triple-celled ones are oval or bottom shaped iv. Microconidia are more than macronidia. The ratio of micro to macroconidia in a field view is 13.1	i. About 57.1% of the macroconidia are three-celled. ii. Macroconidia are boat-shaped
P. 93	i. Macroconidia are stocky ii. Micro and macroconidia have up to six cells within iii. About 64% of the microconidia are oval-shaped. iv. Macroconidia are more than macrconidia. The ratio of micro to macroconidia in a field view is about 10.2:1	i. Macroconidia are abundant (about 43 in a field view) ii. Macroconidia are slightly boat-shaped and ends at a point. iii. There are three to six cells in a macroconidium iv. There was no sporulation on dehydrated PDA
P. 459	i. Micro and macroconidia have up to four cells within ii. About 11.5% of the microconidia are slightly boatshaped. The remaining are stocky iii. There are more micro than macroconidia. The ratio of micro to macroconidia in a field view is about 10.2:1.	i. Macroconidia are convex or slightly boatshaped ii. Macroconidia are abundant (about twelve in a field view)
P. 568	i. Microconidia are stocky ii. Micro and macroconidia have up to six cells within iii. There are more micro than macroconidia. The ratio of micro to macroconidia in a field view is about 105:1	i. Macroconidia are convex or slightly boat shaped

three, four, five or six cells while those for P.36, P.50 and P.459 had three or four cells, respectively. All the isolates had tip cells with smaller widths than the centre cells and in most cases, longer cells at the tips. This gave them the characteristic boat or convex-shaped structure. The differences in size of the tip and centre cells of the macroconidia, and differences in culture

TABLE 3

Mean size (μ) of Macroconidia of Five Isolates of Fofse

	Isolates				
	P. 38	*P. 50*	*P. 93*	*P. 459*	*P. 568*
Mean width of 50 macroconidia (μ)	3.6	3.7	3.4	3.3	3.5
Range					
Mean diameter of chlamydospores (μ)	3.0-4.4	3.0-4.8	3.0-4.4	3.0-4.1	3.0-4.1
Range	4.8-8.4	12.0-19.2	4.8-6.2	4.8-12.0	4.8-14.4
Full length of macroconidia (μ)	33.2	15.4	28.4	24.4	30.3
Range	30.7-36.6	14.1-17.4	15.5-42.1	22.2-30.3	22.9-43.2
Mean number of cells in a macroconidia	3.5	3.5	4.5	3.5	4.5
Range	3.4	3.4	3.6	3.4	3.6

TABLE 4

Size (μ) of Individual Cells within a Macroconidia on PSA at 26±2 °C

Isolates	*Tip cell*		*Centre cell*	
	Length + SE	*Bredth + SE*	*Length + SE*	*Bredth + SE*
P. 38	7.73 ± 0.27	2.87 ± 0.05	9.09 ± 0.33	3.33 ± 0.05
P. 50	3.91 ± 0.25	3.18 ± 0.13	3.78 ± 0.15	3.96 ± 0.09
P. 93	7.09 ± 0.62	2.98 ± 0.09	5.25 ± 0.45	3.27 ± 0.11
P. 459	5.76 ± 0.37	2.84 ± 0.15	6.46 ± 0.30	3.35 ± 0.17
P. 568	7.60 ± 0.36	3.59 ± 0.12	5.73 ± 0.56	3.38 ± 0.06

characteristics confirm that the isolates are variants.

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