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Research Article

Molecular and morphological diversities among Fusarium wilt pathogens of eggplant in Ghana

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Abstract

The identification of *Fusarium* species associated with Fusarium wilt of eggplant is an important step toward accurate diagnosis and management of the disease. In this study, 32 *Fusarium* isolates recovered from Fusarium wilt-infected eggplants in 15 communities across the Ashanti, Eastern and Volta Regions of Ghana were identified and characterized based on their morphological and molecular properties. Colony and conidia characteristics were used to identify and characterise the isolates morphologically. Genomic DNA of the *Fusarium* isolates was extracted and the Internal Transcribed Spacer (ITS) primer (ITS1F) and Translation Elongation Factor (TEF) primer (Fa-7_Ra-6F) were used to sequence the ITS and TEF regions of the isolates' DNA respectively. Most of the *Fusarium* isolates were morphologically identified as *Fusarium solani, F. oxysporum,* or *F. culmorum* and were confirmed by the ITS and TEF region sequences. Morphological characterisation offers limited distinction among isolates of the same *Fusarium* species. Morphological characteristics, such as conidia shape and size, appeared uniform for the Fusarium species, except for *F. solani,* which was grouped into two based on sporodochia production. Both ITS and TEF region sequences could detect differences among the *Fusarium* isolates. However, there were differences in the phylogenetic grouping between the ITS and TEF region sequences. The TEF sequence showed wider divergence in the *F. oxysporum* and *F. culmorum* isolates but a close relationship in the *F. solani* isolates. *Fusarium oxysporum* isolates were the most phylogenetically diverse.

1. Introduction

The identification of species within the *Fusarium* genus is an important step in diagnosis disease and management. Morphological identification of *Fusarium* species is mainly based on the distinctive characteristics of the shapes and sizes of macro and micro conidia, colony appearances, pigmentation and growth rates on agar media [12]. Morphological identification of *Fusarium* species, although basic, is a difficult step in the detection procedure [18]. Considerable expertise is required to distinguish between closely related species. Morphological methods are time-consuming and have proven to be limited and largely unable to identify differences within species. Molecular techniques have been resourceful in confirming the morphological identification of plant pathogens [18].

Nucleic acid (NA)-based techniques, especially those that rely on polymerase chain reaction (PCR), have

been identified to provide reliable results in plant pathogen detection [21, 11]. According to [20], the diagnostic PCR method was beneficial for the control of Fusarium wilt in strawberries because it quickly determined the identity of the pathogen and could handle many samples simultaneously. PCR-based methods are also rapid because pathogens do not need to culture before identification. These methods are specific because identification of the species is based on genotypic differences and are highly sensitive, as target DNA molecules are detected in complex mixtures even when the mycelia are no longer viable [3]. DNA sequencing and species-specific polymerase chain reaction (PCR) assays are recommended [22].

Sequences that have been valuable in distinguishing species and origins of *Fusarium* include the internal transcribed spacer (ITS) region from the conserved ribosomal RNA genes, intergenic spacer (IGS), translation elongation factor, β-tubulin region, and the mitochondrial small subunit (mtSSU) [19, 4, 15]. This sequence information has been widely used in the taxonomy and phylogenetic studies of *Fusarium* species. It provides sufficient resolution at the sub-species level, as this variability is mainly harbored in the introns [19, 15].

The ITS region has been widely used as a barcode in fungal community studies [13, 17]. However, ITS markers do not provide species-level resolution for many *Fusarium* species that have non-orthologous copies of the ITS2 region [14]. Therefore, ITS markers are limited for studying *Fusarium* communities at the species level.

The translation elongation factor (TEF) gene is a useful phylogenetic marker for *Fusarium*, as it provides species-level discrimination [6]. TEF 1-α consistently presents as a single-copy gene in the *Fusarium* genus. This gene demonstrates a high level of sequence polymorphism among closely associated species of *Fusarium*, even compared with the intron-rich portions of protein-coding genes, such as β-tubulin, calmodulin and histone H3. TEF is a highly recommended marker as a single-locus detection tool in *Fusarium* [9, 6]. This study aimed to identify and characterize isolates of Fusarium wilt pathogens in eggplant using morphological characterize and PCR-based molecular methods with ITS and TEF primers.

2. Materials and methods

2.1. Sampling of wilt-infected eggplants

The purposive sampling method was used to sample eggplant farms with Fusarium wilt incidence. Farmers who reported wilt symptoms were contacted and through a snowball (respondent-driven) sampling approach, other wilt-infected eggplant farms were identified. Ten farms were selected in each eggplant growing community for sampling of infected eggplants. Five communities were sampled each from the Ashanti, Eastern and the Volta regions of Ghana.

Ten plants were sampled from each farm visited: five plants with visible symptoms of wilt and five with no visible wilt symptoms. The systematic sampling method was as follows: starting at the third row in the left-hand corner of the farm facing North, a plant was collected from any other row towards the right-hand side until the 10 samples were collected. Whole plants were uprooted together with some rhizosphere soil, excised at the stem base, placed in a paper envelope, separately labelled and sent to the Plant Pathology Laboratory of the Department of Crop and Soil Sciences, KNUST, Kumasi, Ghana. The samples were air-dried on the laboratory bench at room temperature (25 ± 2 ^{0}C) and later the rhizosphere soil and roots were examined for *Fusarium* species.

2.2. Isolation of Fusarium isolates

Infected eggplant roots were washed under running tap water to remove all soil and debris from the field and subjectively excised to 1.0 cm long and 0.5 cm wide pieces for each plant. The cut pieces were surface sterilized in 0.5% NaOCl for 3 min and in 75% ethanol for another 3 min, rinsed three times in sterile distilled water, and blotted dry. Root pieces were then plated on chloramphenicol (250 mg/L) amended Potato Dextrose Agar medium and incubated at (25 ±2 °C) for 7 days at 12 h photoperiod under fluorescent light.

Fusarium isolates were identified using a stereomicroscope at a magnification of X400 with the help of identification manuals [12, 7]. Single conidia culture of *Fusarium* isolates was prepared by the following protocol described by [8]. A 7-day old single conidia culture of *Fusarium* isolates was used in this

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study.

2.3. Morphological identification of Fusarium isolates

Each *Fusarium* isolate was examined microscopically and identified at the species level based on the morphological characteristics described by [7, 12]. Isolates were identified based on distinctive morphological characteristics such as the shape and size of macroconidia and microconidia the shape of the conidial apical and basal cells, the production and colour of sporodochia on Banana Leaf Agar, and the colony appearance, colour and pigment formation on PDA [7, 12].

2.4. Assessment of cultural and morphological characteristics of Fusarium isolates

Mycelial plugs of 7-mm diameter were taken from the growing margins of seven day-old single spore cultures of *Fusarium* isolates with a sterile cork borer. The plugs were placed separately at the centre of PDA in Petri dishes and incubated at a temperature of 25 ± 2°C at the incubation room. The cultural and morphological characteristics of mycelia on PDA were assessed for 33 isolates of *F. oxysporum*, 45 isolates of *F. solani*, and 11 isolates of *F. culmorum*. The culture characteristics collected were mycelial growth, mycelial colour, pigmentation, odour, presence of sporodochia, sporodochia colour and thickness of mycelia, following the described procedure [12].

Two perpendicular lines were marked with a ruler at the under-side of each 9 cm Petri dish to measure the mycelial growth. Mycelial growth was determined by measuring two colony diameters for each *Fusarium* isolate along the perpendicular lines with a ruler daily until the Petri dish was fully covered with mycelia. The means of the two colony diameters were computed for each isolate and used to determine the growth rate of the *Fusarium* isolates.

Mycelial colour and pigment production in the agar were determined by observing the upper and lower surfaces of the Petri dishes, respectively after 14 days of incubation at a temperature of 25 ± 2°C. Mycelial odour was determined by smelling the mycelium after 14 days of incubation at 25 ± 2°C. Other characteristics such as sporodochia colour and thickness, were also assessed by observing the upper surface of the Petri dishes.

The morphological characteristics of macroconidia and microconidia were assessed after 21 days of inoculation of the *Fusarium* isolates on banana leaf agar at 25 ± 2°C. Macroconidia were assessed for size, shape, septation and apical and basal cell shapes. Microconidia were observed for abundance, size, and septation as the described method [12].

2.5. Molecular characterization of Fusarium isolates

2.5.1. Genomic DNA extraction

Total genomic DNA of the *Fusarium* isolates was extracted separately from lyophilised seven-day old mycelia of single conidia cultures. A modified CTAB-based protocol was used for DNA extraction. It was carried out at the Molecular Biology Laboratory of the CSIR- Crops Research Institute, Kumasi, Ghana. Mycelia were collected from the Petri dishes with a sterile glass rod placed in liquid nitrogen in a 2.0 mL Eppendorf tube and ground into a fine powder with a rod. Each tissue powder was mixed with 500 µL CTAB extraction buffer [100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (pH 8), 2% (w/v) CTAB, 2% w/v PVP, (0.1% v/v) β-mercaptoethanol, and homogenized by vortexing. Each homogenate was incubated at 65 °C for 30 min with intermittent vortexing.

After cooling to room temperature, an equal volume of chloroform-isoamyl alcohol (24:1 v/v) was added to the homogenate of each isolate followed by inversions of the tube to ensure complete mixing. Each homogenate was centrifuged at 13000 g for 15 min. Each aqueous phase was hand-picked carefully without disturbing the middle layer and placed into a labelled clean 2 ml tube. The resulted solution of each isolate was re-extracted with chloroform-isoamyl alcohol (24:1). The DNA of each isolate was precipitated at −20 °C overnight by addition of two-thirds volume of ice-cold isopropanol. The DNA of each isolate was pelleted by centrifugation at 13000 g for 5 min followed by washing in 1 mL of 70% ethanol. Ethanol was discarded, and each pellet was dried for 30 min. A 250 µL of 7.5M ammonium acetate was then added to each pellet, mixed and incubated on ice for 5 min. Each sample was then centrifuged at 13000 rpm for 10 min. The supernatant of each isolate was transferred separately into a new tube, 700 µL of cold isopropanol was added, and mixed by inversion and incubated at -20 °C for 1 h. Centrifugation at

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13000 rpm for 10 min was carried out and the pellet of each isolate was washed with 80 % ethanol. It was then dried at 25±2 °C and re-suspended in 20 µl molecular grade water prior to use.

2.5.2. Genomic DNA amplification and sequencing of the ITS and TEF regions

Polymerase Chain Reaction was run using Promega GoTaq Green Master Mix (Functional Biosciences Inc., Madison, WI, USA) with a standard 3-step PCR protocol. The Sanger Sequencing protocol with Big Dye Terminator V3.1 was used to sequence the amplified PCR products. After Sanger cycling, the magnetic bead was used to clean-up and remove excess contaminants and dyes from the reaction. Finally, the cleaned-up product was injected into the ABI 3730XL sequencing analyzer. The primers used for the TEF regions were Fa-7_Ra-6F (AACGTCGTCGTCATCGGCCACGTCGACTCT; AC ATACCAATGACGGTGACATAGTAG CG) and for the ITS regions, ITS1F (CTTGGTCATTTAGAG GAAGTAA) was used [10]. The Sanger Sequencing reactions were performed at the Functional Biosciences Inc. facility in Madison, Wisconsin, USA.

2.6. Experimental design and data analyses

A completely randomised design with three replicates per *Fusarium* isolate (treatment) was used in this study. Mean differences in size of conidia and mycelial growth rate of the isolates of different *Fusarium* isolates were compared using honest significant difference (HSD) of 1% using GenStat 12th edition, VSN International, UK. Qualitative data, such as mycelial colour and shape of conidia were assessed visually and presented with descriptive statistics.

The ITS and TEF region nucleotide sequences for the isolates were compared to the databases of the National Center for Biotechnology Information (NCBI; www.ncbi.nih.gov) using the Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN). Alignment of the ITS and TEF DNA sequences and phylogenetic trees of the sequences were created with the CLC Sequence Viewer Version 8 based on unweighted pair group method for arithmetic analysis (UPGMA).

3. Results

3.1. Morphological identification

The 32 *Fusarium* isolates were grouped into three species based on morphological identification. Fifteen isolates were *Fusarium solani*, 11 were *Fusarium oxysporum* and 6 were *Fusarium culmorum* (Table 1).

3.2. Morphological characterisation of the Fusarium isolates

3.2.1 Fusarium solani isolates

Two morpho-types were identified in the *F. solani* isolates based on the presence of sporodochia in the cultures. The morpho-type A which included the isolates A1, E3, V1, V4 and V5 produced sporodochia that were yellow. Isolates with sporodochia also produced thin mycelia. The morpho-type B produced no sporodochia. Mycelial colour and pigment varied among the isolates (Table 2).

There were significant differences ($P \leq 0.01$) in mycelial growth among the *F. solani* isolates. Mycelial growth was slowest in isolate A1 and fastest in isolate V1 (Table 2). Generally, the mycelial growth rate of the isolates was indicative of fast growth, which could aid in the quick distribution of the pathogen in the eggplant and the environment.

The shapes of the macroconidia and microconidia were uniform for all *F. solani* isolates. However, the number of septa in the macroconidia differed and ranged from two to four (Table 3; Plate 1). The dorsal part of the macroconidia was more curved than the ventral part, had a blunt apical end and barely had a notched basal end. The microconidia were oval and two-celled.

The size of the conidia differed significantly ($P \leq 0.01$) among the isolates. There were also significant ($P \leq 0.01$) variations in conidia sizes among the isolate. The smallest and largest macroconidia were recorded in isolates E3 and V1, respectively (Table 4).

The culture characteristics that distinguished the *F. oxysporum* isolates were the colour of the mycelia and culture thickness. No sporodochium was produced by any of the isolates (Table 5).

Mycelial growth differed significantly ($P \leq 0.01$) among the isolates. The slowest growth rate was recorded for-isolate E6, whereas isolate V8 recorded the fastest rate (Table 5). The growth rates of the isolates were generally fast and could be useful for the prolific invasion of eggplants.

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Table 1. Morphological identification of the *Fusarium* isolates from Ashanti, Eastern and Volta regions used.

Isolate code	Morphological identification	Source of isolates	Region
A1	*Fusarium solani*	Offinso	Ashanti
A2	*F. solani*	Nsuta	Ashanti
A3	*F. solani*	Juaso	Ashanti
A4	*F. solani*	Besoro	Ashanti
A5	*F. solani*	Abofour	Ashanti
E1	*F. solani*	Asiakwa	Eastern
E2	*F. solani*	Nkurakan	Eastern
E3	*F. solani*	Kwaho Praso	Eastern
E4	*F. solani*	Enyerisi	Eastern
E5	*F. solani*	Huhunya	Eastern
V1	*F. solani*	Yordan	Volta
V2	*F. solani*	Vapko	Volta
V3	*F. solani*	Aneta	Volta
V4	*F. solani*	Tafi	Volta
V5	*F. solani*	Have	Volta
A6	*F. oxysporum*	Nsuta	Ashanti
A7	*F. oxysporum*	Juaso	Ashanti
A8	*F. oxysporum*	Besoro	Ashanti
E6	*F. oxysporum*	Asiakwa	Eastern
E7	*F. oxysporum*	Nkurakan	Eastern
E8	*F. oxysporum*	Kwaho Praso	Eastern
E9	*F. oxysporum*	Enyerisi	Eastern
E10	*F. oxysporum*	Huhunya	Eastern
V6	*F. oxysporum*	Vapko	Volta
V7	*F. oxysporum*	Aneta	Volta
V8	*F. oxysporum*	Have	Volta
A9	*F. culmorum*	Offinso	Ashanti
A10	*F. culmorum*	Nsuta	Ashanti
A11	*F. culmorum*	Abofour	Ashanti
E12	*F. culmorum*	Kwaho Praso	Eastern
V9	*F. culmorum*	Vapko	Volta
V10	*F. culmorum*	Have	Volta

Table 2. Culture characteristics of *Fusarium solani* isolates from Ashanti, Eastern and Volta regions used.

Morpho-type	*Fusarium solani* codes	Mycelial colour	Pigment	Sporodochia	Sporodochia colour	Thickness of mycelium (μm)	Growth rate (cm/day)
A	A1	Light cream	Orange	Present	Yellow	Thin	0.52
	E3	Light cream	Orange	Present	Yellow	Thin	0.66
	V1	Light cream	Orange	Present	Yellow	Thin	1.13
	V4	Creamy white	Brown	Present	Yellow	Thin	0.82
	E5	Cream	Brown	Present	Yellow	Thin	0.97
B	A2	Light orange	Orange	Absent	-	Thick	0.59
	A3	Creamy White	Orange	Absent	-	Thin	0.71
	A4	White	Light Orange	Absent	-	Thick	0.49
	A5	Light cream	Light Orange	Absent	-	Thick	0.80
	E1	Light pink	Brown	Absent	-	Thick	0.94
	E2	Light cream	Light Orange	Absent	-	Thick	0.93

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Table 2. *(Continued).*

Morpho-type	*Fusarium solani* codes	Mycelial colour	Pigment	Sporodochia	Sporodochia colour	Thickness of mycelium (µm)	Growth rate (cm/day)
	E4	Light orange	Orange	Absent	-	Thick	0.77
	V5	Creamy white	Orange	Absent	-	Thin	0.85
	V2	Light orange	Orange	Absent	-	Thick	0.85
	V3	Creamy white	Orange	Absent	-	Thin	0.94
HSD (0.01)							*0.09*
CV (%)							*0.22*

Table 3. Conidia characteristics of *Fusarium solani* isolates from Ashanti, Eastern and Volta regions used.

Fusarium solani code	Macroconidia				Microconidia
	Shape	Apical	Basal	Septum	Shape
A1	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
A2	Dorsal more curved than Ventral	Blunt	Barely notched	2	Two-celled oval
A3	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
A4	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
A5	Dorsal more curved than Ventral	Blunt	Barely notched	2	Two-celled oval
E1	Dorsal more curved than Ventral	Blunt	Barely notched	4	Two-celled oval
E2	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
E3	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
E4	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
E5	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
V1	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
V2	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval
V3	Dorsal more curved than Ventral	Blunt	Barely notched	2	Two-celled oval
V4	Dorsal more curved than Ventral	Blunt	Barely notched	2	Two-celled oval
V5	Dorsal more curved than Ventral	Blunt	Barely notched	3	Two-celled oval

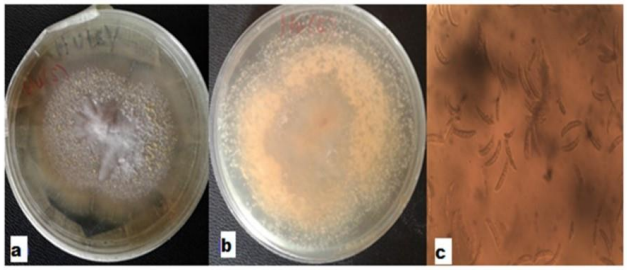

Plate 1. *Fusarium solani*: a. mycelia colour; b. pigment; c. macroconidia (x400) *Fusarium oxysporum* Isolates.

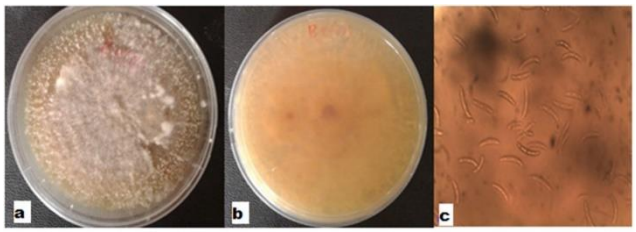

Plate 2. *Fusarium oxysporum*: a. mycelia colour; b. pigment; c. macroconidia (x400).

The shapes of the macroconidia and microconidia were similar for all *Fusarium oxysporum* isolates. The number of septa in the macroconidia, however, differed and ranged from three to five (Table 6). The macroconidia were slender shaped with a hooked apical end and a distinctly notched basal end (Plate 2). The microconidia were all reniform shaped.

Conidia size differed significantly ($P \leq 0.01$) between the isolates. There were also significant variations in the conidia size for each isolate. The smallest and biggest macroconidia were recorded in isolates E7 and E10, respectively (Table 7).

3.2.2. Fusarium culmorum isolates

The culture characteristic that distinguished the *F. culmorum* isolates was the mycelial thickness. No sporodochium was produced by any of the isolates (Table 8). Mycelial growth differed significantly ($P \leq 0.01$)

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Table 4. Conidia size of *Fusarium solani* isolates from Ashanti, Eastern and Volta regions used.

Morpho-type	*Fusarium solani* isolates	Macroconidia		Microconidia	
		Mean length (µm)	Mean width (µm)	Mean length (µm)	Mean width (µm)
A	A1	25.23 (23.90 - 26.60)	3.43 (3.20 - 3.80)	6.93 (6.30 - 7.70)	3.13 (2.80 - 3.30)
	E3	16.13 (14.20 - 18.50)	3.96 (3.80 - 4.20)	6.00 (5.40 - 6.60)	2.63 (2.30 - 3.30)
	V1	23.46 (22.60 - 24.50)	5.00 (4.20 - 5.80)	8.86 (8.20 - 9.20)	3.06 (2.60 - 3.70)
	V4	25.53 (24.60 - 27.10)	4.63 (3.40 - 5.70)	6.83 (6.50 - 7.40)	3.80 (3.4 - 4.00)
	E5	24.93 (24.00 - 25.70)	5.16 (4.70 - 5.50)	4.73 (4.40 - 5.20)	2.53 (2.4 - 2.6)
B	A2	25.56 (25.00 - 26.10)	4.40 (3.90 - 4.90)	6.93 (6.20 - 7.90)	4.46 (4.40 - 4.60)
	A3	25.63 (23.00 - 28.00)	3.93 (3.60 - 4.10)	11.83 (11.30 - 12.80)	3.33 (2.90 - 3.70)
	A4	23.10 (22.80 - 23.30)	4.13 (4.10 - 4.20)	9.63 (9.00 - 10.30)	4.13 (4.00 - 4.40)
	A5	18.50 (17.80 - 19.00)	4.26 (3.60 - 5.20)	6.63 (6.30 - 7.10)	2.93 (2.80 - 3.00)
	E1	22.70 (21.80 - 23.60)	4.86 (4.30 - 5.40)	8.86 (8.40 - 9.60)	3.23 (2.80 - 3.60)
	E2	24.30 (20.60 - 26.70)	5.43 (4.90 - 5.80)	7.66 (7.00 - 8.60)	2.76 (2.10 - 3.10)
	E4	23.93 (21.80 - 27.6)	5.16 (5.00 - 5.50)	11.73 (10.00 - 13.00)	3.76 (3.30 - 4.20)
	V5	25.60 (24.60 - 25.70)	4.40 (3.80 - 4.80)	12.76 (12.00 - 13.50)	4.60 (4.20 - 4.80)
	V2	21.80 (19.60 - 23.80)	4.60 (4.30 - 5.00)	9.06 (8.40 - 9.80)	3.06 (2.80 - 3.20)
	V3	23.96 (22.60 - 25.60)	4.23 (4.10 - 4.50)	7.03 (6.50 - 7.60)	4.03 (3.50 - 4.30)
	HSD (0.01)	*1.52*	*0.3*	*1.28*	*0.36*
	CV (%)	*0.11*	*0.12*	*0.27*	*0.19*

The values in parenthesis ( ) are the minimum and maximum conidial measurements.

Table 5. Culture characteristics of *Fusarium oxysporum* isolates from Ashanti, Eastern and Volta regions used.

Fusarium oxysporum isolates	Mycelial colour	Pigment	Thickness of mycelium (µm)	Growth rate (cm/day)
A6	White	Orange	Thick	0.97
A7	Light orange	Orange	Thick	0.87
A8	Whitish orange	Orange	Thin	0.85
E6	Whitish orange	Orange	Thick	0.63
E7	Whitish orange	Orange	Thin	0.71
E8	White	Brown	Thick	0.91
E9	Orange	Brown	Thin	0.68
E10	Light pink	Pink	Thick	0.72
V6	Light pinkish white	Deep Brown	Thick	1.30
V7	Whitish orange	Orange	Thick	1.01
V8	Light pink	Red	Thick	1.08
HSD (0.01)				*0.13*
CV (%)				*0.23*

Table 6. Conidia characteristics of *Fusarium oxysporum* isolates from Ashanti, Eastern and Volta regions used.

Fusarium oxysporum isolate (code)	Macroconidia				Microconidia
	Shape	Apical	Basal	Septum	Shape
A6	Slender	Hooked	Distinctly notched	5	Reniform
A7	Slender	Hooked	Distinctly notched	3	Reniform
A8	Slender	Hooked	Distinctly notched	4	Reniform
E6	Slender	Hooked	Distinctly notched	4	Reniform
E7	Slender	Hooked	Distinctly notched	4	Reniform
E8	Slender	Hooked	Distinctly notched	3	Reniform

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Table 6. (Continued).

Fusarium oxysporum isolate (code)	Macroconidia				Microconidia
	Shape	Apical	Basal	Septum	Shape
E9	Slender	Hooked	Distinctly notched	5	Reniform
E10	Slender	Hooked	Distinctly notched	3	Reniform
V6	Slender	Hooked	Distinctly notched	3	Reniform
V7	Slender	Hooked	Distinctly notched	5	Reniform
V8	Slender	Hooked	Distinctly notched	3	Reniform

Table 7. Conidia size of *Fusarium oxysporum* isolates from Ashanti, Eastern and Volta regions used.

Fusarium oxysporum isolates (code)	Macroconidia		Microconidia	
	Mean length (µm)	Mean width (µm)	Mean length (µm)	Mean width (µm)
A6	25.13 (25.00 - 25.40)	4.00 (3.70 - 4.30)	8.83 (8.50 - 9.40)	3.56 (3.50 - 3.60)
A7	19.53 (17.70 - 22.00)	3.16 (2.80 - 3.70)	13.76 (12.80 - 14.40)	3.96 (3.20 - 4.40)
A8	24.33 (22.70 - 28.40)	4.50 (3.50 - 5.60)	7.83 (7.80 - 7.90)	5.26 (5.20 - 5.40)
E6	19.96 (18.40 - 21.50)	3.80 (3.20 - 4.20)	4.50 (4.20 - 4.70)	2.46 (2.20 - 2.60)
E7	16.33 (15.90 - 16.90)	2.7 (2.60 - 2.90)	8.86 (8.50 - 9.30)	3.23 (3.10 - 3.50)
E8	25.20 (24.30 - 26.70)	4.36 (4.00 - 4.70)	10.46 (9.30 - 11.70)	4.23 (4.00 - 44.00)
E9	17.73 (17.10 - 18.30)	3.56 (3.30 - 3.80)	7.20 (6.40 - 7.90)	2.93 (2.60 - 3.20)
E10	27.86 (27.10 - 28.50)	4.86 (4.70 - 5.00)	12.90 (12.20 - 13.40)	4.46 (3.70 - 5.40)
V6	26.96 (25.30 - 27.90)	4.30 (4.10 - 4.50)	7.70 (6.30 - 8.60)	3.36 (3.20 - 3.70)
V7	22.93 (18.60 - 28.00)	3.93 (3.10 - 4.7)	12.00 (11.9 - 12.3)	4.63 (4.50 - 4.70)
V8	26.83 (25.50 - 28.50)	4.53 (4.30 - 4.90)	10.43 (8.50 - 11.50)	4.26 (4.20 - 4.40)
HSD (0.01)	*2.67*	*0.42*	*1.83*	*0.55*
CV (%)	*0.17*	*0.15*	*0.28*	*0.21*

The values in parenthesis ( ) are the minimum and maximum conidial measurements.

Table 8. Culture characteristics of *Fusarium culmorum* isolates from Ashanti, Eastern and Volta regions used.

Fusarium culmorum isolates (code)	Mycelial colour	Pigment	Thickness of mycelium(µm)	Growth rate (cm/day)
A9	Pink	Red	Thick	0.69a
A10	Pink	Red	Thick	0.68a
A11	Pink	Red	Thin	0.63a
E11	Pink	Dark red	Thin	1.26b
V9	Red	Red	Thin	0.64a
V10	Dark pink	Reddish Brown	Thin	0.75a
HSD (0.01)				*0.25*
CV (%)				*0.31*

among the isolates. The slowest growth rate was recorded for isolate A11, whereas, isolate E11 recorded the fastest rate (Table 8). The growth rates of the isolates were generally moderate, except for isolate KPFC, which was fast-growing. The moderate growth rate of the isolates could have resulted in slower infection and disease progression, compared to isolates of *Fusarium solani* and *Fusarium oxysporum* that comparatively grew faster.

The shapes of the macroconidia were similar for all *F. culmorum* isolates. The number of septa in the macroconidia, however, differed and ranged from three to four (Table 9). The dorsal part of the macroconidia was more curved than the ventral part and had a blunt apical end and barely notched basal end.

The size of the macroconidia differed significantly ($P \leq 0.01$) between the isolates. There were also

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Table 9. Conidia characteristics of *Fusarium culmorum* isolates from Ashanti, Eastern and Volta regions used.

Fusarium culmorum isolate	Macroconidia			
	Shape	Apical	Basal	Septum
A9	dorsal side more curved than ventral side	Blunt	Barely notched	4
A10	dorsal side more curved than ventral side	Blunt	Barely notched	3
A11	dorsal side more curved than ventral side	Blunt	Barely notched	3
E11	dorsal side more curved than ventral side	Blunt	Barely notched	3
V9	dorsal side more curved than ventral side	Blunt	Barely notched	3
V10	dorsal side more curved than ventral side	Blunt	Barely notched	3

Table 10. Conidia size of *Fusarium culmorum* isolates from Ashanti, Eastern and Volta regions used.

Fusarium culmorum isolate (code)	Macroconidia	
	Mean length (µm)	Mean width(µm)
A9	30.53 (25.80 - 33.80)	4.46 (3.90 - 5.10)
A10	23.66 (21.20 - 26.50)	4.03 (3.80 - 4.20)
A11	22.50 (22.10 - 23.20)	4.76 (4.70 - 4.90)
E11	24.8 (22.90 - 28.20)	4.40 (4.20 - 5.00)
V9	24.26 (23.70 - 25.2)	4.46 (3.90 - 5.00)
V10	19.86 (14.50 - 23.20)	4.46 (3.20 - 5.20)
HSD (0.01)	*3.30*	*0.24*
CV (%)	*0.14*	*0.05*

The values in parenthesis () are the minimum and maximum conidial measurements.

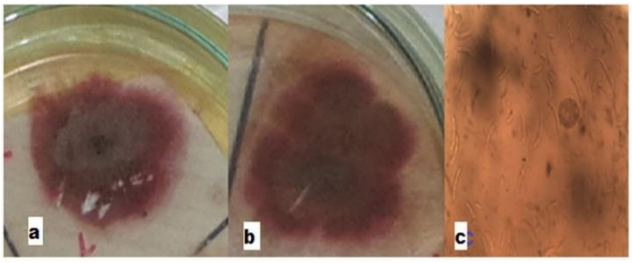

Plate 3. *Fusarium culmorum*: a. mycelia colour; b. pigment; c. macroconidia (x400).

significant variations in conidia size for each isolate. The smallest and biggest macroconidia were recorded in isolates V10 and A9, respectively (Table 10; Plate 3).

3.3. Molecular identification of Fusarium isolates with ITS primer

3.3.1. Fusarium solani isolates

Fifteen of the *Fusarium* isolates were identified as *F. solani* with the ITS1 primer when the DNA sequence was compared with the NCBI database using the BLASTN. The *F. solani* isolates differed in the number of base-pairs (bp) amplified in the ITS regions of the genomic DNA. The amplified region ranged from 324 to 446 bp (Table 11). The identification of ITS1F in the *F. solani* isolates, confirmed the morphological identification.

The phylogenetic tree of *F. solani* isolates consisted of two main clusters. Cluster I comprised two isolates (A1 and A2) and Cluster II comprised E5 and sub-clusters, as presented in (Fig. 1). The clustering pattern of the *F. solani* isolates indicated relatedness, except for the two main clusters, which showed wide variation.

3.3.2. *Fusarium oxysporum* isolates

Eleven of the *Fusarium* isolates were identified as *F. oxysporum* the ITS1 primer when the DNA sequence was compared with the NCBI database using the BLASTN. The *F. oxysporum* isolates differed in the number of base-pairs (bp) amplified in the ITS regions of the genomic DNA. The amplified region ranged from 322 to 332 bp (Table 12). The identification of ITS1F in the *F. oxysporum* isolates confirmed morphological identification.

The phylogenetic tree of *F. oxysporum* isolates consisted of two main clusters (Fig. 2). Cluster I comprised isolate E10, and Cluster II comprised two sub-clusters: Cluster II A, which comprised (A7 and E6) and Cluster II B, which comprised isolate V6 and

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Table 11. Identification of *Fusarium solani* isolates using ITS (ITS1F).

Fusarium isolate code	ITS identification	No. bp	Accuracy (%)	GeneBank accession No.
A1	*F. solani*	325	100.00	ON899848.1
A2	*F. solani*	325	99.70	ON890821.1
A3	*F. solani*	446	100.00	ON899848.1
A4	*F. solani*	327	100.00	ON899848.1
A5	*F. solani*	330	100.00	ON738704.1
E1	*F. solani*	325	99.34	MN173143.1
E2	*F. solani*	324	99.70	MH890688.1
E3	*F. solani*	326	100.00	ON899842.1
E4	*F. solani*	446	99.31	ON899848.1
E5	*F. solani*	325	99.70	ON899848.1
V1	*F. solani*	324	99.70	MN173137.1
V2	*F. solani*	324	100	ON899848.1
V3	*F. solani*	332	100	ON899848.1
V4	*F. solani*	330	100	ON899848.1
V5	*F. solani*	329	100	ON745555.1

Table 12. Identification of *Fusarium oxysporum* isolates using ITS (ITS1F).

Fusarium isolate code	ITS identification	No. bp	Accuracy (%)	GeneBank accession No.
A6	*F. oxysporum*	326	100.00	ON890821.1
A7	*F. oxysporum*	326	100.00	OM406348.1
A8	*F. oxysporum*	325	100.00	ON668120.1
E6	*F. oxysporum*	330	100.00	OM406348.1
E7	*F. oxysporum*	325	100.00	ON117317.1
E8	*F. oxysporum*	328	100.00	ON803511.1
E9	*F. oxysporum*	328	99.70	ON890821.1
E10	*F. oxysporum*	322	100.00	OM956392.1
V6	*F. oxysporum*	328	100.00	ON890820.1
V7	*F. oxysporum*	332	95.71	ON890820.1
V8	*F. oxysporum*	327	99.70	ON803511.1

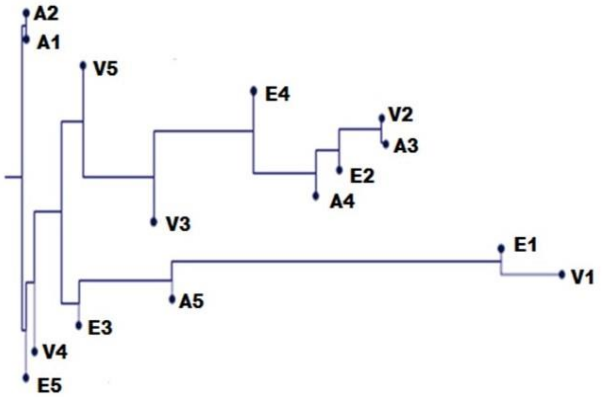

Figure 1. ITS phylogenetic tree of *Fusarium solani* isolates.

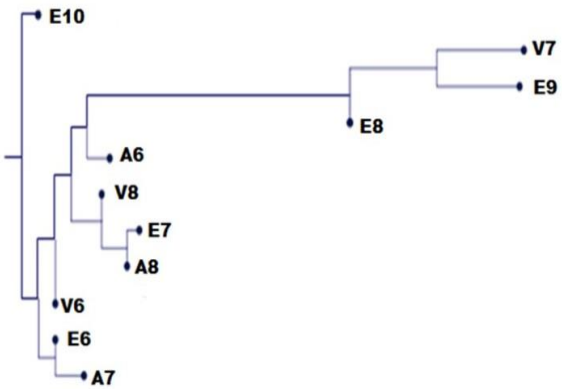

Figure 2. ITS phylogenetic tree of *Fusarium oxysporum* isolates.

other sub-clusters (Fig. 2). The clustering pattern of the *F. oxysporum* isolates indicated close relatedness of the majority of the isolates in Cluster II, except for isolates E8, E9 and V7, which had wide variation.

3.3.3. *Fusarium culmorum* isolates

Six of the *Fusarium* isolates were identified as *F.*

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Table 13. Identification of *Fusarium culmorum* isolates using ITS (ITS1F).

Fusarium isolate code	ITS identification	No. bp	Accuracy (%)	GeneBank accession No.
A9	*F. culmorum*	325	100.00	MW898129.1
A10	*F. culmorum*	308	99.70	ON117408.1
A11	*F. culmorum*	324	99.70	ON117408.1
E11	*F. culmorum*	657	99.31	ON117409.1
V9	*F. culmorum*	331	100.00	OL699879.1
V10	*F. culmorum*	322	100.00	MW898129.1

Table 14. Identification of *Fusarium solani* isolates using TEF (Fa-7_Ra-6F).

Fusarium isolate code	TEF identification	No. bp	Accuracy (%)	GeneBank accession No.
A1	*F. solani*	581	100.00	DQ247396.1
A2	*F. solani*	580	99.81	MN689879.1
A3	*F. solani*	584	100.00	DQ247396.1
A4	*F. solani*	577	100.00	LN828007.1
A5	*F. solani*	579	100.00	DQ247396.1
E1	*F. solani*	590	100.00	MN0689879.1
E2	*F. solani*	571	95.96	HE647945.1
E3	*F. solani*	587	99.64	DQ247396.1
E4	*F. solani*	576	100.00	DQ247396.1
E5	*F. solani*	581	100.00	DQ247396.1
V1	*F. solani*	580	99.82	DQ247396.1
V2	*F. solani*	582	99.64	MF327659.1
V3	*F. solani*	584	99.64	MF327650.1
V4	*F. solani*	576	99.81	MN689879.1
V5	*F. solani*	584	99.81	KY586223.1

culmorum using the ITS1 primer when the DNA sequence was compared with the NCBI database using BLASTN. The *F. culmorum* isolates differed in the number of base-pairs (bp) amplified in the ITS regions of the genomic DNA. The amplified regions ranged from 308 to 657 bp (Table 13). The identification of ITS1F in the *F. culmorum* isolates confirmed morphological identification.

The phylogenetic tree of *Fusarium culmorum* isolates consisted of two main clusters. Cluster I (isolates E11 and V10), Cluster II.: Cluster II comprised two sub-clusters, Cluster II A (isolates V9 and A10) and Cluster II B (isolates A11 and A9) as illustrated in (Fig. 3). The clustering pattern indicated a wide variation in the *F. culmorum* isolates.

3.4. Molecular identification of Fusarium isolates with TEF primer

3.4.1. Fusarium solani isolates

Fifteen of the *Fusarium* isolates were identified as *F. solani* using the TEF primer (Fa-7_Ra-6F) when the DNA sequence was compared with the NCBI database using BLASTN. The *F. solani* isolates differed in the number of base-pairs (bp) amplified in the TEF regions of the genomic DNA. The amplified region ranged from 571 to 590 bp (Table 14). The TEF identification of the *Fusarium solani* isolates confirmed the morphological identification.

The phylogenetic tree of *F. solani* isolates consisted of

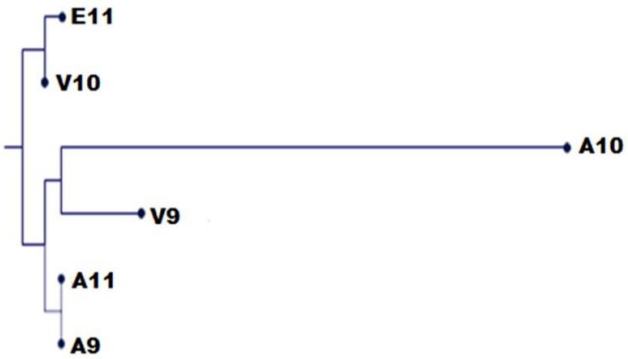

Figure 3. ITS phylogenetic tree of *Fusarium culmorum* isolates.

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Table 15. Identification of *Fusarium oxysporum* isolates using TEF (Fa-7_Ra-6F).

Molecular isolate code	TEF identification	No. bp	Accuracy (%)	GeneBank accession no.(sequence ID)
A6	*F. oxysporum*	592	99.82	OK631814.1
A7	*F. oxysporum*	587	100.00	MN689879.1
A8	*F. oxysporum*	548	99.81	MG733183.1
E6	*F. oxysporum*	572	99.81	MG733183.1
E7	*F. oxysporum*	574	100.00	MN689879.1
E8	*F. oxysporum*	580	100.00	MG733183.1
E9	*F. oxysporum*	580	99.81	MG733183.1
E10	*F. oxysporum*	570	99.82	OK631814.1
V6	*F. oxysporum*	578	99.63	MK752504.1
V7	*F. oxysporum*	583	99.63	MT5766471
V8	*F. oxysporum*	581	100.00	MN411314.1

Table 16. Identification of *Fusarium culmorum* isolates using TEF (Fa-7_Ra-6F).

Fusarium isolate code	TEF identification	No. bp	Accuracy (%)	GeneBank accession No.
A9	*F. culmorum*	579	99.82	MG733183.1
A10	*F. culmorum*	585	92.40	MF974551.1
A11	*F. culmorum*	580	99.82	MG733183.1
E11	*F. culmorum*	583	99.82	MG733183.1
V9	*F. culmorum*	582	99.62	KM401894.1
V10	*F. culmorum*	577	92.40	MF974551.1

Figure 4. TEF phylogenetic tree of *Fusarium solani* isolates.

two main clusters. Cluster I had two sub-clusters and Cluster II comprised two sub-clusters, as presented in Fig. 4. The clustering pattern of the *F. solani* isolates indicated a close relationship between the isolates in clusters I and II. However, isolate A4 in cluster II was very diverse from the other isolates in the cluster.

3.4.2. Fusarium oxysporum isolates

Eleven of the *Fusarium* isolates were identified as *F. oxysporum* using the TEF primer (Fa-7_Ra-6F) when the DNA sequence was compared with the NCBI database using BLASTN. The *F. oxysporum* isolates differed in the number of base-pairs (bp) amplified in the TEF regions of the genomic DNA. The amplified region ranged from 548 to 592 bp (Table 15). The TEF identification of the *Fusarium oxysporum* isolates confirmed the morphological identification.

The phylogenetic tree of *F. oxysporum* isolates consisted of two main clusters (Fig. 5). Cluster I comprised E9 and E7 isolates. Cluster II was divergent, with wide variations in the component

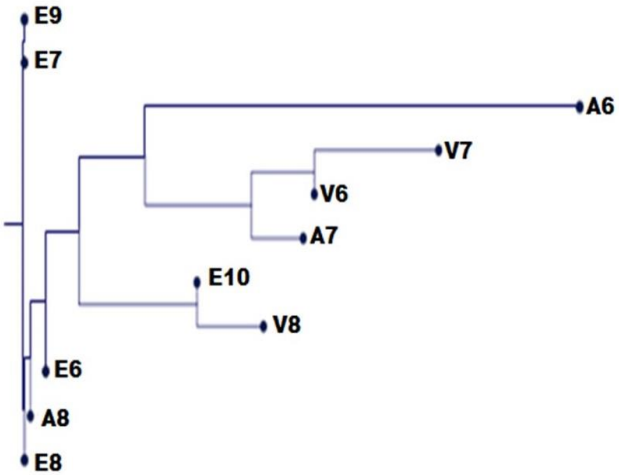

Figure 5. TEF phylogenetic tree of *Fusarium oxysporum* isolates.

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isolates, as illustrated in (Fig. 5). The clustering pattern of the *F. oxysporum* isolates indicated wide variations the among isolates.

3.4.3. Fusarium culmorum isolates

Six of the *Fusarium* isolates were identified as *F. culmorum* using the TEF primer (Fa-7_Ra-6F) when the DNA sequence was compared with the NCBI database using the BLASTN. The *Fusarium culmorum* isolates differed in the number of base-pairs (bp) amplified in the TEF regions of the genomic DNA. The amplified region ranged from 577 to 585 bp (Table 16). The TEF identification of the *F. culmorum* isolates confirmed their morphological identification.

The phylogenetic tree of *F. culmorum* isolates consisted of two main clusters. Cluster I comprised closely related isolates A10 and V9. Cluster II showed wide variations in the isolates, as shown in Fig. 6. The clustering pattern of *F. culmorum* isolates indicated wide variations among the isolates.

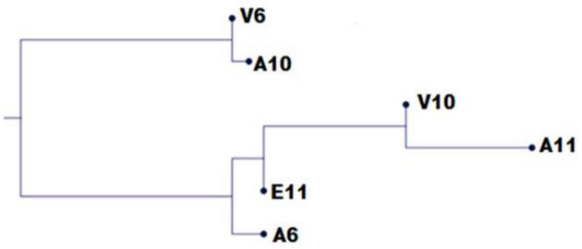

Figure 6. TEF phylogenetic tree of *Fusarium culmorum* isolates.

4. Discussion

This study reports the morphological and molecular phylogenetic characteristics of Fusarium wilt pathogens of eggplant isolated from 15 major growing communities across the Ashanti, Eastern and Volta Regions of Ghana. Thirty-two *Fusarium* isolates recovered from Fusarium wilt infected eggplants were morphologically identified as three species. Fifteen of the isolates were *F. solani*, 11 were *F. oxysporum* and 6 were *F. culmorum*. The *F. solani* isolates were divergent in cultural appearance and were primarily distinguished by the presence of sporodochia and secondarily by the mycelial colour and pigmentation. Conidial characteristics did not show any significant variations among the *F. solani* isolates. There were significant differences in the growth rate as well as conidia size among the *F. solani* isolates. The internal transcribed spacer (ITS) region sequences showed differences within the *F. solani* isolates, as depicted by morphological characterisation. However, the translation elongation factor (TEF) region sequences showed close relatedness in most *F. solani* isolates.

The *F. oxysporum* isolates were mostly uniform in mycelial colour, pigmentation and conidial characteristics. The sources of variation among the *F. oxysporum* isolates were growth rate and conidia size. Variations in the ITS region sequences were similar to those in TEF region sequences. The *F. oxysporum* isolates were divergent at the molecular level but uniform at the morphological level.

The *F. culmorum* isolates were uniform in mycelial colour, pigmentation and conidial characteristics. The growth rates and conidia sizes were also not significantly different among the isolates. However, molecular characterisation showed significant variations in the *F. culmorum* isolates. The divergence between the TEF region sequences was greater than that between the ITS region sequences. The TEF primer was more efficient in identifying differences among the *F. culmorum* isolates.

Morphological characterization offers limited distinction among isolates of the same *Fusarium* species. Differences in conidia size were unreliable due to the wide range of conidia sizes. Growth rate is not a good parameter for differentiation because it can be influenced by factors such as poor culture and growth medium. According to [18], many morphological features used for *Fusarium* species identification are unstable and provide false estimates. In phylogenetically diverse fungi such as *Fusarium* [3], morphological identification should be limited to the genus level, although some species can be easily identified morphologically with selective media and experience [18].

Fusarium identification based on the ITS region sequence has been widely used. In this study, the ITS region sequence distinctly identified *Fusarium* species and their variations within the species. The credibility of the ITS region sequence in the identification of *Fusarium* has been challenged [16], who reported that DNA sequences of the ITS regions were uninformative for *Fusarium*, although they are useful

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in distinguishing species in many eukaryotic organisms. O'Donnell et al. [16] observed a wide divergence in *Fusarium* sequences with different ITS primers. According to the previous reports [1, 5], the ITS region represents a small portion of the total genome; therefore, other segments of the genome must be studied to ascertain the true structure of the genome of the organism being investigated. Singha et al. [18] further argued that segments of the ITS regions of *Fusarium* species are non-orthologous. Therefore, ITS regions are limited in the phylogenetic analysis of *Fusarium* species.

According to [22, 10, 6, 3], the TEF gene is a useful phylogenetic marker for *Fusarium*, as it consistently presents a single-copy gene in the *Fusarium* genus, which provides species-level discrimination. In the present study, the TEF primer produced a larger genome sequence of the *Fusarium* isolates which identified more interrelations among the *Fusarium* isolates. *Fusarium solani* isolates were identified as closely related by the TEF sequence compared to the ITS sequence, which showed wide divergence. The phylogenetic grouping of the TEF sequences of the *F. oxysporum* isolates was differed from that of the ITS sequence grouping. The TEF sequence showed a wider divergence in *F. oxysporum* isolates. The phylogenetic grouping of the TEF sequence of the *F. culmorum* isolates was also different from that of the ITS sequence grouping, with the later showing wider divergence among the isolates.

5. Conclusions

Most of the Fusarium wilt pathogens of eggplant in Ghana were morphologically and molecularly identified as three *Fusarium* species, namely *F. solani*, *F. oxysporum* and *F. culmorum.* Morphological characteristics were largely uniform within the *Fusarium* species. However, clear distinctions were identified by the molecular characterisation. Both ITS and TEF region sequences could detect differences among the *Fusarium* isolates. However, there were differences in the phylogenetic grouping between the ITS and TEF region sequences. *Fusarium oxysporum* isolates were the most phylogenetically diverse.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Visualization, methodology, writing– original draft, E.O.O.; writing – review & editing, C.K.K., E. O.; protocol writing, R.P., E.A.A.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflicting interest.

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