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Identification and Characterization of *Fusarium* Species Associated With Amaranth (*Amaranthus* Species) Wilt Disease in the Semi-deciduous and Guinea Savannah Agro-ecological Zones of Ghana

Zippora Appiah-Kubi[1], Joseph Adomako[1], Bernard Armooh[3], David Appaih-Kubi[1], Allen Oppong[1], Marian Quian[1], Enoch A. Osekre[2], Joseph Nii Lamptey[1] & Charles Kwoseh[2]

[1] Plant Health Division, CSIR-Crops Research Institute, Kumasi, Ghana

[2] Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[3] Cocoa Research Institute of Ghana, New-Tafo Akim, Ghana

Correspondence: Zippora Appiah-Kubi, Plant Health Division, CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana. E-mail: zipak1@yahoo.com

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Abstract

Fusarium wilt is a major constraint in amaranth production in Africa; the disease can lead to total crop failure. However, few studies have identified *Fusarium* species associated with amaranth diseases in Ghana. The study was conducted to identify *Fusarium* species causing wilt in amaranth in the Semi-deciduous and Guinea Savannah Agro-ecological zones of Ghana and determine variations in isolates. Using standard laboratory procedures, fungal pathogens were isolated and culture characteristics studied. Variations in virulence were determined using root dip method. Sequence analysis of the internal transcribed spacer region of isolates was carried out for species identfication. Based on morphological features complemented by sequence analysis; *Fusarium equiseti*, *F. oxysporum*, *F. solani* and *F. proliferatum* were identified. *Fusarium equiseti* was the dominant species appearing in 82% of isolates. All the isolates were pathogenic. Based on virulence level, 9% of the isolates were classified as very highly virulent whilst 56% were weakly virulent. Genetically, isolates clustered into four groups irrespective of origin. The work identified and classified *Fusarium* species causing amaranth wilt in Ghana.

Keywords: *Fusarium* species, isolate variations, pathogenic, sequencing, virulence, wilt

1. Introduction

Amaranth (*Amaranthus* species) is an indigenous leafy vegetable crop grown and consumed in several tropical African countries. It thrives well under minimum rainfall of about 500 mm and on marginal soils (Jimoh et al., 2018; Vidhi, 2020). Nutritionally, amaranth is rich in proteins, fat, carbohydrate, calcium and vitamins (Alegbejo et al., 2013; Achigan-Dako et al., 2014; Ochieng et al., 2019). It has the great potential of providing the nutritional needs of consumers, combat food insecurity and reduce poverty among rural poor farmers (Mekonnen et al., 2018).

The production of Amaranth is however; constrained by numerous pathogenic fungi causing several diseases such as leaf spots, wilting, root rot, dumping-off and dieback. Among the various diseases associated with the crop, diseases caused by *Fusarium* are highly prevalent because, this fungal pathogen is known to be cosmopolitan (Rampersad, 2020). The pathogen has a wide of host range; survives in soils and plant debris; and infects crops usually through the roots (Agrios, 2005). It may be spread by water, contaminated working equipment or seed (Lamichhane et al., 2017). *Fusarium* species causing wilt are soil-borne and survive in the soil debris as chlamydospores without a host plant for many years (Mwaniki et al., 2011; Worku & Sahe, 2018). *Fusarium* species attack on vegetables leads to vascular wilt, root rot, chlorotic leaves and damping-off (Hibar et al., 2007; Mwaniki et al., 2011; Osdaghi, 2020; Oljira & Berta, 2020). According to Blodgett et al. (2000) and National Research Council (2006), wilting and damping-off diseases caused by *Fusarium* can lead to total crop failure in Amaranth. Azil et al. (2020) citing Hwang and Evans (1995) reported that when the pathogen invades its host, it

colonizes the xylem vessels and causes the plant to show symptoms of leaf chlorosis, vascular discoloration, wilting, stunting and death of plant.

Previous research studies have identified and reported multiple species of *Fusarium* responsible for or involved in different diseases and disease complexes. According to Barros et al. (2014) and Šišić et al. (2018), both *F. solani* and *F. graminearum* were associated with wilting and damping-off diseases of soybean and pea. Similarly, Daami-Remadi and El Mahjoub (2004) found that *F. sambucinum*, *F. oxysporum* and *F. solani* were responsible for potato wilt disease. Several authors (Blodgett et al., 1998; Barros et al., 2014; Kuzdraliński et al., 2014) have generally associated *Fusarium* species with multiple diseases attacking several vegetables, including amaranth. In Ghana, Ministry of Agriculture (2016) and Agbaglo et al. (2020) have reported that multiple species of *Fusarium* induce diseases and limit production of vegetables such as tomato, eggplant, pepper, and okra. However, a few have identified *Fusarium* species involved in amaranth wilt disease. To overcome this challenge, there is the need for more research to properly identify and understand the population difference in the pathogen. This is crucial for the development of efficient disease management strategies. This study was therefore carried out with the objectives to (i) identify and characterize *Fusarium* species associated with amaranth wilt disease in Ghana (ii) determine differences in pathogenicity of the isolates.

2. Materials and Methods

2.1 Sampling and Fungal Isolation

Amaranth seeds, diseased plants, and soils were collected from 43 surveyed farms in the peri-urban communities of Kumasi and Tamale in the semi-deciduous and Guinea Savannah agro-ecological zones of Ghana respectively. Isolation of seed-borne fungal pathogens was conducted following the procedure described by the International Seed Testing Association (ISTA, 2015). Amaranth seeds were surfaced sterilized with 3% sodium hypochlorite (NaOCl) for 3 min and serially rinsed with sterilized distilled water three times. It was then placed on Potato Dextrose Agar (PDA) and incubated for seven days under alternating circles of 12 h light and 12 h darkness under near-ultraviolet (NUV) light. To isolate fungi from diseased plant tissues, root tissues were washed under tap water to remove debris and then cut into small pieces. Leaf tissues were cut into pieces to contain both symptomatic and asymptomatic portions and surface sterilized with 5% sodium hypochlorite for 1 min, and then rinsed with sterilized distilled water three times. The sterilized specimens were placed on PDA and incubated for five days at 26±2 °C. Serial dilution method was used to isolate fungal species from soil (Kumar et al., 2015). One gram of each soil sample was suspended in 10 ml distilled water and serial dilutions of 10^{-1} to 10^{-5} were made using sterile distilled water. From dilutions of 10^{-4} and 10^{-5}, 1 ml aliquots were picked with a pipette and spread on streptomycin amended PDA plates. Plates were then incubated at 26±2 °C for four days.

After the period of incubation, fungal colonies that appeared on all plates were transferred onto fresh PDA and purified by single spore culturing. Fungi were identified based on habit and morphological characteristics of the fruiting bodies and slide examination with an identification manual (Barnett & Hunter, 1972; Mohd Nazri et al., 2020). Pure cultures of fungi growing in media were obtained by single spore isolation, stored in slant universal bottles, and kept in the refrigerator at 4 °C for use (Choi et al., 1999).

2.2 Morphological Characterization

All purified fungal isolates were placed separately in the center of 90-mm diameter Half Strength Potato Dextrose Agar (HSPDA) plates and incubated at 26±2 °C for seven days for examination of colony form, elevation, margin, and colour (Watanabe, 2010; Miyashira et al., 2010). Colony growth of the different *Fusarium* isolates were measured daily along two perpendicular lines drawn beneath the plates. Mean colony diameter was then calculated by the average of the two colony diameters for each plate; the growth rate was calculated as the average of the 7-day mean daily growth (Than et al., 2008; Mukuma, 2016). The experiment was laid out in a completely randomized design with four replications. Conidia shapes and sizes produced by representative isolates were observed and measured with an Amscope microscope for 30 conidia of each isolate.

2.3 Fusarium Pathogenicity Tests

Thirty-four *Fusarium* isolates were used for pathogenicity assays. The pathogenicity tests were performed using the root dip inoculation method. Inoculum of each *Fusarium* species was prepared by flooding the surface of 7-day-old culture of each isolate with water. The surface was scraped with sterile glass rod to dislodge the spores. The spore-mycelium-water suspension was filtered through a four layered cheese cloth to remove the mycelia debris. The spore concentration of the suspension was determined and standardized by counting with a heamocytometre (Fuchs-Rosenthal). The resultant suspension was adjusted to contain 1×10^8 spores/ml and used for the assays. Seeds of amaranth were sterilized with 5% sodium hypochlorite (NaOCl) for 3 min and serially

rinsed with sterilized distilled water three times before sowing in sterilized soil for seedling growth. After 21 days, seedlings of amaranth were uprooted and tip of roots trimmed with sterilized scissors. The wounded roots were dipped into separate *Fusarium* inoculum for 30 min and then transplanted into 12-liter plastic buckets filled with sterilized soil (Korolev et al., 2000). Wounded roots immersed in sterilized distilled water for 30 min served as a control. Inoculated plants were covered with transparent polythene bags for 48 hours to build up high humidity for disease development. Wilt Disease severity was recorded weekly for six weeks. The plants were rated for disease severity on a 1 to 5 disease severity scale, where, 1 = no lesion or damage; 2 = 1-25% damage; 3 = 26-50% damage; 4 = 51-75% damage; 5 = more than 75% damage or total destruction. The disease severity index (DSI) was calculated for each isolate as:

$$DSI = \frac{\sum(nv)}{NS} \times 100 \quad (1)$$

where, n = Number of plants rated in each grade category; v = Numerical value of disease grade; N = Total number of plants rated; S = Highest disease grade (Asma et al., 2018). Means for the six weeks severity score were used in determining the pathological differences among the isolates (Zhu et al., 2014; Kalman et al., 2020). The experiment was laid in a completely randomized design with three replications. At the end of the assays, fungal pathogens were re-isolated to confirm Koch's postulate.

2.4 Genomic DNA Isolation, Polymerase Chain Reaction, Amplification and Sequencing

Genomic DNA of each fungal isolates was extracted from 7-day-old cultures using the modified Cetyl trimethylammonium bromide (CTAB) method (Tripathy et al., 2017; Weiland, 2019). At the Functional Bioscience Laboratory, Madison, WI, USA, Polymerase chain reaction (PCR) was carried out with the DNA extracted from isolates as templates. The primer pair ITS1/ITS4, (ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'and ITS4: 5'-TCCTCCGCTTATTGATATGC-3') designed to amplify the internally transcribed spacer (ITS) region of isolates (White et al., 1990) was used in the PCR. The mixture was comprised 5 µl of template DNA, 2.5 µL each of forward and reverse primers, 1.25 µl of 2 mM $MgCl_2$, 25 µl of master mix (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5% Glycerol, 0.08% IGEPAL® CA-630, 0.05% Tween® 20, 25 units/ml Taq DNA Polymerase, pH 8.6 at 25°C) (New England Biolabs, UK) and 13.75 µl of deionized autoclaved water. The PCR cycles were as follows: initial denaturation at 95 °C for 30 s, followed by 35 cycles of denaturing, annealing and extension at 95 °C for 10 s, 59 °C for 15 s and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The Amplified products were separated by 1.5% w/v agarose gel (Invitrogen, Carlsbad, CA), stained with Ethidium bromide alongside 1.0 kb marker at 100 V for one hour. Bands were observed under UV light. The amplified PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, USA) and analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems, USA).

2.5 Analysis of Data

Data collected from isolate colony diameter, daily growth, conidia length and width, and the severity scores were subjected to Analysis of variance using the Genstat statistical software version 12.0. (Lawes Agricultural Trust, VSN International, UK). Differences in means were compared using the Least Significant Difference (LSD) where significant treatment differences were found. Virulence of isolates was grouped according to their severity scores as: 2-weakly virulent; 3-moderately virulent; 4-highly virulent; 5-very highly virulent.

2.6 Phylogenetic Analysis

Obtained sequenced data were manually edited and assembled using the BioEdit software, then queried for similarities with strains (Rahjoo et al., 2008; Chen et al., 2017) deposited at the Genebank of the National Centre for Biotechnology Information (NCBI) using the BLAST search tool. Sequences were aligned using the Clustal W package in MEGA software (Tamura et al., 2013). A phylogenetic tree was constructed using maximum likelihood (ML) in MEGA 7 software.

3. Results

3.1 Fungal Identification and Morphological Characterization

Thirty-four isolates obtained and examined fit the description of *Fusarium* species based on their morphological features. Three, twelve, thirteen, and six of the strains were isolated from leaf, seeds, roots, and rhizosphere soil, respectively (Table 1). The isolates obtained, however, exhibited diverse cultural characteristics in colony forms, elevations, margins, and colour irrespective of where they originated from. Two colony forms namely circular and irregular were observed (Figure 1). Majority (56%) of the isolates exhibited circular colony with smooth edges whilst 44% of the isolates produced irregular colony form with wavy edges. Eight and twenty-six of the isolates exhibited flat and raised elevations respectively (Figure 2). Culture margins produced by the isolates were either

smooth or undulate whilst four main colony colours namely white, cream, orange and pink were observed (Figure 3). Conidia shape of the isolates was crescent with multiple septations (Figure 4). The length and width of *Fusarium* conidia differed significantly ($P \leq 0.01$) from each other. The length ranged from 9.41µm (Boad Soil 1) to 41.5 µm (AsoM 3R) all from Kumasi metropolis while the width ranged from 1.97 µm (ChoMa 39L) to 6.87 µm (AsoM 3R) isolated from Tamale and Kumasi metropolises respectively.

Colony diameter and growth rate for *Fusarium* isolates showed significant difference ($P \leq 0.01$) among the isolates. Mean colony diameter and growth rate for *Fusarium* isolates ranged from 12.10 to 46.5 mm and 2.04 to 11.21 mm/day respectively (Table 1). For both colony diameter and growth rate of *Fusarium* isolates, highest and the lowest records were registered by isolates 'Emin Seed 13' and 'ChoLo Seed 40' obtained from Kumasi and Tamale metropolises respectively.

Table 1. Morphological characteristics of *Fusarium* isolates from Semi-deciduous and Guinea savannah Agro-ecological zones

Agro-ecological	Location	Isolate code	Culture				Mean colony diameter (mm)	Daily growth rate (mm/day)	Conidia Length (µm)	Conidia Width (µm)
			Form	Elevation	Margin	Colour				
Semi-deciduous	Kumasi	AsoM 3R	Circular	Raised	Smooth	Orange	29.52	7.23	41.5	6.87
Semi-deciduous	Kumasi	AsoM 4R	Circular	Raised	Smooth	Orange	29.62	5.22	17.3	3.08
Semi-deciduous	Kumasi	AsoM Soil 3	Circular	Raised	Undulate	Cream	12.88	3.28	18.31	3.4
Semi-deciduous	Kumasi	Boad Soil 1	Circular	Raised	Undulate	Pinkish	28.1	6.58	9.41	3.53
Guinea savannah	Tamale	ChoIa Seed 30	Irregular	Raised	Undulate	Orange	33.62	8.21	30.81	4.34
Guinea savannah	Tamale	ChoLo 43R	Irregular	Raised	Undulate	Orange	13.95	2.79	15.64	2.92
Guinea savannah	Tamale	ChoLo Seed 40	Circular	Raised	Undulate	Orange	46.52	11.21	36.31	5
Guinea savannah	Tamale	Cholo Seed 43	Irregular	Raised	Undulate	White	43.93	10.89	21.02	3.5
Guinea savannah	Tamale	ChoMa 39L	Irregular	Raised	Smooth	Cream	18.45	4.54	20.6	1.97
Guinea savannah	Tamale	ChoNa 35L	Irregular	Flat	Undulate	White	28.5	6.94	29.28	4.46
Guinea savannah	Tamale	ChoNa Seed 36	Irregular	Raised	Undulate	Cream	32.17	7.47	27.11	3.6
Guinea savannah	Tamale	ChoNa Soil 37	Circular	Raised	Undulate	Cream	18.57	4.58	20.94	4.17
Guinea savannah	Tamale	ChoYa Seed 31	Circular	Raised	Undulate	Cream	26.71	6.57	10.66	3.12
Semi-deciduous	Kumasi	Ded 5Rr	Irregular	Raised	undulate	White	40.5	10.21	13.85	3.97
Semi-deciduous	Kumasi	Ded 6Rr	Irregular	Raised	Undulate	White	21.47	4.34	18.97	2.96
Semi-deciduous	Kumasi	Ded 7Rr	Irregular	Flat	Undulate	Orange	12.12	2.43	10.76	2.83
Semi-deciduous	Kumasi	Ded Seed 6	Circular	Raised	Undulate	White	30.88	8.2	26	3.92
Semi-deciduous	Kumasi	Ded Seed 7	Circular	Raised	Smooth	White	30.52	7.83	25.57	3.69
Semi-deciduous	Kumasi	Ded Soil 6	Circular	Flat	Smooth	White	37.86	9.1	16.94	3.68
Semi-deciduous	Kumasi	Emin 10Ll	Irregular	Flat	Smooth	White	36.64	9.27	28.06	3.14
Semi-deciduous	Kumasi	Emin 8Rr	Irregular	Raised	Undulate	Cream	25.61	5.53	28.58	4
Semi-deciduous	Kumasi	Emin Seed 13	Circular	Flat	Smooth	Pinkish	12.1	2.04	17.87	2.3
Semi-deciduous	Kumasi	Emin Seed 8	Circular	Flat	Undulate	Orange	22.41	6.09	23.39	3.32
Guinea savannah	Tamale	Gumb 20Rr	Circular	Flat	Smooth	Orange	33.17	7.67	24.47	4.15
Guinea savannah	Tamale	Gumb Seed 18	Irregular	Raised	Smooth	White	36.8	9.35	25.95	3.94
Guinea savannah	Tamale	Gumb Seed 19	Circular	Raised	Undulate	Pinkish	21.34	4.98	18.55	3.52
Guinea savannah	Tamale	Gumb Seed 21	Irregular	Raised	Undulate	Cream	29.02	7.06	24	3.77
Guinea savannah	Tamale	Gumb Soil 18	Circular	Raised	Undulate	White	40.55	10.07	26.04	4.68
Guinea savannah	Tamale	Kaku Soil 22	Circular	Raised	Smooth	White	27.93	8.22	12.28	2.07
Semi-deciduous	Kumasi	Kent 2Rr	Circular	Flat	Undulate	Pinkish	16.05	4.08	21.26	3.62
Guinea savannah	Tamale	SagD 23Rr	Irregular	Raised	Undulate	Cream	35.93	8.47	23.48	3.92
Guinea savannah	Tamale	SagD 24Rr	Circular	Raised	Smooth	Pinkish	35.69	8.64	18.9	3.66
Guinea savannah	Tamale	SagD Seed 24	Circular	Raised	Undulate	Orange	34.69	9.13	20.86	3.55
Semi-deciduous	Kumasi	TeckP 15Rr	Irregular	Raised	undulate	Orange	24.76	5.92	40.81	3.37
L.S.D. ($p \leq 0.05$)							6.41	1.91	0.748	0.43
CV							13.8	17.1	2.2	7.3

Note. l and r represent isolates from leaf and root respectively.

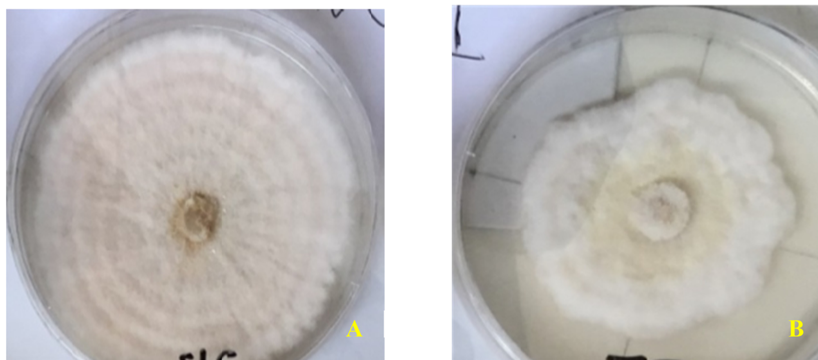

Figure 1. Colony forms of *Fusarium* cultures (A) Circular, (B) Irregular

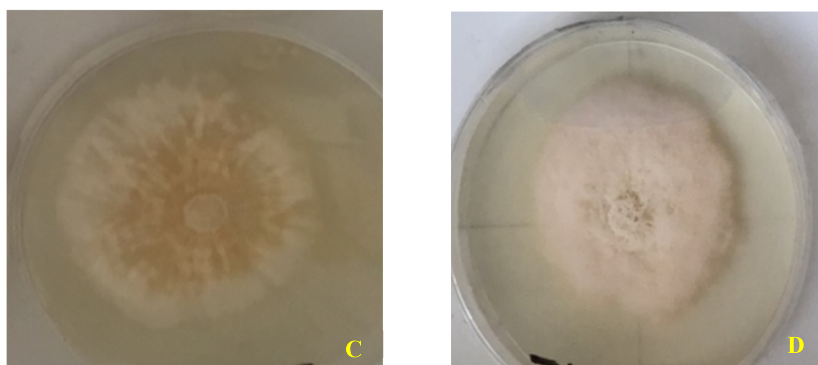

Figure 2. Elevations of *Fusarium* cultures (C) Flat, (D) Raise

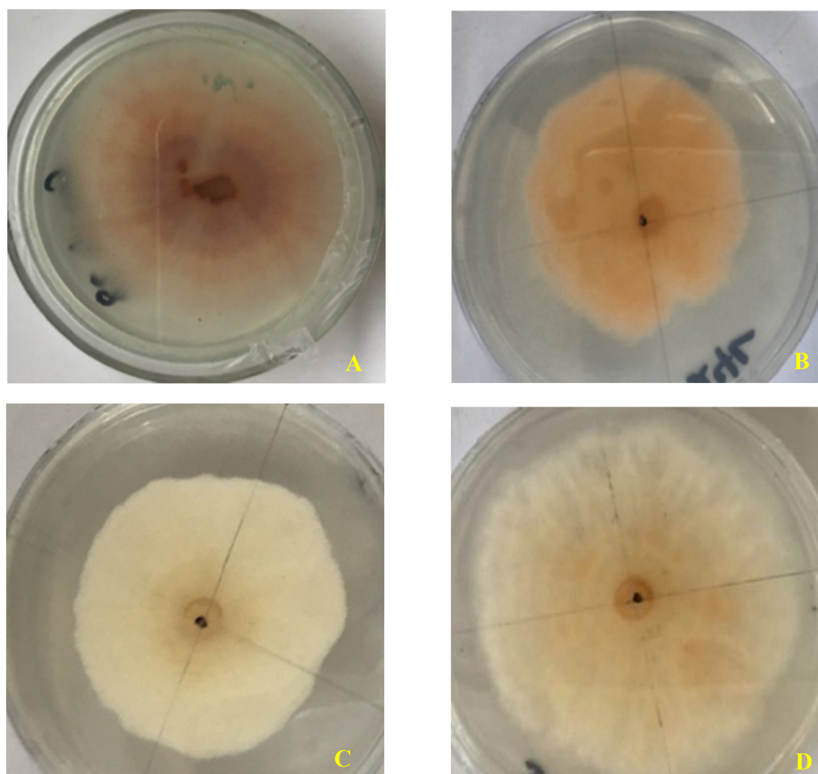

Figure 3. Colours and margins of *Fusarium* cultures. (A) Pink colour with smooth edge (B) Orange colour with undulating edge; (C) White colour with smooth edge; (D) Cream colour and undulating edge

Figure 4. Conidia of *Fusarium* species

3.2 Pathological Variations Among Fusarium Isolates

Results of root dip experiment showed that all 34 *Fusarium* isolates were pathogenic on Amaranth causing symptoms of chlorosis and wilting of the plant (Figure 5). It was observed that isolates varied in the extent of damage caused on Amaranth plants. One isolate Ded 6R from Kumasi metropolis and two isolates Gumb Soil 18 and ChoNa Soil 37 from Tamale produced the highest wilt severity score of 5.0 (Table 2). Nine isolates obtained from Kumasi and ten from Tamale metropolis recorded least severity of 2.0 Significant differences ($P \leq 0.05$) in wilting severity was observed among the isolates. Wilting severity scores grouped the isolates into four classes as: very highly virulent, highly virulent, moderately virulent and weakly virulent. Generally, isolates clustered into groups irrespective of origin. Nine isolates obtained from Kumasi and ten Tamale metropolis were classified as weakly virulent.

Figure 5. Symptoms shown by Amaranth plants inoculated with *Fusarium* isolates.
(A) Healthy; (B) Chlorosis; (C) Wilting

Table 2. Pathological variation among *Fusarium* isolates from Semi-deciduous and Guinea savannah Agro-ecological zones

Agro-ecological	Location	Fusarium Isolate	Severity Scale (1-5)	Virulence
Semi-deciduous	Kumasi	Emin 8R	2	Weakly virulent
Semi-deciduous	Kumasi	AsoM 4R	2	Weakly virulent
Semi-deciduous	Kumasi	Ded 5R	2	Weakly virulent
Semi-deciduous	Kumasi	Emin Seed 13	2	Weakly virulent
Semi-deciduous	Kumasi	Kent 2R	2	Weakly virulent
Semi-deciduous	Kumasi	Ded Seed 6	2	Weakly virulent
Semi-deciduous	Kumasi	TeckP 15L	2	Weakly virulent
Semi-deciduous	Kumasi	AsoM 3R	2	Weakly virulent
Semi-deciduous	Kumasi	Ded Seed 7	2	Weakly virulent
Guinea savannah	Tamale	Gumb 20R	2	Weakly virulent
Guinea savannah	Tamale	SagD 23R	2	Weakly virulent
Guinea savannah	Tamale	ChoLo 43R	2	Weakly virulent
Guinea savannah	Tamale	ChoNa Seed 36	2	Weakly virulent
Guinea savannah	Tamale	ChoLo Seed 40	2	Weakly virulent
Guinea savannah	Tamale	ChoMa 39L	2	Weakly virulent
Guinea savannah	Tamale	Gumb Seed 21	2	Weakly virulent
Guinea savannah	Tamale	Gumb Seed 18	2	Weakly virulent
Guinea savannah	Tamale	SagD 24R	2	Weakly virulent
Guinea savannah	Tamale	ChoNa 35L	2	Weakly virulent
Semi-deciduous	Kumasi	Boad Soil 1	3	Moderate violent
Semi-deciduous	Kumasi	AsoM Soil 3	3	Moderate violent
Semi-deciduous	Kumasi	Emin 10L	3	Moderate violent
Semi-deciduous	Kumasi	Ded Soil 6	3	Moderate violent
Guinea savannah	Tamale	Cholo Seed 43	3	Moderate violent
Guinea savannah	Tamale	ChoYa Seed 31	3	Moderate violent
Guinea savannah	Tamale	Kaku Soil 22	3	Moderate violent
Guinea savannah	Tamale	ChoIa Seed 30	3	Moderate violent
Guinea savannah	Tamale	SagD Seed 24	3	Moderate violent
Semi-deciduous	Kumasi	Ded 7R	4	Highly virulent
Semi-deciduous	Kumasi	Emin Seed 8	4	Highly virulent
Guinea savannah	Tamale	Gumb Seed 19	4	Highly virulent
Semi-deciduous	Kumasi	Ded 6R	5	Very highly virulent
Guinea savannah	Tamale	ChoNa Soil 37	5	Very highly virulent
Guinea savannah	Tamale	Gumb Soil 18	5	Very highly virulent

3.3 Molecular Characterization

All DNA sequences generated for this study have been deposited in the Genebank with accession numbers OL998416-OL998447. Blast search matched sequence data from this study with those deposited at the NCBI GenBank and confirmed identity of isolates to the species level based on high similarity score of between 96-100% and E-value of 0.0. The search results confirmed that 28 isolates of the 34 isolates were *Fusarium equiseti* and a single isolate of *F. oxysporum*, *F. solani* and *F. proliferatum* (Figure 6). Three of the isolates (Cholo seed 40, choNa seed 39 and SegD seed 24) were only identified as *Fusarium* species.

Four main clusters were formed by the phylogenic tree constructed from the sequence analysis (Figure 6). Isolates clustered into these groups irrespective of origin. Cluster A was the biggest group consisting of 21 *F. equiseti* isolates and single isolates of *F. solani* and *F. proliferatum*. Very highly virulent *Fusarium* isolates observed in the pathogenicity assay were contained in this cluster. The cluster A is subdivided into three-sub clusters A1, A2, and A3, Sub-cluster A1 contained seven isolates of *F. equiseti* and single isolates of *F. solani* and *F. proliferatum*. Sub-clusters A2 and A3 were made up of nine and three isolates of *F. equiseti* respectively. Cluster B, consist of ten isolates with two-sub clusters B1 and B2. Sub-cluster B1 consisted of four highly virulent isolates, made up of

three isolates of *F. equiseti* and an isolate of *F. oxysporum* whilst sub-cluster B2 comprised three each of moderately and weakly virulent isolates of *F. equiseti*. Cluster C of the phylogenic tree consist of two moderately virulent *Fusarium* isolates from seed. Cluster D was made up of a single highly virulent isolate and showed no close relationship with the rest of the isolates.

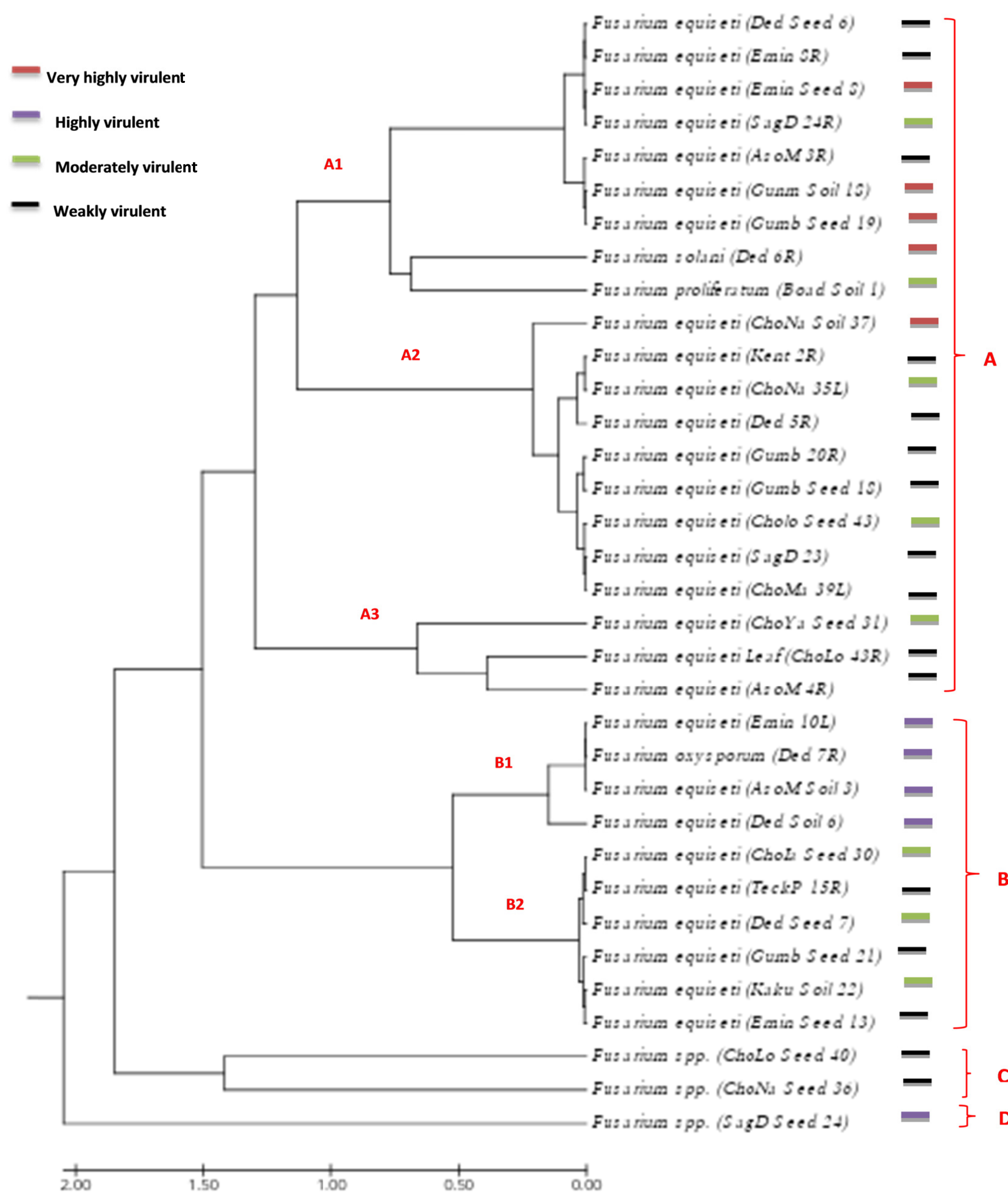

Figure 6. Phylogenic tree showing relationship between 34 *Fusarium* isolates

4. Discussion

In this study, combined applications of morphological, pathological, and molecular tools were used to identify and differentiate species of *Fusarium,* causing amaranth wilt disease. This study to the best of our knowledge represents the first attempt to identify and characterize pathogens of *Fusarium* wilt of amaranth in Ghana.

Morphological traits employed in this study successfully identified all fungal pathogens isolated as *Fusarium.* The isolates showed variations in culture characteristics such as colony diameter, colour, elevations, margins and form irrespective of origin. The observed differences in colony morphology agree with Lazarotto et al. (2014), Mukuma (2016), who found differences in several morphological traits of *Fusarium*; as well as difference in conidia sizes

(Akbar et al., 2018; Bechem & Afanga, 2018). The observed differences in morphplogy of the isolates could be due to movement of *Fusarium* species carried in seeds across farms in the country. Morphological characteristics, however, could not detect the different *Fusarium* involved to the species level due to the limitation associated with traditional markers for pathogen identification. These limitations include high level of overlapping features such as colony colour, colony diameter and conidia shapes. The inability of the current study to morphologically identify *Fusarium* to the species level is in line with previous studies (Leslie & Summerell, 2006; Dita et al., 2010; Kalman et al., 2020), who found that overlap of several morphological features among *Fusarium* limits its use to separate the pathogen to species level. To overcome the limitation associated with morphological identification, this study relied on the use of molecular tools to properly identify the pathogens to species level without any ambiguity or misdiagnosis. Application of sequence analysis of the ITS region made it possible to properly identify and confirm *F. equiseti*, *F. oxysporum*, *F. solani*, and *F. proliferatum* as major *Fusarium* species associated with amaranth wilt in the study area. The ITS region has successfully been relied on to identify species of unknown fungal isolates due to its ability to delineate interspecific variations among closely related species (Lin et al., 2011). The blast search results showed that of the four species, *Fusarium equiseti*, was the dominant species responsible for amaranth wilt and chlorosis. This finding agrees with several studies that *Fusarium equiseti* is the major *Fusarium* species that causes wilt in several vegetable crops such as tomato and eggplant (Mwaniki et al., 2011; Akbar et al., 2018). Identification of single isolates of *F. oxysporum, F. solani,* and *F. proliferatum* in this study however, suggest these species are of minor economic importance in amaranth wilt disease; although, *F. oxysporum and F. solani* are well known for causing serious diseases in vegetables and other crops (Mukuma, 2016; Kalman et al., 2020). Identification of *Fusarium proliferatum* however, raises great concern in amaranth more especially since the crop is a vegetable requiring minimal heating before consumption. *Fusarium proliferatum* is reported to be a major source of mycotoxins such as fumonisin, fusaric acid, beauvericin, fusarins, fusaproliferin and moniliformin. Fumonisin is known to be highly thermostable, conserving its compound during heating or processing (Leslie & Summerell, 2006). The identities of three *Fusarium* species were not confirmed in this study. This could possibly be due to the use of limited number of ITS primers for sequencing as several authors (Mukuma, 2016; Alsohaili & Bani-Hasan, 2018), have reported the limitations associated with the use of few ITS primers to identify closely related fungi species.

All 34 *Fusarium* isolates evaluated within the present study were found to be pathogenic with varied disease severity. *Fusarium* species were classified as very highly virulent, highly virulent, moderately virulent, or weakly virulent which agrees with Hirayama et al. (2018) and Kim et al. (2020); who found variations in pathogenicity of different *Fusarium* species involved in wilt diseases of several crops. Although the current study did not find the basis underlining the causes of differences in virulence of the isolates, previous studies (Akbar et al., 2018; Asma et al., 2018) have reported that variations in virulence of fungal isolates are genetically controlled. Phylogenetic analysis of the isolates revealed the relatedness of *Fusarium* species involved in amaranth wilt. Clustering of the isolates was not based on their origin but it was interesting to observe that isolate designated as very highly and highly virulent in the pathogenicity assay all clustered in clade A, apart from one highly virulent isolate (Ded 7R) which was grouped in clade B. Clustering of isolates from different origins in the same clades according to Rampersad (2020) could be due to the adaptive ability of the fungus resulting from the spread of fungal spores across fields and vegetative materials. Results of this study further revealed that *F. equiseti* clustered with other *Fusarium* species which according to O'Donnell et al. (2015) suggests *F. exquiseti* involved in amaranth diseases may be polyphyletic expressing close relatedness to different isolates.

5. Conclusion

The study identified that *Fusarium equiseti, F. solani, F. proliferatum,* and *F. oxysporum* were associated with amaranth wilt disease in the Semi-deciduous and Guinea Savannah agro-ecological zones of Ghana with *Fusarium equiseti* identified as the dominant *Fusarium* species in the study area. The work further showed variations in genetic, virulence and cultural characteristics among *Fusarium* isolates. Identification of multiple *Fusarium* species involved in amaranth wilt disease is newly reported in Ghana. It is recommended that a holistic approach is employed to manage *Fusarium* diseases in Amaranth.

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