

*Full Length Research Paper*

# Genetic diversity of *Fusarium* isolates infecting potato (*Solanum tuberosum*) and tobacco (*Nicotiana tabacum*) crops in Zimbabwe

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Potato (*Solanum tuberosum*) and tobacco (*Nicotiana tabacum*) are important crops that contribute significantly to Zimbabwe's economic development. However, they are attacked by *Fusarium* species leading to reduced yields, quality and profitability for farmers. A study was conducted to investigate the diversity of *Fusarium* spp. causing wilt and rots in both crops in Zimbabwe. Plants displaying symptoms of wilting, root and tuber rots were collected during nationwide disease surveys. *Fusarium* spp. were isolated and characterized morphologically, using pathogenicity tests and DNA sequencing targeting the internal transcribed spacer (ITS) region. Morphological characterization revealed the presence of *Fusarium* spp. that produced brown, grey, white and pink colonies on PDA. They also produced conidia of different shapes. All the *Fusarium* isolates induced some rotting, yellowing, and wilting in potatoes. Seven *Fusarium* spp., namely *Fusarium falciforme*, *Fusarium foetens*, *Fusarium fujikuroi*, *Fusarium perseae*, *Fusarium longifundum*, *Fusarium nygamai*, and *Fusarium chlamydosporum*, were identified through DNA sequencing. Furthermore, *F. falciforme* was most (41.67%) prevalent on tobacco, while *F. foetens* (25%) and *F. fujikuroi* (12.5%) were detected in both crops. This study highlighted the wide diversity of *Fusarium* spp. infecting potatoes and tobacco in Zimbabwe thus providing a base for developing management strategies against the pathogens. The 24 partial ITS sequences deposited in the GenBank database will be used in future studies to understand the diversity of *Fusarium* spp.

**Key words:** *Fusarium*, Internal-Transcribed-Spacer Region, mycotoxins, potato, tobacco, Zimbabwe.

## INTRODUCTION

The family Solanaceae contains economically important crops like tobacco (*Nicotiana tabacum* L.) and potato (*Solanum tuberosum* L.) that are widely grown in

Zimbabwe. In the 2021-2022 farming season, Zimbabwean farmers produced 211 million kilograms of flue-cured tobacco worth US\$650 million and over 475

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000 metric tonnes of potatoes worth over US\$390 million. However, several abiotic and biotic factors can significantly reduce the yield and quality of both crops. Amongst the biotic factors are ascomycetous fungi belonging to the genus *Fusarium* (Aoki et al., 2014).

*Fusarium* species' infection of potatoes and tobacco can result in root and tuber rots and plant wilting. In tobacco, the pathogen induces foliage yellowing that precedes wilting, while sudden wilting can occur without any foliage yellowing (Ntui et al., 2011). If *Fusarium*-infected potato tubers are planted, they can rot, leading to reduced crop stand in the field. Tuber rots can also occur in storage, giving rise to potato dry rot, a major postharvest disease worldwide (Stefanczyk et al., 2016). Some *Fusarium* spp. produce mycotoxins such as beauvericin, moniliformin, fumonisins, trichothecenes and zearalenone that can contaminate potatoes, making them unsuitable for human and livestock consumption (Nicholson et al., 2004; Wu, 2007; Mohd Zainudin et al., 2008; Stepien, 2013).

The genus *Fusarium* includes over 400 phylogenetically distinct species nested within 23 species complexes. Six species complexes, namely *Fusarium fujikuroi* species complex (FFSC), *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium oxysporum* species complex (FOSC), *Fusarium sambucinum* species complex (FSAMSC), *Fusarium solani* species complex (FSSC), and *Fusarium tricinctum* species complex (FTSC), are of particular interest to plant pathologists as they account for approximately 75% of the known genealogically exclusive phylospecies (O'Donnell et al., 2022). Some *Fusarium* spp. are highly pathogenic on crops while others are weak saprophytes (Peters et al., 2008).

The range and identity of *Fusarium* spp. that infect crops vary depending on geographical location and time of year (Dongzhen et al., 2020; Azil et al., 2021), with some highly pathogenic on crops and others acting as weak saprophytes (Peters et al., 2008). *F. oxysporum* f. sp. *nicotianae* is commonly reported in many tobacco-growing countries. In Michigan, USA, *F. oxysporum* was identified as the primary agent responsible for potato dry rot among 11 species (Gachango et al., 2012). The number of *Fusarium* spp. associated with potato dry rot is increasing globally, with at least 17 species reported by Dongzhen et al. (2020). This upsurge in reported species may stem from recent improvements in disease diagnostics, allowing for more precise identification and characterization of *Fusarium* spp. infecting crops worldwide.

In recent times, the incidence of *Fusarium*-induced disease cases has increased in Zimbabwe. According to records at the Kutsaga Research Station Plant Clinic, 45% of potato and tobacco plant samples brought in for diagnostics in the 2021-2022 farming season were infected with *Fusarium* spp. However, morphological characterization that is used for diagnoses at the clinic is time-consuming and limited. Therefore, robust DNA-

based studies are required for precise and speedy pathogen identification and characterization.

Molecular assays are a powerful tool for inter- and intra-species identification of fungal species (Pinaría et al., 2015; Chehri, 2016). Anonymous markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and single sequence repeats (SSR) (Baysal et al., 2010) and sequence-specific markers like the internal transcribed spacer (ITS), intergenic spacer (IGS), transposons and translation elongation factor-1 $\alpha$  (TEF-1 $\alpha$ ), are widely employed in fungal diagnostics. Spacer regions, such as ITS and IGS, are targeted to identify genetic variability of various *Fusarium* spp., and polymorphism in these regions is more stable and reliable than in anonymous markers (Gashgari and Gherbawy, 2013).

This study aimed at investigating the diversity of *Fusarium* spp. associated with wilts and rots in potato and tobacco crops in Zimbabwe, using morphological and pathogenicity assays alongside DNA sequencing targeting the ITS region. Accurate pathogen characterization is critical in developing and implementing effective disease management strategies for improved crop yield and quality.

## MATERIALS AND METHODS

### Pathogen isolation

*Fusarium* spp. were isolated from tobacco and potato plant samples that were collected during disease surveys and submissions made by growers to the Kutsaga Research Station Plant Clinic in the 2021-2022 farming season. Symptomatic plant materials consisting of stems, leaves, roots and tubers were surface sterilized with 1% sodium hypochlorite for 2 min, blot-dried on filter paper and plated on chloramphenicol-amended potato dextrose agar (PDA) media. After incubation at 24  $\pm$  2°C for 5 days, mycelial growth was observed, and pure *Fusarium* cultures obtained through sub-culturing. The cultures were stored on agar slants in a refrigerator until use. A total of 24 *Fusarium* isolates were used in this study.

### Morphological identification

The colours and patterns of fungal colonies that developed on PDA after 5 days of incubation were observed and recorded. Slide cultures of *Fusarium* isolates were prepared, and the mycelia and spores were examined under the ZEISS Primo Star compound microscope [Manufacturer, City/Country] at 400X magnification.

### Pathogenicity tests

#### *In vitro* pathogenicity test

The experiment was conducted following the procedure described by Gashgari and Gherbawy (2013). The study was designed as a one-factor experiment in a completely randomized design with six replications. Uniform-sized healthy-looking potato tubers (cultivar Diamond) were selected, washed in water to remove excess soil, and surface-sterilized in 1% sodium hypochlorite. The tubers were

then air-dried, and wounded with a sterile 5 mm diameter cork borer to a depth of 5 mm. Then, a 5 mm diameter agar plug containing actively-growing *Fusarium* isolates' mycelium was placed into the wound, and sealed with the excised plug of tuber tissue. The control treatment consisted of potato tubers that were inoculated with plain PDA. The inoculated tubers were wrapped in black polyethylene plastic and incubated at 24°C for three weeks. Disease development was measured by cutting the tubers longitudinally from the point of inoculation and measuring the depth of internal necrosis at 7, 14, and 21 days after inoculation.

#### **Preparation of *Fusarium* inoculum for in vivo pathogenicity tests**

To prepare conidial suspensions, conidia from *Fusarium* cultures grown on PDA for 21 days at 18°C were scrapped into sterile distilled water. Mycelia and spores were retrieved by aseptically scraping the PDA surface and transferred into flasks containing 200 ml sterile distilled water. The inoculum was sieved to remove the mycelia and the conidia present in the supernatant were adjusted to a concentration of  $10^6$  conidia ml<sup>-1</sup> by dilution.

#### **In vivo pathogenicity test**

Individual *Fusarium* isolates were evaluated for their pathogenicity on potato plants that were grown in 15 cm diameter plastic pots in a greenhouse. The experiment was designed as a one-factor experiment using a completely randomized design with five replications. Three weeks after emergence, plants were inoculated by pouring 5 ml of a fungal inoculum suspension ( $10^6$  conidia ml<sup>-1</sup>) into the soil at the base of the potato plants in the pots. The control treatment was inoculated with distilled water. Disease progress was assessed at two-week intervals for six weeks by counting and recording the number of yellowing leaves against the total number of leaves per plant. Additionally, for root disease progress assessment, plants were uprooted, and the tubers and roots were scored for discolorations, using the scale where: 0 = No visible disease symptom; 1 = Less than 15% of roots affected; 2 = 15-35% of roots affected; 3 = 36-49% of roots affected; 4 = 50-74% of roots affected; and 5 = more than 75% of roots affected as well as plants which no longer had any roots at all.

#### **Molecular characterization of *Fusarium* wilt isolates**

##### **Fungal DNA extraction**

DNA was extracted from the *Fusarium* isolates using the modified Cetyltrimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987). Firstly, a 3% CTAB solution was heated in a microwave for at least 2 min to dissolve it. Then, the solution was added into a 2 mL Eppendorf tube and vortexed rigorously. Thereafter, the contents were incubated in a shaking incubator for 1 h at 125 rpm and 65°C. Afterward, the tubes were centrifuged for 10 min at 12,500 rpm for 10 min to sediment the debris. The supernatant was collected and an equal volume of chloroform was added, followed by another centrifugation step. The aqueous phase was collected and, to it an equal volume of chloroform was added, followed by another centrifugation step at 12,500 rpm for 10 min. Next, 1% CTAB was added to the upper aqueous phase and incubated at ambient temperature for 1 h. The tubes were again centrifuged for 10 min at 12,500 rpm and the supernatant was discarded. The now visible pellet was treated with 1 M CsCl and isopropanol and incubated. The solution was centrifuged, and supernatant discarded. The pellet was then precipitated using

isopropanol, washed in 70% ethanol, and finally dissolved in ultra-pure water. DNA quality and quantity were assessed for using a spectrophotometer (BioDrop®, UK).

#### **ITS region amplification and sequencing of *Fusarium* isolates**

The genomic DNA samples were sent to Inqaba Laboratories (Pty) Ltd. (Pretoria, South Africa) for amplification of the ITS-rDNA region using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGTTATTGATATGC-3') (White et al., 1990). The amplification reaction was performed using 2 µl of DNA template, 5 µl of 10X Standard Taq Reaction Buffer, 3 µl forward primer, 3 µl reverse primer, 1 µl of 10 mM dNTPs, and 4 µl of Taq DNA Polymerase and 32 µl of nuclease-free water, in a total reaction volume of 50 µl. The cycling conditions were as follows: initial denaturation at 94°C for 2 min, followed by 40 cycles denaturation at 94°C for 30 s, annealing at 54°C for 1 min extension at 72°C for 2 min and a final extension step at 72°C for 7 min. A negative control was included in the PCR amplifications. The amplified fragments were analyzed by 1.5% agarose gel electrophoresis, and the amplicons were excised and purified using a Zymoclean™ Gel DNA Recovery Kit (Zymo Research, USA). The purified amplicons were then sequenced in both directions on the ABI Big Dye Terminator Sequencing Kit (v3.1) and read with an ABI13730xl capillary sequencer (Applied Biosystems, Foster City, CA, USA).

#### **Data analysis**

The lesion diameter depths of inoculated potatoes were analyzed using one-way analysis of variance (ANOVA) using Genstat. When significant differences were detected, means were separated using the least significant difference (LSD) at 5% probability level.

For molecular data, consensus sequences were generated from the forward and reverse sequences of the *Fusarium* isolates using the BioEdit software. The consensus sequence for each isolate was blasted into the MEGA 6.0 programme (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The nucleotide sequences of the *Fusarium* isolates reported in this study have been deposited into the GenBank under the accession numbers shown in Table 2.

The Maximum Likelihood method based on the Jukes-Cantor model (Jukes and Cantor, 1969) was used to infer the evolutionary history of isolates generated in this study. Partial ITS sequences of *Fusarium* spp. reported from other parts of the world were obtained from GenBank and used for comparison using MEGA 6.0. The data from a ClustalW alignment of the ITS were used as input for the phylogenetic tree construction using MEGA 6.0 software (Tamura et al., 2013). The bootstrap consensus tree inferred 1000 replicates were taken to represent the evolutionary history of the analyzed taxa. *Colletotrichum sojae* (NR 158358.1) was used as the outgroup.

## **RESULTS**

### **Morphological characteristics of the *Fusarium* isolates**

The *Fusarium* isolates produced different colony colours when cultured on PDA as shown in Figure 1a to f. Some isolates produced white mycelia with concentric rings. Others produced white, creamy white, pink, and gray colourations. The isolates had typically septate hyphal strands and produced canoe-shaped, rice-shaped, and

**Table 1.** Pathogenicity of *Fusarium* isolates, as measured by first and second assessments' means of the lesion depths (mm) of the inoculated potato tubers. The lesion depths were measured at 7, 14 and 21 days after inoculation.

Isolate	Lesion depth (mm) (mean $\pm$ SD)		
	1st assessment	2nd assessment	3rd assessment
Control	16.33 $\pm$ 3.37	17.53 $\pm$ 2.27	8.07 <sup>a</sup> $\pm$ 1.60
<i>Fusarium perseae</i>	13.67 $\pm$ 4.10	14.17 $\pm$ 3.13	13.10 <sup>ab</sup> $\pm$ 3.41
<i>Fusarium foetens</i>	14.27 $\pm$ 3.58	14.60 $\pm$ 0.12	14.80 <sup>bc</sup> $\pm$ 4.51
<i>Fusarium nygamai</i>	12.97 $\pm$ 4.35	13.67 $\pm$ 3.42	14.95 <sup>bc</sup> $\pm$ 1.62
<i>Fusarium longifundum</i>	12.60 $\pm$ 4.04	14.92 $\pm$ 2.17	16.80 <sup>bc</sup> $\pm$ 3.36
<i>Fusarium fujikuroi</i>	14.25 $\pm$ 3.13	14.38 $\pm$ 2.18	12.50 <sup>ab</sup> $\pm$ 2.05
<i>Fusarium falciforme</i>	18.40 $\pm$ 0.72	18.82 $\pm$ 0.10	19.60 <sup>c</sup> $\pm$ 2.27
<i>Fusarium chlamyosporum</i>	11.68 $\pm$ 5.71	13.33 $\pm$ 4.52	8.47 <sup>a</sup> $\pm$ 2.12
P- value	0.529	0.467	0.004
S.E.D.	2.90	2.61	2.49
LSD	6.00	6.38	5.17
CV (%)	24.80	20.08	22.00

\*Lesion depths followed by the same letter in a column are not statistically different at  $p = 0.05$ .

bullet-shaped conidia (Figure 1g to i).

## Pathogenicity tests

### In vitro tests

Some rotting were observed in the potato tubers that were inoculated with PDA plugs containing mycelia of different *Fusarium* isolates (Figure 2). No significant differences ( $p = 0.529$ ) were observed in the lesion depths of the potato tubers in the first and second assessments. Significant differences ( $p = 0.004$ ) were observed in the third assessment with *Fusarium chlamyosporum* (8.47  $\pm$  2.12 mm) having the smallest lesion depth while *F. falciforme* (19.6  $\pm$  2.27 mm) had the biggest lesion depth (Table 1).

### In vivo tests

After inoculation with the fungal isolates, the potato plants were observed daily to follow disease progress. There were no significant differences ( $p > 0.05$ ) in disease development in the potato plants that were inoculated with the *Fusarium* isolates, as measured by the amount of wilting and yellowing of leaves. Symptoms of wilting and leaf yellowing were first observed on the lower leaves of the plant at 12 days after inoculation. In some plants, the wilting and yellowing occurred on one side of the plant. As the disease progressed, there was leaf curling (Figure 3). By the fifth week (that is, three weeks after inoculation), most potato plants had wilted and died. The control plants showed no symptoms of wilting and/or yellowing.

## Molecular characterization of *Fusarium* isolates obtained from tobacco and potato plants in Zimbabwe

Blastn analysis revealed that ten isolates (41.67%) were most closely related to *F. falciforme* CBS 475.67 (NR\_164424). Another 6 isolates (25%) shared at least 99.57% nucleotide sequence identity to *F. foetens* (NR\_159865) from the Netherlands. Three isolates (FOX003 ITS1, FOX005\_Nyanga and FOX016\_Marondera) shared 100% nucleotide sequence identity to *F. fujikuroi* (NR\_111889), a rice-infecting isolate. Isolates FOX009\_Msasa and FOX012\_Msasa were at least 99.3% related to *F. chlamyosporum* var. *chlamyosporum* from Honduras (NR\_172283). Isolate FOX006\_Nyanga was most closely related (99%) to *Fusarium perseae* from Italy while isolate FOX011\_Kutsaga was most closely related (99.3%) to *Fusarium nygamai* (NR\_130698) (Table 2).

## Phylogenetic analysis

Phylogenetically, the sequences of the *Fusarium* isolates were grouped into four distinct clusters, A-D. Cluster A was made of *F. fujikuroi* and *F. nygamai* isolates while Cluster D had *F. falciforme*, and *F. perseae*. *F. chlamyosporum* was in cluster B while *Fusarium foetens* was in cluster C (Figure 4).

## DISCUSSION

Pathogen characterization is necessary to help understand the biology of a pathogen prior to the

**Table 2.** Identity of the *Fusarium* isolates recovered infecting potato and tobacco in Zimbabwe.

Isolate ID	Host plant	Accession number	Isolate identity
FOX001_ITS1	Potato	OP445592	<i>F. foetens</i>
FOX002_Burma	Tobacco	OP458568	<i>F. foetens</i>
FOX003_ITS1	Tobacco	OP473984	<i>F. fujikuroi</i>
FOX004_Banket	Potato	OP474046	<i>F. foetens</i>
FOX005_Nyanga	Potato	OP474047	<i>F. fujikuroi</i>
FOX006_Nyanga	Potato	OP474048	<i>F. perseae</i>
FOX007_Nyanga	Potato	OP474049	<i>F. foetens</i>
FOX008_Kutsaga	Potato	OP474050	<i>F. foetens</i>
FOX009_Msasa	Potato	OP474051	<i>F. chlamydosporum</i>
FOX010_Mhangura	Potato	OP474052	<i>F. foetens</i>
FOX011_Kutsaga	Tobacco	OP474054	<i>F. nygamai</i>
FOX012_Msasa	Potato	OP474053	<i>F. chlamydosporum</i>
FOX013_Burma 2	Tobacco	OQ423103	<i>F. falciforme</i>
FOX014_Headlands	Tobacco	OQ423104	<i>F. falciforme</i>
FOX015_Rusape	Tobacco	OQ423105	<i>F. falciforme</i>
FOX016_Marondera	Tobacco	OQ423106	<i>F. fujikuroi</i>
FOX017_Trelawney	Tobacco	OQ423107	<i>F. falciforme</i>
FOX018_Mhangura	Tobacco	OQ423108	<i>F. falciforme</i>
FOX019_Chinhoi	Tobacco	OQ423109	<i>F. longifundum</i>
FOX020_Mvurwi	Tobacco	OQ423110	<i>F. falciforme</i>
FOX021_Shamva	Tobacco	OQ423111	<i>F. falciforme</i>
FOX022_Mazowe	Tobacco	OQ423112	<i>F. falciforme</i>
FOX023_Centenary	Tobacco	OQ423113	<i>F. falciforme</i>
FOX024_Guruve	Tobacco	OQ423114	<i>F. falciforme</i>

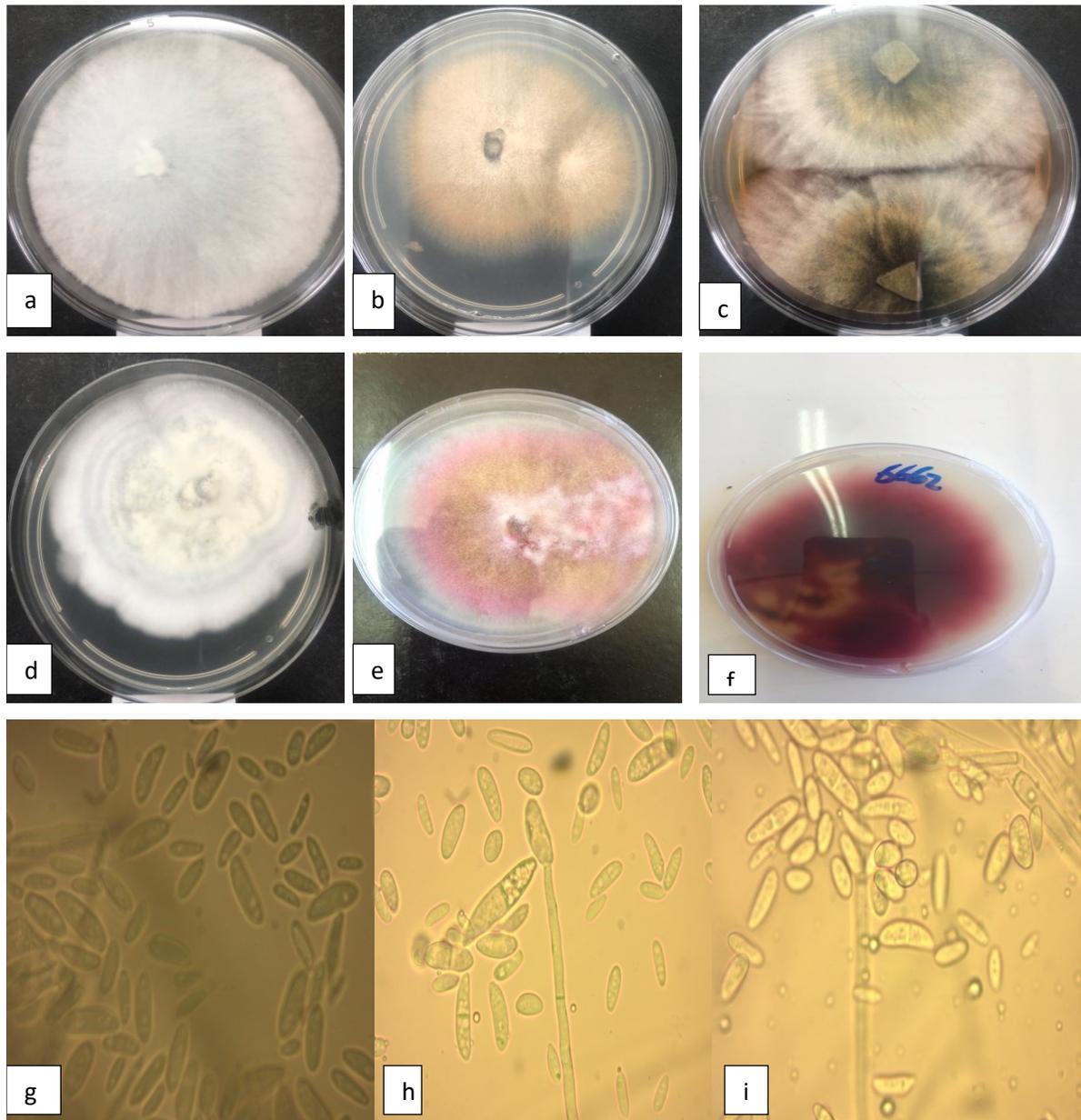
implementation of any disease management strategies. This study was undertaken to characterize *Fusarium* spp. that are associated with wilts and rots of potatoes and tobacco in Zimbabwe. Seven *Fusarium* spp. were reported, with *F. falciforme* dominant in tobacco, while *F. foetens* and *F. fujikuroi* were detected in both potatoes and tobacco. To the best of our knowledge, this is the first time that these seven *Fusarium* spp. have been reported infecting tobacco and potato crops in Zimbabwe. Prior to this study, only *F. oxysporum* f. sp. *nicotianae* and *F. solani* had been reported infecting tobacco while *Fusarium culmorum*, *F. oxysporum*, *Fusarium vasinfectum*, and *F. solani* were reported infecting potato in Zimbabwe (Masuka et al., 1998). This study increased our knowledge of the range of fusaria that infect potatoes and tobacco in Zimbabwe. In addition, it provides protocols that can either be adapted or adopted for use in *Fusarium* characterization. In this study, the ITS region which has been identified as the standard marker for fungal identification (Iwen et al., 2002; Badotti et al., 2017), was utilized to identify the Zimbabwean *Fusarium* isolates.

*F. falciforme* has been reported infecting many crops in different parts of the world. It is an emerging pathogen that has been reported to cause wilt of chrysanthemum in Vietnam (Thao et al., 2021), root rot of chickpea,

cucurbits, tobacco, tomato, and soybean (Vega-Gutierrez et al., 2019; Qiu et al., 2020, 2022; Velarde Felix et al., 2021; Li et al., 2022; Xu et al., 2022), and postharvest fruit rot in watermelon (Balasubramaniam et al., 2023). *F. falciforme* is a trans-kingdom fungal pathogen that has also been reported infecting humans (Edupuganti et al., 2011; Ma et al., 2013). Given that *F. falciforme* is a generalist pathogen, there is high likelihood that it could spread and infect other crops in Zimbabwe and potentially cause serious yield losses.

*F. foetens* was first reported infecting begonia in the Netherlands (Schroers et al., 2004), but has also been reported infecting tea (*Camellia sinsensis*) and rooibos (*Aspalathus linearis*) in South Africa (Lamprecht and Tewoldemedhin, 2017). *F. fujikuroi*, a primary pathogen of cereals such as rice, barley, maize, millet and sugarcane (Hsuan et al., 2011), also infects soybean (Qiu et al., 2020). Both *F. foetens* and *F. fujikuroi* are mycotoxins producers (González-Jartín et al., 2019; Zakaria, 2023). The infection of potato and tobacco by these fungi poses potential health hazards to consumers.

Phylogenetic analysis revealed that the *Fusarium* spp. reported in this study belong to four species complexes: *F. fujikuroi* species complex (FFSC), *F. chlamydosporum* species complex (FCSC), *F. oxysporum* species complex (FOSC), and *Fusarium solani* species complex (FSSC).



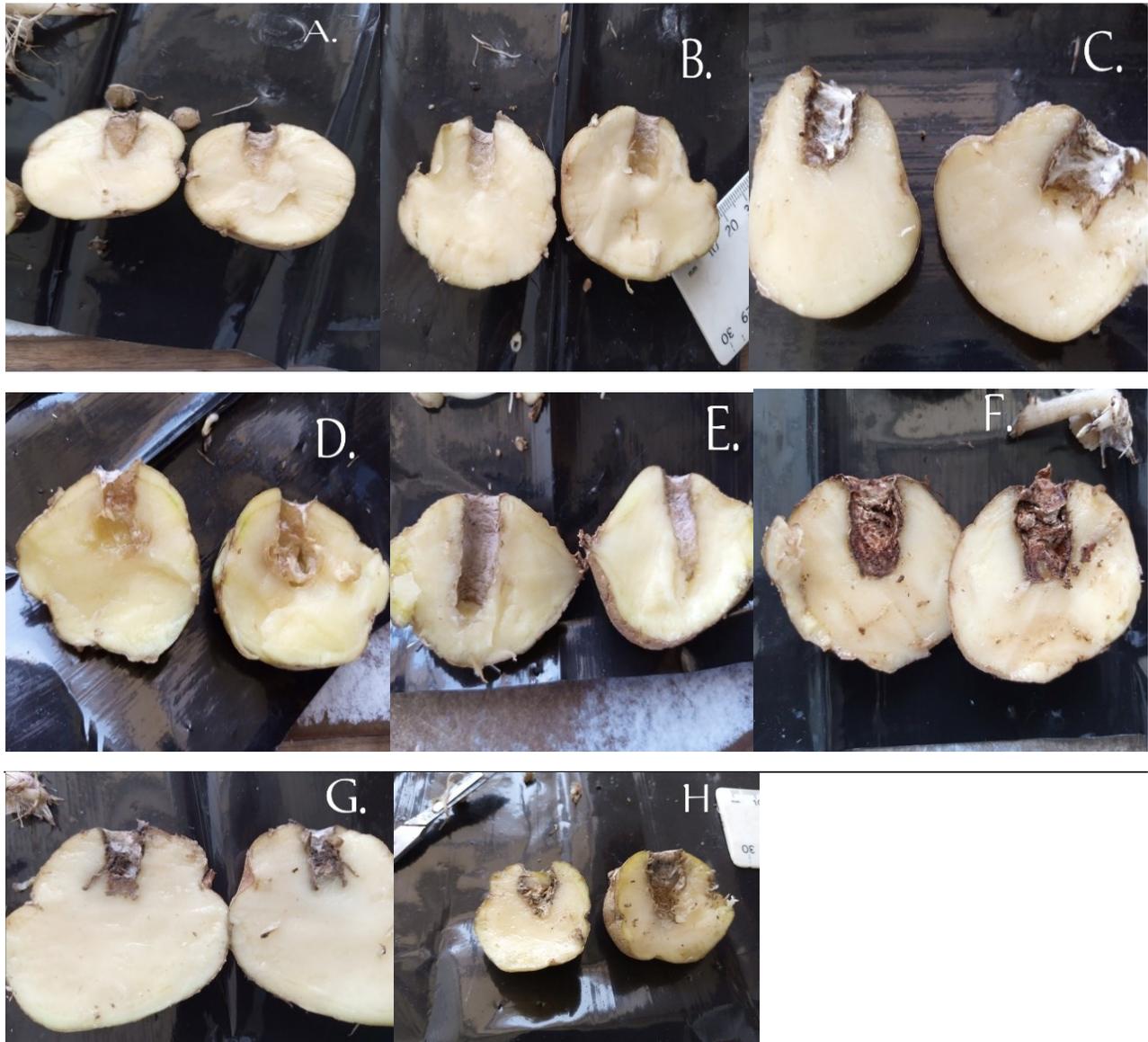
**Figure 1.** Morphological and cultural characteristics used in the characterization of 24 *Fusarium* isolates obtained from potato and tobacco plants. The isolates produced different coloured colonies (a-f) on PDA. There were also different growth patterns as exemplified by concentric rings (d). The *Fusarium* spp. also produced different macroconidia (g-i).

The FFSC, one of the biggest complexes with 65 accepted species (Yilmaz et al., 2021), was represented by *F. fujikuroi* and *F. nygamai*. The FCSC was represented by *F. chlamydosporum*. This species complex is one of the smallest till date, with 9 accepted species (Lombard et al., 2019). Both *F. foetens* and *Fusarium lognifundum* are members of the FOOSC (Navale et al., 2023) while *F. falciforme* and *F. perseae* belong to the FSSC (Gleason et al., 2020).

The detection of these fusaria on potato and tobacco could be evidence of an expanding host range for the

pathogens in the country. It could also be evidence of improved diagnostics as DNA sequencing was used for the first time ever in studying *Fusarium* wilt and rot pathogens of tobacco and potato in Zimbabwe. Molecular assays, though expensive, offer quick and unambiguous identification of pathogens (Luchi et al., 2020). This is critical if effective disease management strategies are to be developed and implemented.

Some of these newly-reported pathogens were probably introduced into Zimbabwe through plant material imports. In the past two decades, Zimbabwe has been



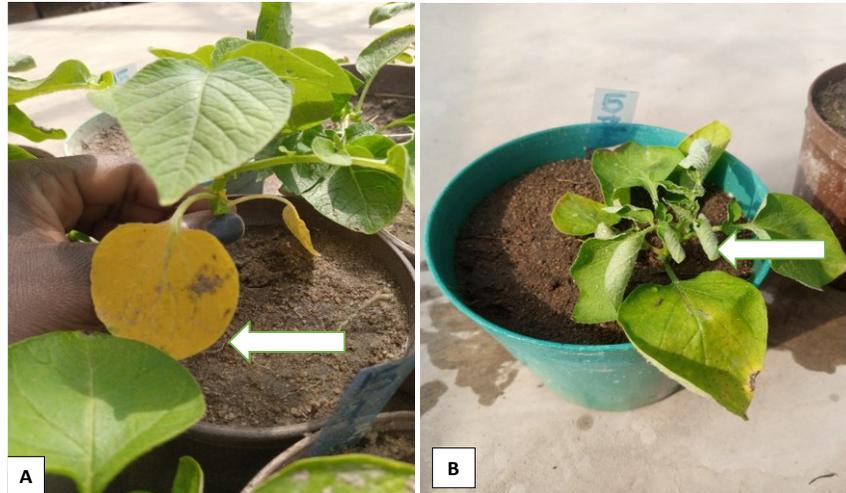
**Figure 2.** Response of potato tubers when inoculated with different *Fusarium* isolates. Potato tubers were inoculated with PDA plugs containing mycelium of A (control- sterile PDA plug), B (*F. nygamai*), C (*F. foetens*), D (*F. perseae*), E (*F. fujikuroi*), F (*F. chlamydosporum*), and G (*F. falciforme*).

importing potatoes from neighbouring countries, most notably South Africa, to meet local demand. A pathogen like *F. foetens* which has been reported on tea and rooibos in South Africa (Lamprecht and Tewoldenedhin, 2017) could have jumped hosts to infect potatoes which were then imported into Zimbabwe. Inadequate screening of imported plant materials at the country's ports of entry and smuggling of agricultural commodities could have aided pathogen introduction into Zimbabwe.

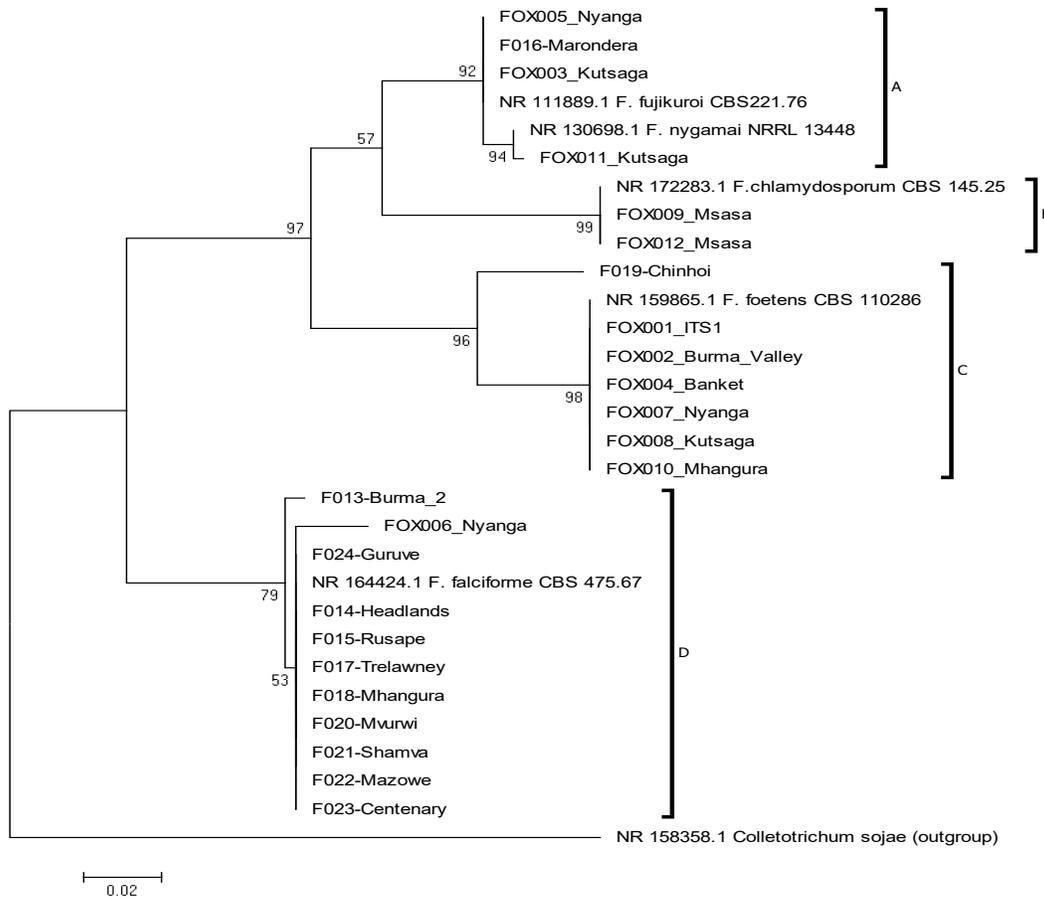
The pathogenicity tests confirmed the capacity of the isolates to cause disease in potatoes. However, the isolates did not show significant differences in pathogenicity in the *in vitro* tests. The severity of *Fusarium*

diseases is conditioned by various factors, including the climate conditions, cultural practices and host plant physiology (Trabelsi et al., 2017). Different host plants represent different selective environments for fungal pathogens and could result in functional trade-offs that would limit the general fitness of the pathogen in the host (Sacristan and Garcia-Arenal, 2008).

This study showed that *Fusarium* has wide diversity in Zimbabwe. This corroborates findings by other researchers on the diversity of *Fusarium* in the world (Singha et al., 2016). Numerous partial ITS sequences are currently present in databases and are regularly used to identify different species of fungi. This study added 24



**Figure 3.** Potato plants showing symptoms of hemiplegic yellowing (A), leaf curling and wilting of the lower leaves (B). These were the common symptoms in all the plants that were inoculated with the *Fusarium* isolates in this study.



**Figure 4.** Phylogenetic analysis of *Fusarium* isolates obtained from Zimbabwe. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. The numbers shown next to branches indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. The analysis was performed with 30 sequences. Evolutionary analyses were conducted in MEGA 6.

partial ITS sequences to these databases for use in future studies to understand the diversity of *Fusarium* spp.

## Conclusion

This study identified *F. chlamydosporum*, *F. falciforme*, *F. foetens*, *F. fujikuroi*, *F. longifundum*, *F. nygamai*, and *F. persea* infecting tobacco and potato in Zimbabwe. *F. falciforme* was reported predominantly infecting tobacco while *F. foetens* and *F. fujikuroi* infected both crops. Knowing the range and diversity of these pathogens is an important step in developing effective disease management strategies against diseases infecting both tobacco and potatoes.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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