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Diversity of *Gibberella fujikuroi* Species Complex Isolated from Maize Produced in Uganda

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ABSTRACT

Fusarium verticillioides (*Gibberella moniliformis*, *Gibberella fujikuroi* mating population A) and *Fusarium proliferatum* (*Gibberella intermedia*, *G. fujikuroi* mating population D) are the major *Fusarium* species contaminating maize worldwide. Occurrence of such species in maize is of great concern due to their ability to produce fumonisins. In this study, we report the diversity of the *G. fujikuroi* species isolated from maize produced in Uganda. Overall, 26 strains belonging to *G. fujikuroi* complex were isolated. The isolates were matched with 9 strains in the NCBI *Fusarium* ID database and formed 4 clusters with seven distinct phylogenetic groups. Majority of the isolates (52%) were identified as *G. moniliformis*. Only, 16% of the isolates were identified as *F. proliferatum*. Six isolates morphologically identified as either *F. verticillioides* or *F. proliferatum* could not be identified to species level. Wide intraspecific variability was observed amongst the strains. *G. moniliformis* exhibited the highest level of intraspecific variability evidenced by formation of 5 distinct groups. Phylogenetic analysis did not show any relationship between clusters/groups and geographical origin. The findings of this study clearly indicated that maize produced in Uganda is infected with strains of *G. fujikuroi* species complex which are highly diverse. The results provide important information for further understanding the relationship between the occurrence of *G. fujikuroi* strains and fumonisin contamination in maize produced in Uganda.

Key words: Maize, staple food, *Gibberella fujikuroi*, *Fusarium*, mycotoxins

INTRODUCTION

Species of the *Gibberella fujikuroi* complex most especially *F. verticillioides* (teleomorph *Gibberella moniliformis*) and *F. proliferatum* are the most common *Fusarium* species associated with maize worldwide (Bush *et al*., 2004; Mukanga *et al*., 2010; De Oliveira Rocha *et al*., 2011). The species have been isolated in maize and maize based products in USA, Europe and South America (Shephard *et al*., 1996; Marasas, 2001; Rocha *et al*., 2009). Studies in some African countries like South Africa (Marasas *et al*., 1988), Benin (Fandohan *et al*., 2005), Kenya (Kedera *et al*., 1999), Ghana (Kpodo *et al*., 2000), Zimbabwe (Gamanya and Sibanda, 2001) and Zambia (Mukanga *et al*., 2010) also reported *F. verticillioides* as the most frequent *Fusarium* specie isolated from maize.

Occurrence of *Fusarium* species in maize and maize products has both economic and health implications. Infection of maize with *Fusarium* species results in reduced yield per acre, reduced grain quality (Wagacha and Muthomi, 2008) and loss in nutritional value of the kernels (Marn *et al.*, 1999). With respect to health, some *Fusarium* species like *F. verticillioides* and *F. proliferatum* have been reported to produce fumonisins which have been associated with several animal and human illnesses (Miller, 2008; Shephard, 2008).

The effects of maize infection with *Fusarium* species and subsequent contamination with fumonisins have attracted much attention in most maize producing countries. In Uganda, very little work has been done despite the importance of maize as a staple for most people in the country. The few studies conducted have only isolated *Fusarium* species from maize (Kaaya and Kyamuhangire, 2006; Bigirwa *et al.*, 2007) with no further attempt to determine the diversity of *Fusarium* species that have been associated with fumonisin production. The aim of this study was to explore the diversity of *G. fujikuroi* species complex associated with freshly harvested maize produced in Uganda. This is the first detailed study focusing on occurrence of *G. fujikuroi* species complex in maize produced in Uganda.

MATERIALS AND METHODS

Study area: The study covered the three major maize producing agro-ecological zones of Uganda namely; the High altitude, Mid altitude (moist) and Mid altitude (dry). The high altitude zone is situated in the mountainous districts of Eastern Uganda. Some of the districts in this zone lie at an altitude of 1650-5000 m above sea level whereas those located in the mountain ranges are more than 5000 m above sea level (Kikafunda-Twine *et al.*, 2001). The High altitude agro-ecological zone has a sub-tropical climate with temperatures ranging between 12-24°C and a unimodal type of rainfall varying between 920-1650 mm annually (Rwabwogo, 2008). It is the most humid zone (relative humidity 86-90%) and has only one maize growing season which starts in March and ends in October each year (Kikafunda-Twine *et al.*, 2001). The Mid altitude (moist) and Mid altitude (dry) agro-ecological zones lie between 900 and 1500 m above sea level (Kikafunda-Twine *et al.*, 2001). Both zones have a bimodal type of rainfall and two maize production seasons. The first season begins in March and ends in July whereas the second season starts in September and ends in January. The Mid altitude (moist) receives 900-1200 mm of rain annually. The relative humidity in this agro-ecological zone varies from 68 to 75% while the average temperature is 25°C. The Mid altitude (dry) receives 875-1600 mm of rain annually with a relative humidity of 60-65%. This zone is characterised by high temperatures of up to 30°C and a long dry season of up to five months in a year (Rwabwogo, 2008).

Sampling procedure: The criteria for selection of farmers who participated in the study followed the method described by Kaaya and Kyamuhangire (2006) with some modifications to suit the purpose of the study. The modifications included: (1) increasing the sample size from 40 to 80 farmers per agro-ecological zone and (2) selecting only farmers with maize that had been harvested two days preceding the survey. In each agro-ecological zone, two major maize producing districts were purposively selected. These included Masindi and Mubende from the mid altitude (moist) zone, Kasese and Nakasongola from the mid altitude (dry) zone and Kapchorwa and Sironko from the high altitude zone. In each district, 40 farmers with maize harvested in the previous two days were randomly selected. Thus, a total of 80 farmers were selected in each agro-ecological zone giving a total of 240 farmers.

During sampling, freshly harvested maize was either found at the farmers' drying yards or in cribs (for farmers in Sironko and Kapchorwa where, maize is directly taken to the cribs after harvesting). In both cases, a sample was obtained by randomly picking 5 cobs of almost same size from different locations. Forty samples were collected from each district thus giving a total of 240 samples for analysis. The samples were packaged in perforated polythene bags and transported within 24 h to the Microbiology Laboratory at the Department of Food Technology and Nutrition, Makerere University and kept at -18°C until analysis.

Isolation of *Fusarium* species: Maize cobs in each sample were hand-shelled and thoroughly mixed. One hundred kernels were randomly picked from each sample and surface sterilised in 3.5% sodium hypochlorite (JIK) solution for 1 min. The sterilised kernels were rinsed twice in distilled water and plated (5 kernels per petri dish) on Malt Extract Agar (MEA) (Difco, Detroit MI). The plates were incubated at 25°C for 5-7 days (Rheeder *et al.*, 1992). The incidence (% of kernels infected with *Fusarium* spp.) and occurrence (% of samples infected with particular *Fusarium* spp.) were determined as recommended by Fandohan *et al.* (2005).

Identification of *Fusarium* species

Morphological identification: Suspected *Fusarium* colonies were single spored and transferred to Potato Dextrose Agar (PDA) (Difco, Detroit MI) and Carnation Leaf Agar (CLA) (*Fusarium* Research Centre, Pennsylvania State University, USA) for further identification. The plates were incubated at 25°C under a combination of cool white fluorescent and "black" near-UV lights for 14-21 days (Rheeder *et al.*, 1992). The species were identified based on morphological features according to the available keys and manuals (Booth, 1971; Nirenberg, 1989; Burgess *et al.*, 1994). Twenty six strains that showed characteristics similar to those belonging to the fumonisin producing spp (*G. fujikuroi* complex) were further subjected to molecular analysis. These isolates were lyophilised and deposited at the culture collection of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council (MRC), South Africa for future reference.

Molecular identification: Genomic DNA extractions of the 26 isolates that were identified as either *F. verticillioides* or *F. proliferatum* (members of the *G. fujikuroi* complex) were performed from scrapings of pure isolates using the ZR Fungal/Bacterial DNA kit (Zymo Research, Irvine, California, USA) according to manufacturer's instructions. The Internal Transcribed Spacer (ITS) region of the genomic DNA was amplified with primers ITS-1 (TCCGTAGGTGAACCTGCGG) and ITS-4 (TCCTCCGCTTATTGATATGC). Amplification was performed in a GeneAMP PCR System 9700 series thermo cyler (Applied Biosystems, Foster City, Califonia, USA) programmed for initial denaturation at 94°C for 5 min followed by 50°C for 30 sec, 72°C for 1 min, 44 cycles of 30 sec at 94°C and a final extension of 10 min at 72°C. Individual PCR amplicons were purified using exonuclease and shrimp alkaline phosphatase (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, United Kingdom). The sequence of each individual isolate was determined using primer ITS 4 and Bigdye™ (ABI) Terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA) according to the manufacture's specifications. The labeled fragments were purified using the ZR-96 DNA sequencing clean up kit (Zymo Research, Irvine, California, USA) according to manufacturer's specifications. The purified, labelled fragments were then separated on an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, Califonia, USA). The sequences were

aligned using CLC Bio software (Aarhus, Denmark) followed by visual inspection and manual adjustment. The sequences were aligned to the entire *Fusarium* ID database located at National Centre for Biotechnology Information (NCBI) using BLAST software to search for similarities. The species with the highest percentage of homology to the sequence of each isolate were recorded.

Phylogenetic analysis: DNA alignments of all the 26 isolates were assembled using the CLC Bio software (Aarhus, Denmark). The alignments were used to construct a maximum likelihood tree using the HKY85 model. The support of individual nodes was assessed by bootstrap analysis of 1000 replicates. The phylogenetic distance was defined as the probability of nucleotide substitutions per site.

Statistical analysis: SPSS for windows version 15 (SPSS, Chicago IL) was used to analyse the data. Means of fungal incidence and occurrence were compared by Analysis of Variance (ANOVA). The p-values ≤0.05 were considered significant.

RESULTS

Incidence of *Fusarium* species in maize produced in Uganda: Table 1 shows the incidence of *Fusarium* species in freshly harvested maize obtained from farmers in six major maize producing districts of Uganda. The results indicated a high incidence of *Fusarium* species in maize from all the districts. The mean incidence of *Fusarium* varied between 91.0 and 99.2%in all the districts. Sironko district had the highest mean *Fusarium* incidence (99.2%) followed by Kapchorwa (99.0%) while Kasese district had the least mean *Fusarium* incidence (91.0%). *Fusarium* incidence was significantly higher ($p<0.05$) in Mubende, Kapchorwa and Sironko districts than Kasese, Masindi and Nakasongola.

Occurrence of *Fusarium* species in freshly harvested maize: Results of the occurrence of *Fusarium* species in freshly harvested maize are presented in Table 2. Overall, 9 *Fusarium* species; *F. verticillioides*, *F. proliferatum*, *F. graminearum*, *F. solani*, *F. culmorum*, *F. clamydosporum*, *F. fugikuroi*, *F. pseudonygamai* and *F. equiseti* were isolated. The *Fusarium* species most frequently isolated from freshly harvested maize were *F. verticillioides*, *F. graminearum*, *F. proliferatum* and *F. culmorum*. All the maize samples (100%) obtained from the Masindi, Sironko and Kapchorwa districts were infected with *F. verticillioides* (Table 2). The lowest occurrence (89%) of *F. verticillioides* was recorded in Nakasongola district. *F. proliferatum* was only isolated from

Table 1: Incidence of *Fusarium* species in freshly harvested maize from the three agroecological zones of Uganda

		% Infected kernels	
Agro-ecological zone	District	Range	Mean[a]±SE
Mid altitude (dry)	Kasese	60-100	91.0[a]±2.01
	Nakasongola	48-100	94.3[a]±1.52
Mid altitude (moist)	Masindi	40-100	94.4[a]±2.03
	Mubende	65-100	97.3[b]±1.20
High altitude	Kapchorwa	68-100	99.0[b]±0.82
	Sironko	84-100	99.2[b]±0.47

[a]Values are mean percentages (± standard error) of infected kernels from each district. Means with different superscripts are significantly different ($p<0.05$)

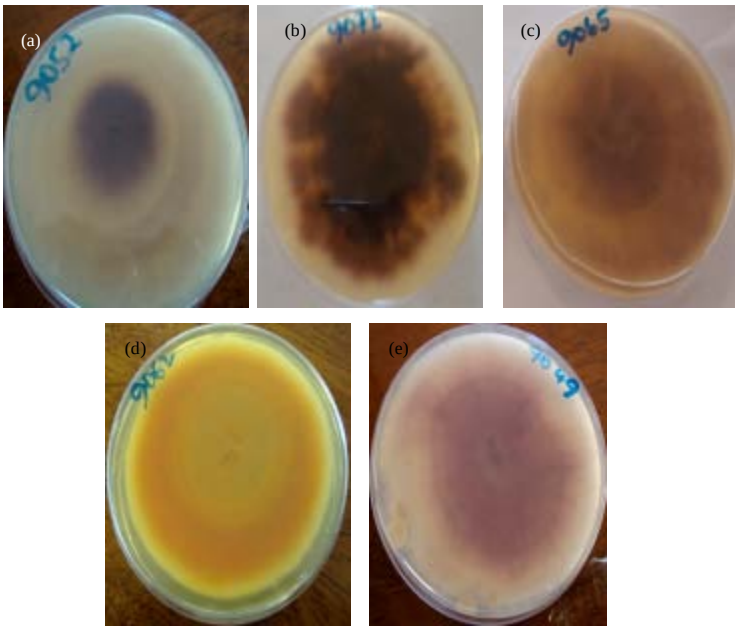

Fig. 1 (a-e): Reverse colour of selected *Fusarium* strains on PDA: (a) Typical *G. moniliformis* (GU257904.1) purple in the centre with well defined concentric rings, (b) *G. moniliformis* (99% identical to GU257904.1) dark purple in colour with no defined concentric rings, (c) *G. moniliformis* (FJ54074.1) light purple with concentric rings (d) *F. proliferatum* (GU066714.1) with purple orange colour and (e) unidentified *Fusarium* species, purple in colour with concentric ring isolated from maize obtained from Kapchorwa, Mubende, Masindi, Sironko and Kasese districts

Table 2: Occurrence of *Fusarium* species in freshly harvested maize from the three agroecological zones of Uganda

	Occurrence (%)					
	Mid altitude (dry)		Mid altitude (moist)		Highland	
Species	Kasese	Nakasongola	Masindi	Mubende	Kapchorwa	Sironko
F. verticillioides	93	89	100	95	100	100
F. proliferatum	nd	51	73	nd	0	48
F. graminearum	12	44	4	31	42	43
F. culmorum	15	19	0	25	0	40
F. clamydosporum	nd	nd	18	10	5	5
F. solani	nd	34	nd	nd	13	26
F. equiseti	nd	nd	nd	nd	10	nd
F. pseudonygamai	nd	5	nd	nd	nd	nd
F. fugikuroi	nd	16	nd	nd	nd	nd
Others[b]	5	5	10	10	15	10

nd: Not detected. [b]*Fusarium* species which could not be identified to species level

maize samples obtained from Masindi, Nakasongola and Sironko with occurrence levels of 73, 51 and 48%, respectively. *F. fujikuroi* was only isolated from 16% of the maize samples obtained from Nakasongola district.

Table 3: Morphological features of strains of *Gibberella fujikuroi complex* isolated from maize produced in the three agroecological zones of Uganda

Agro ecological zone	District	Isolate No.	Colony colour	Microconidia	Microconidia chains	Macroconidia	Monophialides	Polyphialides
Mid altitude (dry)	Kasese	MRC 9048	White/purple	+	+	-	+	-
		MRC 9049	White/purple	+	+	-	+	+
		MRC 9050	White/purple	+	+	+	+	-
		MRC 9051	White/purple	+	+	-	+	-
	Nakasongola	MRC 9054	White/purple	+	-	-	+	-
		MRC 9055	White/purple	+	+	-	+	-
		MRC 9056	White/purple	+	+	+	+	-
		MRC 9057	White/purple	+	+	-	+	-
		MRC 9058	White/Purple	+	-	+	+	+
		MRC 9059	White/purple	+	-	-	+	+
Mid altitude (moist)	Masindi	MRC 9063	White/Purple	+	-	-	+	-
		MRC 9064	White/purple	+	+	+	+	+
		MRC 9065	White/purple	+	-	+	+	-
		MRC 9066	White/Purple	+	+	-	+	-
		MRC 9067	White/purple	+	-	-	+	-
		MRC 9068	White/Purple	+	-	+	+	-
		MRC 9069	White/Purple	+	+	-	+	-
		MRC 9071	White/Purple	+	+	-	+	+
		MRC 9073	White/Purple	+	+	-	+	+
	Mubende	MRC 9070	White/Purple	+	+	-	+	-
		MRC 9072	White/Purple	+	+	-	+	-
High altitude	Kapchorwa	MRC 9052	White/purple	+	+	-	+	-
		MRC 9053	White/orange	+	-	+	+	-
	Sironko	MRC 9060	White/Purple	+	+	-	+	-
		MRC 9061	White/Purple	+	+	-	+	-
		MRC 9062	White/Purple/ Orange	+	+	+	+	+

MRC: Medical Research Centre, + indicates presence of the morphological feature, - indicates the absence of the feature

Morphological characteristics of *G. fujikuroi* species isolated from freshly harvested maize: The different morphological features of the strains of *G. fujikuroi* complex isolated from freshly harvested maize are presented in Table 3. All the isolates exhibited the morphological features characteristic of species in the section *Liseola*. Colonies on PDA generally had white/purple aerial mycelium interspersed with rings of different shades of purple/white colours. The reverse colony colours ranged from light to dark purple and/or orange/yellow as indicated in Fig. 1a-e. Globular or fusiform microconidia with 0-2 septate, mesoconidia with 0-3 septate and falcate to cylindrical macroconidia with 3-5 septate were observed. Microconidia were abundant forming false heads in some strains.

Of the 26 strains, 18 formed microconidia chains on Carnation Leaf Agar (Table 3). Conidia were born on both mono and polyphialides (Fig. 2c, d). Only 8 strains formed macroconidia on CLA (Table 3) with varying sizes and shapes (Fig. 2e, f). Only *F. proliferatum* species formed polyphialides (Fig. 2d). No clamydospores were observed amongst all the strains.

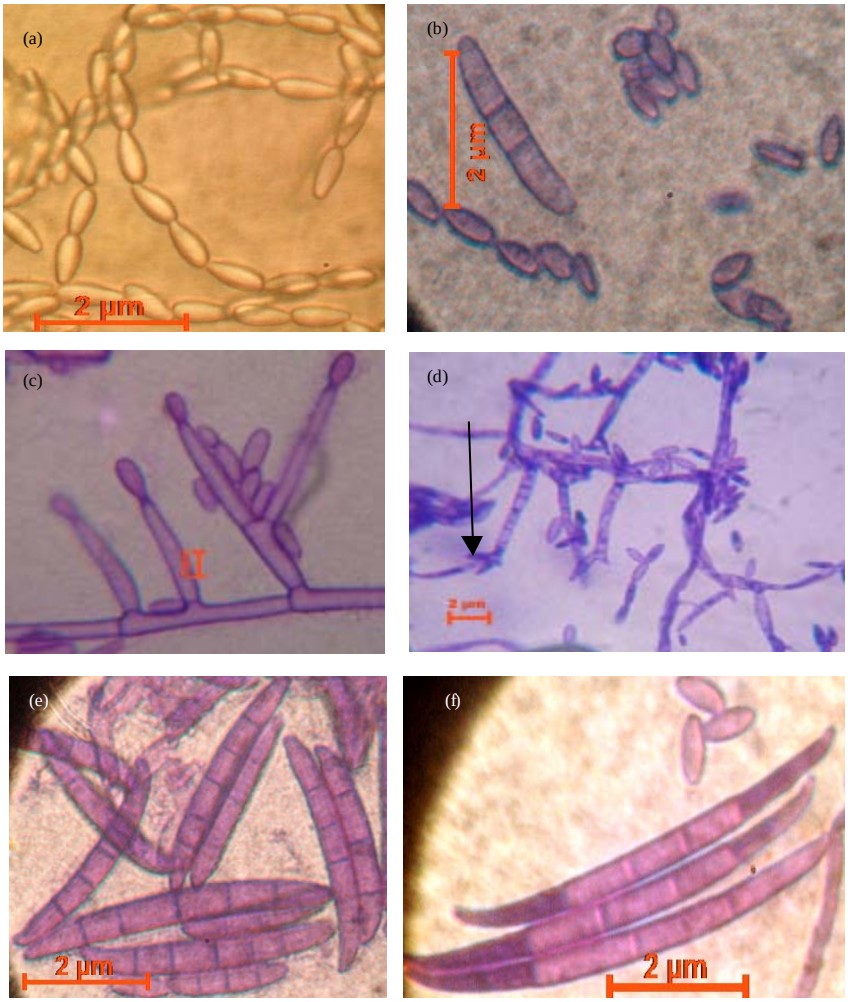

Fig. 2 (a-f): Distinguishing morphological features of *Fusarium* species isolated from freshly harvested maize: (a) Microconidia chains, (b) mesoconidia with 2 septa, (c) conidiophores in aerial mycelium, (d) polyphialides and (e, f) macroconidia with different shapes and sizes

Molecular characterisation of *G. fujikuroi* species isolated from freshly harvested maize: Molecular characteristics of the 26 isolates as determined by partial DNA sequencing of the ITS-4 primer and aligning to the entire NCBI database are indicated in Table 4. The isolates were matched with 9 strains in the NCBI *Fusarium* ID database with nucleotide identities ranging from 98 to 100% with the exception of strain MRC 9051 which recorded the lowest identity of 91% with an unknown *Fusarium* specie accession number GQ141219.1. Thirteen (13) isolates were identified as *G. moniliformis* which is a teleomorph of *F. verticillioides*. Three isolates (MRC 9067, MRC 9069 and MRC 9052) showed 100% nucleotide identity with a strain of *G. moniliformis* (Accession number GU257904.1). Four strains (MRC 9059, MRC9071, MRC9073 and MRC9062) were identified as *F. proliferatum* (accession number GU066714.1) with a nucleotide identity of 99% each. These strains were only isolated from maize collected from the districts of Nakasongola,

Table 4: ITS-4 primer based molecular characterisation of strains of *Gibberella fujikuroi complex* isolated from freshly harvested maize obtained from three agroecological zones of Uganda

Agro-ecological zone	District	Isolates	Sequence based identification	Sequence with best match	Identity (%)
Mid altitude (dry)	Kasese	MRC 9048	*G. moniliformis**	GU257904.1	99
		MRC 9049	*Fusarium* spp.	EF680754.1	99
		MRC 9050	*G. moniliformis*	FJ154074.1	99
		MRC 9051	*Fusarium* spp.	GQ141219.1	91
	Nakasongola	MRC 9054	*G. fujikuroi***	EU979565.1	99
		MRC 9055	*G. fujikuroi*	EU979565.1	99
		MRC 9056	*F. pseudonygamai*	FJ154075.1	99
		MRC 9057	*Fusarium* spp.	EF680759.1	99
		MRC 9058	*Fusarium* spp.	EF680754.1	99
		MRC 9059	*F. proliferatum*	GU066714.1	99
Mid altitude (dry)	Masindi	MRC 9063	*G. moniliformis*	GU257904.1	99
Mid altitude (dry)		MRC 9064	*Fusarium* spp.	EF680754.1	98
		MRC 9065	*G. moniliformis*	FJ154074.1	99
		MRC 9066	*G. moniliformis*	GU257904.1	99
		MRC 9067	*G. moniliformis*	GU257904.1	100
		MRC 9068	*G. moniliformis*	GU257904.1	99
		MRC 9069	*G. moniliformis*	GU257904.1	99
		MRC 9071	*F. proliferatum*	GU066714.1	99
		MRC 9073	*F. proliferatum*	GU066714.1	99
	Mubende	MRC 9070	*G. moniliformis*	FJ154074.1	99
		MRC 9072	*G. moniliformis*	GU257904.1	99
High altitude	Kapchorwa	MRC 9052	*G. moniliformis*	GU257904.1	100
		MRC 9053	*G. moniliformis*	GU257904.1	99
	Sironko	MRC 9060	*G. moniliformis*	FJ154074.1	100
		MRC 9061	*Fusarium* spp.	EF680751.1	99
		MRC 9062	*F. proliferatum*	GU066714.1	99

*: Teleomorph of *Fusarium verticillioides* **: Teleomorph of *Fusarium fujikuroi*

Masindi and Sironko. The strains MRC 9054 and MRC 9055 were identified as *G. fujikuroi* (accession number EU 979565.1). Only MRC 9056 was identified as *F. pseudonygamai* (accession number FJ154074).

Six isolates which had been morphologically identified as either *F. verticillioides* or *F. proliferatum* could not be identified to species level using the ITS-4 primer. Three of these (MRC 9049, MRC 9058 and MRC 9064) isolated from maize samples obtained from Kasese, Nakasongola and Masindi, respectively were matched with an unidentified *Fusarium* specie accession number EF680754.1. The other isolates, MRC 9051, MRC 9061 and MRC 9057 were matched with different *Fusarium* spp. of accession numbers GQ 141219.1, EF680751.1 and EF680759.1, respectively. These strains were isolated from maize obtained from Kasese, Sironko and Nakasongola, respectively.

Phylogenetic relationships amongst the *G. fujikuroi complex* strains isolated from freshly harvested maize: The genetic relationship amongst the strains isolated from freshly harvested maize as inferred by ML technique is indicated in Fig. 3. The species formed 4 clusters with 7 distinct groups (G1-G7). Strain MRC 9065 identified as *G. moniliformis* (accession number FJ154074.1) formed its own cluster. The clusters showed a clear separation between

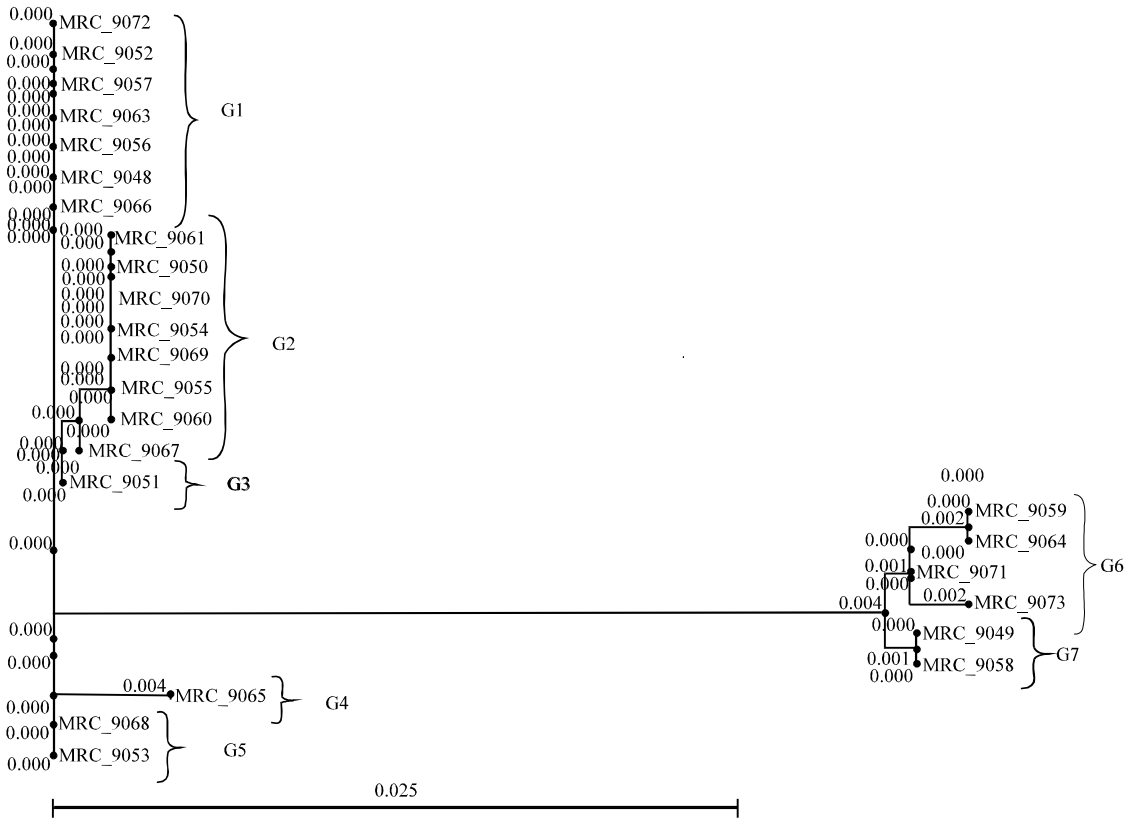

Fig. 3: Maximum Likelihood (ML) tree based on sequences generated by amplified ITS 4 primers of *Fusarium* species isolated from freshly harvest maize using the HKY85 model. G1-G7 refers to the inferred groups

G. moniliformis (Teleomorph of *F. verticillioides*) and *F. proliferatum* species with some unidentified strains grouping with either of the two. The only *F. pseudonygamai* strain isolated from Nakasongola was grouped with *G. moniliformis*. Intraspecific diversity was observed among the species. *G. moniliformis* showed the highest level of intraspecific diversity by forming 5 groups (G1-G5). The geographic origin of the isolates seemed not to have any impact on the formation of clusters or groups.

DISCUSSION

Results from the study indicated high incidence of *Fusarium* species in maize produced in Uganda. These findings are in agreement with the most recent study conducted in 10 districts of Uganda which reported high incidence and severity of *Fusarium* species in maize (Bigirwa *et al.*, 2007). The results also agree with several other studies conducted in African countries including Zambia (Mukanga *et al.*, 2010), South Africa (Marasas *et al.*, 1979), Ghana (Kpodo, *et al.*, 2000), Zimbabwe (Gamanya and Sibanda, 2001), Benin (Fandohan *et al.*, 2005) and Kenya (Kedera *et al.*, 1999) which reported dominance of *F. verticillioides* over other *Fusarium* species in maize. *F. verticillioides* is regarded as a cosmopolitan pathogen of maize and is usually found wherever maize is grown in the world (Leslie and Summerell, 2006).

The high incidence of *Fusarium* in maize produced in Uganda could be attributed to a number of factors. For example, due to land shortage, majority of the farmers in Uganda plant maize on the

same plot for consecutive seasons (Doss *et al.*, 2002). Repeated planting of maize on the same plot does not allow for a break in the cycle of Fusarium diseases associated with the previous crop which increases *Fusarium* inoculum in the soil (Lipps and Deep, 1991; Bilgrami and Choudhary, 1998). Related to repeated planting is the practice of leaving maize stover in the field. Most farmers in Uganda leave maize stover in the field until the next crop is planted (Bigirwa *et al.*, 1999). Such plant residues are reported to be the most important source of inoculum for *F. verticillioides*, *F. proliferatum* and *F. subglutinans* (CAST, 2003). Studies in Uganda have also indicated that mould incidence increased when maize harvesting was delayed for three weeks after physiological maturity (Kaaya *et al.*, 2005). In the current study, majority of the farmers who provided samples reported that they left maize to dry in the field for more than three weeks.

This study isolated *G. fujikuroi* strains from maize obtained from districts representing different agro-ecological zones with different climatic conditions. However, phylogenetic analysis showed that the strains formed clusters/groups irrespective of the geographical origin. The occurrence of strains with similar genetic lineages in different agro-ecological zones could be attributed to the fact that *F. verticillioides* and *F. proliferatum* are capable of adapting to different environments where maize is grown (De Oliveira Rocha *et al.*, 2011). In addition, these fungi are easily dispersed in nature and their spores can survive in soil which partly explains their wide distribution (Gong *et al.*, 2009). The results of this study are in agreement with the recently published studies in Brazil (De Oliveira Rocha *et al.*, 2011) in which phylogenetic analysis of *F. verticillioides* and *F. proliferatum* strains isolated from freshly harvested maize grains obtained from four regions did not show any relationship between strains and geographical origin.

Morphological and molecular analysis demonstrated a high intraspecific variability within *F. verticillioides*. This was further confirmed by phylogenetic analysis as evidenced by the formation of 5 distinct phylogenetic groups. The high intraspecific diversity within *F. verticillioides* was expected since majority of the strains were identified as *G. moniliformis* by molecular analysis. *G moniliformis*, a teleomorph of *F. verticillioides* is capable of sexual reproduction thus accounting for the high intraspecific variability (Leslie *et al.*, 2007). Similar observations were reported in maize from South Africa (Belgrove *et al.*, 2011) and Brazil (De Oliveira Rocha *et al.*, 2011).

In this study, six isolates which had been morphologically identified as either *F. verticillioides* or *F. proliferatum* could not be identified to species levels using the ITS-4 primer. However, phylogenetic analysis indicated that three strains (MRC 9048, MRC 9058 and MRC 9064) were closely related to *F. proliferatum* whereas the rest we closely related to *G. moniliformis*. Geiser *et al.* (2004) explained that failure to obtain perfectly matching sequences in a data bank could be due to a number of reasons which include; (1) the sequences may be allelic variants that are not present in the *Fusarium* data base used, (2) the species could be new whose sequences are not yet deposited in the *Fusarium* ID database , (3) the query sequence could be from a known species whose sequence is not in the database and (4) the sequence may be corresponding to a species that is poorly defined. In order to resolve the identification of such species, the same authors recommended the use of DNA sequences from a number of genes (multi-gene phylogeny). We therefore recommend further studies on the use of other molecular markers like the translation elongation factor (TEF-1β), the intergenic spacer region (IGS), the α-tubulin and the use of the fumonisin encoding genes which may provide perfect matching sequences that will be very useful in the identification of the strains that could not be resolved by ITS primer 4.

CONCLUSION

We have reported and characterized for the first time the diversity of the strains of *G. fujikuroi* species complex isolated from maize produced in Uganda. Our findings clearly indicated a high intraspecific diversity amongst the isolates of *G. fujikuroi* complex associated with maize produced in Uganda. Since members of the *G. fujikuroi* complex are known to be the major producers of fumonisins in maize, the results of this study provide important information for further understanding the relationship between the occurrence of such strains and fumonisin contamination in maize produced and consumed in Uganda.

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