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Research Article

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Morphological and pathogenic characterization of *Fusarium* species causing common bean root rot in Uganda

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ABSTRACT

Fusarium root rot (FRR) of common bean occurs in Africa, Central and South America, and causes yield losses of up to 86%. Recently, FRR-like wilt symptoms were observed in Uganda's agroecology zones. To identify the causal pathogen, we conducted surveys in seven agroecology zones to determine the prevalence and incidence of the reported disease. During the surveys, diseased roots were collected for pathogen isolation. Fungal strains were characterized using colony color, mycelial growth rate and microscopic structures such as conidia and microconidia. The pathogenicity of 99 strains on five bean varieties was determined in artificially inoculated soils in the screenhouse. Based on field symptoms, the observed wilting was identified to be *Fusarium* root rot, the prevalence of which varied across agroecologies, with the highest (95%) in the Karamoja pastoral zone (KP) and the lowest (40%) in West Nile farming system (WN). Similarly, the incidence of FRR was highest (87%) in KP, and lowest (20%) in WN. *Fusarium* strains differed in growth rate, colony color, shape and size of microscopic structures. All evaluated strains were pathogenic on common bean and caused severities of 0.9 to 98.3%. Further studies are required to identify the isolated strains at the species level using molecular tools.

KEYWORDS: Common bean, Disease incidence, Disease prevalence, *Fusarium* root rot, Pathogenicity

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the third most important food legume crop in the world after soybean (*Glycine max* L.) and peanuts (*Arachis hypogaea* L.) (Broughton *et al.*, 2003). However, the production of the crop is constrained by soilborne diseases (Miklas *et al.*, 2006), key among which in Uganda are Southern blight and Fusarium root rot (FRR) (Paparu *et al.*, 2018). *Fusarium* root rot of common bean is an important disease in African countries such as Uganda, Rwanda, Burundi, the Democratic Republic of Congo, Kenya and South Africa; and in Central and South America (Abawi & Corrales, 1990). The symptoms of FRR include longitudinal reddish-brown lesions on hypocotyls accompanied by longitudinal fissures or cracks with dying root tissues turning reddish brown. Infected plants are chlorotic beginning with the primary leaves, stunted and plants may wilt completely or undergo premature senescence. Bean yield losses due to FRR may reach 86% in severely infected soils (Abawi & Corrales, 1990).

In a study by Paparu *et al.* (2018), FRR disease was found to be the second most important bean root rot disease in Uganda after Southern blight caused by *Sclerotium rolfsii* Sacc. (teleomorph *Arthelia rolfsii* (Curzi) C. C. Tu & Kimbr.). In the above study, root rots caused by *Pythium* and *Rhizoctonia* species were also reported. Disease prevalence (defined as the number of gardens with a particular disease in a defined geographical area) and incidence (the proportion of diseased plants relative to the total number of plants sampled, usually expressed as a percentage) of FRR of common bean has been reported to vary by agroecology (Nutter *et al.*, 1991; Moya-Elizondo *et al.*, 2011). Factors such as elevation, soil type, agronomic practices, temperature and relative humidity among others, contribute to the variation of pathogen species in farmers' gardens (Moya-Elizondo *et al.*, 2011; Trabelis *et al.*, 2017). In Tunisia, *Fusarium solani* was

recovered mostly from gardens that previously had solanaceous crops like pepper (*Capiscum* spp.), potatoes (*Ipomea* spp.) and tomatoes (*Lycopersicum esculentum* L.) (Trabelis *et al.*, 2017). Good crop production practices such as crop rotation tend to reduce inoculum levels in soil, resulting in reduced root rot disease severity (Abawi & Corrales, 1990).

Fungal strains may be characterized morphologically using colony characteristics such as growth rate, color, texture, shape of colony edges, and shape and size of microscopic structures (Mandal *et al.*, 2018; Kiprop *et al.*, 2002). Where resources are limited, the above features can be useful for the preliminary identification of disease causal agents (Kristensen *et al.*, 2005; Chopada *et al.*, 2015).

Pathogenicity is the quantitative ability of an organism to cause disease. Related to it is virulence which is the extent to which a strain can cause disease relative to other strains (Weiland *et al.*, 2013). Virulence is quantified by measuring severity, the latter being the degree of damage to individual plants (Chiang *et al.*, 2017). Previous researchers have observed differences in pathogenicity among *Fusarium* spp. causing root rots in different crop species. For example, in olive trees (*Olea europaea*) Trabelsi *et al.* (2017) found that among 104 isolates, 23 were pathogenic, and *F. solani* was the most pathogenic species compared to *F. oxysporum*, *F. chalmydosporum* and *F. brachygibbosum*. Meanwhile in Soybean, nine species of *Fusarium* were observed to vary significantly in their pathogenicity with *F. graminearum* causing the most severe disease followed by *F. proliferatum*, *F. orochotrichoides* and *F. solani* (Arias *et al.*, 2013). In a related study, the more virulent strains of *Fusarium* spp. in sugar beet (*Beta vulgaris* L.) induced foliar symptoms earlier than the less virulent ones (Burlakoti *et al.*, 2012).

The study by Paparu *et al.* (2018) showed an increasing significance of FRR in Uganda's agroecology zones, yet there is limited information on the diversity of *Fusarium* spp. causing the observed disease in common beans. To fill this knowledge gap, our study sought to; 1) determine the prevalence and incidence of FRR on common bean in seven agroecology zones of Uganda, and 2) collect wilting roots, isolate and morphologically characterize *Fusarium* spp. strains obtained. During the surveys, we documented varieties grown by farmers since the former is reported to affect the incidence and prevalence of Fusarium root rot (Moya-Elizondo *et al.*, 2011; Trabelis *et al.*, 2017; Paparu *et al.*, 2018).

MATERIALS AND METHODS

Root Rot Disease Surveys and Sample Collection of *Fusarium* spp.

Surveys to determine the prevalence and incidence of FRR disease were carried out between September 2017 and November 2019 in 32 districts representing seven agroecology zones in Uganda (Figure 1). The surveys focused on smallholder farmers fields where the common beans were grown. The surveyed districts were chosen based on the bean production records published by Kalyebara *et al.* (2006). Common bean agroecology zones have varying annual precipitation, altitude and farming systems. We therefore surveyed the following agroecology zones; West Nile Mixed Farming System zone (WN) with annual rainfall of 1340-1371 mm and altitude 778-1409 m, Karamoja Pastoral zone (KP) with annual rainfall <1000 mm and altitude 1164-1475 m, Northern Mixed Farming System zone (NM) with annual rainfall >1197 mm and altitude of 1010-1176 m, Western Mixed Farming System zone (WM) with an annual rainfall of 1000-1200 mm and altitude of 1020-1880 m, Southwestern Highlands (SWH) with annual rainfall >1200 mm and altitude 1800-1855 m, Lake Victoria Crescent and Mbale Farmlands (LVC) with annual rainfall of 1215-1328 mm and altitude 1100-1536 m, and Eastern Highlands (EH) with annual rainfall >1200 mm and altitude 1369-2125 m. Rainfall data were obtained from the head office of Uganda National Meteorological Authority (UNMA) while altitudes were recorded during surveys using a global positioning system (GPS).

Surveys were conducted during the bean growing season. In each district, we surveyed 15 gardens, randomly chosen at a minimum interval of 1km along the route of the survey. However, in some districts, we surveyed fewer gardens because the crop was not at a suitable stage for assessing FRR disease. In sampled gardens, bean growth stages ranged from V2 (primary leaf stage) to R7 (pod formation). To determine the prevalence of bean root rot disease (the incidence of gardens with diseased plants in a defined geographic area), we walked along a Z-line transect and observed the presence or absence of wilting plants. *Fusarium* root rot disease incidence (the number of diseased plants expressed as a percentage of the total number sampled) was determined from a maximum of 30 wilting plants showing symptoms of the different root rot diseases. The number of wilting plants picked was not the same across gardens, as this depended on the number of wilting plants along the Z transect for every garden sampled. We used the guidelines by Buruchara *et al.*, 2010) to identify the different bean root rot diseases. During the surveys, we collected information on varieties grown and the previous crop(s) in the gardens surveyed. Roots from wilting plants showing typical FRR symptoms were collected and placed in paper bags and brought to the Pathology Laboratory at the National Crops Resources Research Institute for pathogen isolation.

Isolation of *Fusarium* spp.

To isolate *Fusarium* spp. a total of 1,496 diseased root samples were collected from 196 gardens in six agroecology zones. These samples were collected from a total of 25 bean varieties (Supplementary Table S1). Two whole roots showing typical symptoms of FRR (reddish brown longitudinal lesions) were sampled for isolation per garden. Once in the laboratory, root pieces of approximately 0.5 cm were cut and sterilized first in 15% Sodium hypochlorite (Jik) for 1 min and then in 70% ethanol for another min. Pieces were rinsed thrice in sterile water and bloat dried on sterile tissue and plated on full strength potato dextrose agar (PDA) (39 g of PDA in 1000 mL of water)

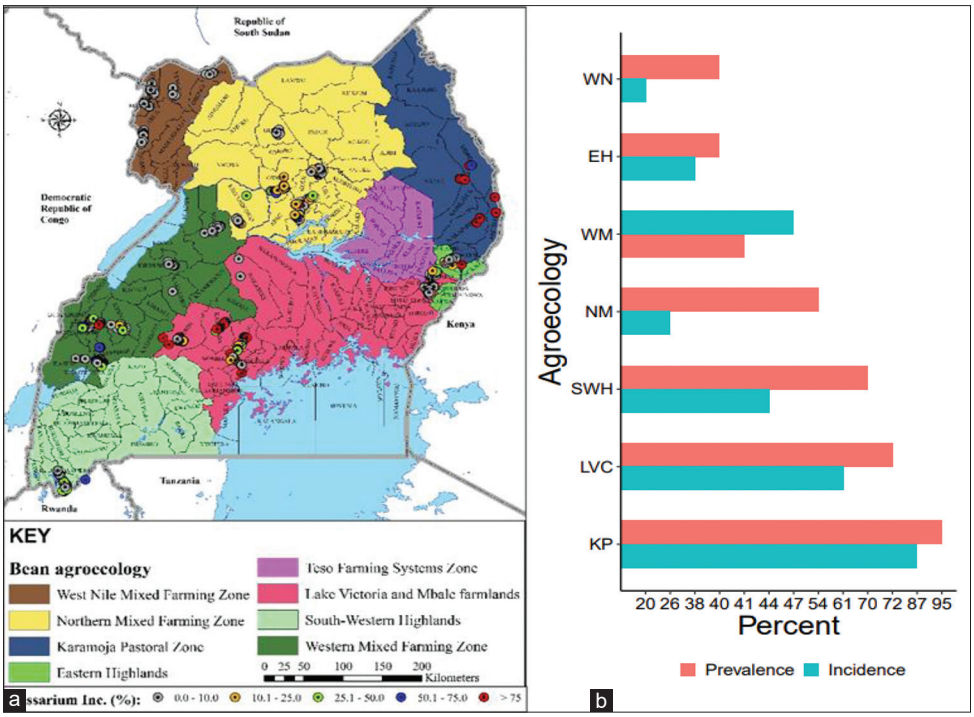

Figure 1: Prevalence and incidence of Fusarium root rot disease of common bean in selected districts of Uganda grouped into agroecology zones that were surveyed between September 2017 and November 2019. a) Geographical distribution of the surveyed locations (circles) within agroecology zones and the corresponding incidence of the disease. b) Comparison of the prevalence and incidence of FRR between agroecology zones. Where, WN is Western Mixed Farming System zone, EH is Eastern Highlands, WM is Western Mixed Farming System Zone, NM is Northern Mixed Farming System zone, SWH is Southwestern Highlands, LVC is Lake Victoria Crescent and Mbale Farmland and KP is Karamoja Pastoral zone

following amendment with 0.3 g of streptomycin sulphate. After five days, *Fusarium* spp.-like fungi growing from cultured root pieces were subcultured on fresh PDA. Pure strains were prepared through hyphal tipping. Strains were then stored as described by Paparu *et al.* (2020) on sterile filter papers.

Morphological Characterization of *Fusarium* spp. Strains

One hundred and ninety-six strains grown on full strength PDA were assessed for growth rate, colony color and texture, and microscopic structures such as the hyphae, the size and shape of conidia. Growth rate was determined using the procedure by Paparu *et al.* (2020). Each sample was replicated thrice. Petridishes were inoculated with the different strains singly, arranged in a completely randomized design in the dark and incubated at 25 °C. Colony growth rate was measured daily between days three and eight post inoculation. This was because from day one to two, the mycelia did not grow significantly enough to be measured, and after day 8, most strains had completely covered the Petridish. The colony color, texture and shape of colony edges were all recorded on day 8. On the final day of growth measurement, mycelia from the different cultures were teased using a sterile needle and transferred into a drop of water on a microscope slide, mashed to loosen conidia and observed under a light microscope (Brunel Microscopes Ltd. UK) connected to a monitor at x40 magnification to determine the presence/absence of macro- and microconidia, their sizes and associated features such as septa and conidiophores.

Pathogenicity of *Fusarium* spp. Strains on Common Bean

Pathogenicity studies were conducted in a greenhouse at the National Crops Resources Research Institute (NaCRRI). Ninety-nine representative strains with different morphologies and growth rates were selected for pathogenicity studies. The pathogenicity of the selected strains was tested on five common bean varieties with known reactions to bean root rot. These included MLB49-89A (FRR tolerant), RWR719 (Pythium root rot tolerant but susceptible to FRR), ALB171 (hybrid of *P. coccineus* and *P. vulgaris*, susceptible to FRR), NABE19 (a released variety, susceptible to FRR) and CAL96 (a universal root rot susceptible check also commonly known as K132). The preparation of inoculum on millet grain was done following the protocol by Paparu *et al.* (2020). Discs of 1 cm were cut from two-week-old cultures and inoculated on 10 g of sterile millet grains in conical flasks, and incubated at 25 °C on benches in the laboratory. When the fungus had fully colonized the millet grain, 10 g of the inoculum for each strain was mixed with approximately 20 kg of moist heat sterilized soil (loam soil and sand mixed in a ratio of 2:1) in 70 x 35 x 10 cm wooden trays in the screenhouse. Each strain was replicated twice. The 99 strains were screened in four different experiments since the screenhouse space was limited. In each experiment, one tray of sterile soil was not inoculated and used as a negative control. To increase soil inoculum levels, the susceptible variety CAL96 was planted in all trays including the non-inoculated control tray and left to grow for 3 weeks during which plants were watered

twice daily with bore hole water. After three weeks, plants were uprooted and assessed for *Fusarium* root rot disease.

After the removal of CAL96, the 5 test varieties were planted in the trays with well grown inoculum (where the susceptible variety was previously planted). All the varieties were planted in a single tray containing a single strain, with 16 seeds of each variety planted in two rows. All 10 rows within a tray were completely randomized. Sixteen seeds of each variety planted in a tray with non-inoculated soil acted as controls. Trays were arranged in a completely randomized design and each strain was replicated twice. Plants were watered twice daily until 28 days after planting.

The 99 isolates were evaluated in four different experiments, during which daily average screenhouse temperatures were between 24 and 30 °C. Germination and FRR incidence were determined at 14 days after planting, and FRR severity at 28 days after planting (at trial harvest). FRR incidence was determined by counting the number of infected plants, and severity was estimated using a scale of 1-9 (Abawi & Corrales, 1990), where: 1=No visible symptoms, 3=Light discoloration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions, 5=Approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system 7=Approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting and reduction of root system 9=Approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system.

Data Analysis

The mean percentage prevalence and incidence of FRR were computed and analysed using STATA statistical software (Version 15.1). Differences in the prevalence and incidence of FRR across agroecology zones were tested using Chi-square. Analysis of variance was used to determine differences in growth rate among strains. Statistical differences between means of growth rate for the different agroecology zones were tested using Tukey's Studentized range test. Growth rate analysis was done using SAS (version 9.1; SAS Institute, Cary, NC, USA). Disease severity index (DSI) was calculated from disease scores according to the method by Chiang *et al.* (2017). DSI were analyzed per experiment using the PROC GLM procedure in SAS. Mean differences for the different variables within each experiment were analyzed using Tukey's Studentized range test.

$$\text{DSI}=\frac{\Sigma(\text{Class frequency}\times \text{Score rating of class})}{(\text{Total number of observation})\times(\text{Maximum disease index})}\times 100$$

Where class frequency is the number of plants at a particular score, score rating is the assigned disease severity of the plant, total number of observations is the total number of plants assessed and maximum disease index is the maximum score.

RESULTS

Prevalence and Incidence of FRR in Surveyed Agroecology Zones

The prevalence of FRR in surveyed agroecology zones was 40-95% (Figure 1a). Following the grouping of disease samples by plant growth stage, the prevalence at the primary leaf stage (young plants) was 43% and that in plants at R7 (pod formation) was 95%. The effect of climatic factors on the prevalence and incidence of FRR was analyzed and a negative correlation was observed between prevalence and mean annual temperature (MAT) (r=-0.67), and prevalence and annual rainfall (AR) (r=-0.87). In contrast, there was a very strong positive correlation between prevalence and mean annual relative humidity (MARH) (r=0.90) (Table 1).

Similarly, there was a negative correlation between FRR incidence and average annual rainfall (r=-0.81) and incidence and average annual temperature (r=-0.31). Just as prevalence, we observed a strong positive correlation between incidence and average annual relative humidity.

Chi-square analysis showed that the incidence of FRR varied significantly among common bean agroecology zones ($X^2=231$, $P<0.01$), ranging from 20 to 87%) (Figure 1b). Just like prevalence, incidence among older plants was twofold higher than that of the younger plants (33%). The incidence and prevalence of FRR were higher when the previous crop grown was beans or a bean intercrop and lowest when the previous crop was cassava or the field had been under fallow.

Morphology of *Fusarium* spp. Strains on PDA Media

A total of 196 *Fusarium* spp. isolates were obtained by selecting cultures with growth characteristics specific to *Fusarium* spp. on potato dextrose agar (Supplementary Table S2). The least number of isolates (3) was obtained from the EH zone whereas the greatest (70) came from the LVC zone. There

Table 1: Prevalence of *Fusarium* root rot and climatic factors in surveyed Ugandan agroecology zones

Agroecology[1]	Prevalence (%)	Incidence (%)	AR (mm/year)	MAT(°C)	MARH (%)
NM	42	26	1467	24.7	65
WM	54	47	1196	23.2	67
LVC	72	61	623	21.8	72
SWH	70	44	1079	18.5	75
WN	40	20	1,216	22	67
KP	95	87	-	-	-
EH	40	38	-	-	-

[1]NM=Northern Mixed Farming System Zone, WM=Western Mixed Farming System zone, LVC=Lake Victoria Crescent and Mbale Farmlands, SWH=Southwestern Highlands, WNM=West Nile Mixed Farming System zone, KP=Karamoja Pastoral Zone, EH=Eastern Highlands. Weather data was obtained from National Meteorological Department Head Quarters, Entebbe, Uganda. However, there was no weather information for Karamoja Pastoral Zone and the Eastern Highlands.

were no isolates from the WN agroecology zone because the surveys in this region were done before a decision was taken to collect diseased roots for isolation of the pathogen. *Fusarium* spp. strains (pure cultures) had contrasting colorations of the mycelia (colony surface) and substrate (bottom of the Petri dish). Selected surface and substrate colorations by *Fusarium* spp. strains can be seen in Figure 2.

Additionally, the total number of strains exhibiting the different surface and substrate colorations is summarized in Table 2. However, detailed information regarding colorations by each strain can be found in Supplementary Table S2. Colony textures included cottony aerial (129), cottony low (39) and compact (28) whereas the edges were either smooth or serrated (Figure 2).

All strains produced septate hyphae. Microconidia were spherical shaped, whereas the macroconidia were oval, rod or curved with 1 to 6 septa (Figure 3). Microconidia were not observed in some strains. The size of macroconidia ranged from 20 to 60 µm. The microconidia was less than 10 µm (Figure 3 a-d).

The daily growth rates of strains were categorized as low (0.17 to 0.53 cm), moderate (0.54 to 0.89 cm) and high (0.9 to 1.27 cm) (Supplementary Table S2). Growth rates were significantly different among agroecology zones ($F=15.81$, $P<0.01$). For two agroecology zones where several districts were surveyed, growth rates for strains from the different districts were not significantly different ($F=0.74$, $P=0.5934$ and $F=0.98$, $P=0.4516$ for LVC and WM zones, respectively). On the contrary, in the KP and NM agroecology zones, strains from different districts had significantly different growth rates ($F=7.92$, $P<0.01$ and $=5.01$, $P<0.01$ for KP and NM zones, respectively). The majority of strains showed moderate growth rates with means of 0.67 ± 0.1, 0.76 ± 0.02, 0.85 ± 0.01 and 0.89 ± 0.02 cm/day for the EH, KP, LVC and WM zones respectively. The highest mean growth rates were observed in the NM (0.93 ± 0.02 cm/day) and SWH agroecology zones (0.99 ± 0.09 cm/day).

Pathogenicity of *Fusarium* spp. Strains on Common Bean

All the 99 strains tested were pathogenic on common bean. There were no root rot symptoms on all plants in control

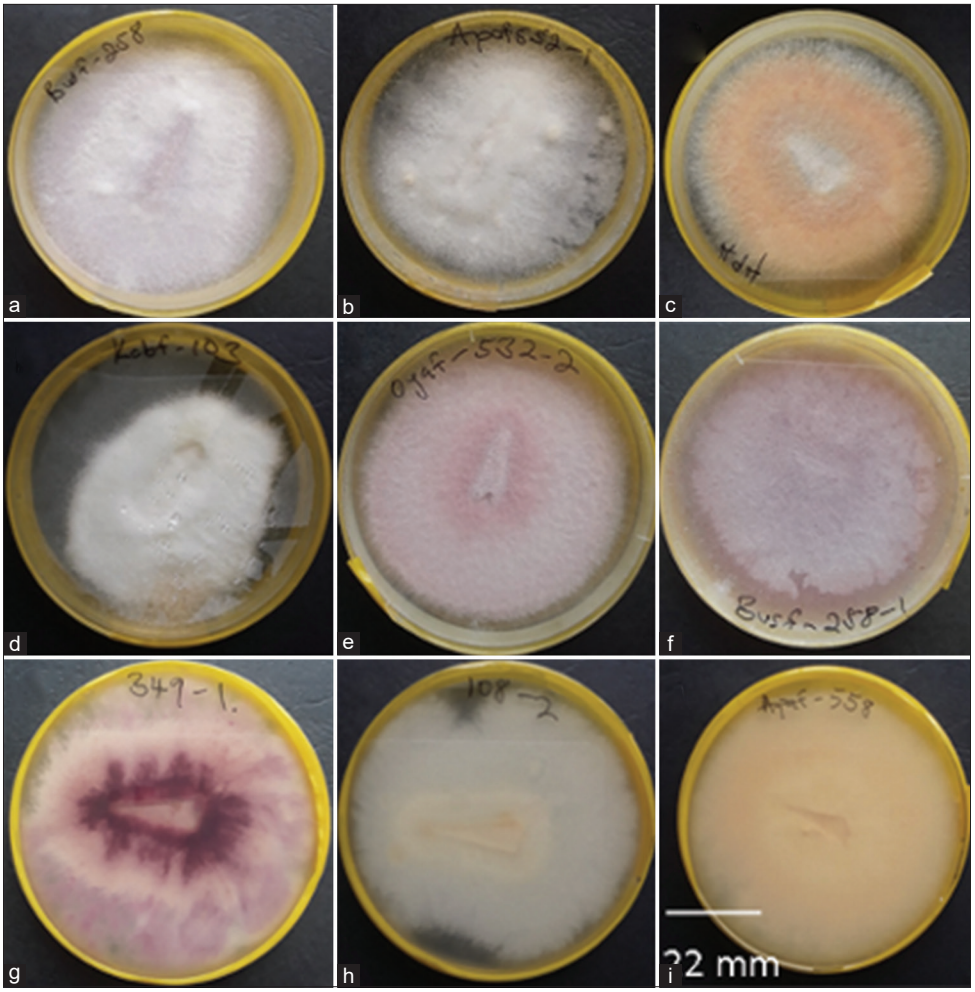

Figure 2: Colony characteristics of selected *Fusarium* spp. strains from Ugandan agroecology zones 14 days after growth on Potato Dextrose Agar. a-f) show surface mycelia color and texture, where a and b=White cottony low mycelia, c=White/Orange cottony low mycelia, d=White cottony aerial mycelia, e=White/Purple cottony low mycelia, and f=White/Purple low mycelia. g, h and i show substrate coloration, where g=White/Purple, h=White and i=Cream

Table 2: Summary of the different surface and substrate colorations exhibited by *Fusarium* spp. strains obtained from Ugandan bean agroecology zones

Coloration	Number of *Fusarium* spp. Strains	
	Surface coloration	Substrate coloration
White	74	80
White/Purple	55	72
White/Pink	23	10
Cream	22	20
Cream/White	13	10
White/Yellow	6	0
White/Orange	2	0
White/Green	1	4
Total number of strains	196	196

trays where *Fusarium* spp. strains were not inoculated (Supplementary Table S3). The disease severity index for all strains was between 0.9 and 98.3%. The 10 most virulent strains had DSI values of ≥70% and of these 6 were from the LVC agroecology zone. The three most virulent strains were KabF-103 (from the SW zone), MitF-490 (from LVC zone) and BusF-258.1 (from WM zone) with average DSI values of 98.3, 88.3 and 87.0%, respectively. The 10 least virulent strains had DSI values ≤20% across all tested varieties and included strains mostly from the KP and WM zones. HoiF-385 (from the WM zone) was the least virulent strain, and caused an average DSI of 0.9%. Other strains with low virulence were GulF-451.1 (from the NM zone) and MubF-460 (LVC zone) with average DSI values of 8.1 and 10.7%, respectively.

Susceptibility of Common Bean Varieties to *Fusarium* spp. Strains

Significant differences were observed in FRR severity among common bean varieties in all four experiments ($F=6.40$, $P=0.0001$; $F=10.40$. $P=0.0021$; $F=6.47$, $P=0.0021$ and $F=9.37$, $P=0.0012$ for experiments 1, 2, 3 and 4, respectively) (Figure 4). The susceptible variety CAL96 showed the greatest DSI in all experiments, while other varieties consistently showed low to moderate FRR severity. In experiment 1, there was a significant difference in the severity of FRR among different bean varieties ($F=6.4$, $P=0.0001$). However, there were no significant differences in the virulence of the strains on varieties MLB-49-89A, RWR719 and NABE19; but these three varieties had significantly lower DSI values compared to ALB171 and CAL96. In experiments 2 and 3, there was still a significant difference in severity of root rot on bean varieties ($F=10.4$. $P=0.0001$ and $F=6.47$, $P=0.0001$ for experiments two and three respectively). The disease severity index for CAL 96 was significantly higher than that for the other varieties screened. In experiment 4, ALB171 showed the lowest DSI which was significantly different from that for the other bean varieties tested ($F=9.37$, $P=0.0001$).

The impact of *Fusarium* spp. strains on the five common bean varieties were assessed by dividing the DSI values into four major categories (0-25%, 25-50%, 51-75% and 76 to 100%), corresponding to low, moderate, high and very high, respectively. Among the five common bean varieties, CAL96 was the most susceptible with an average DSI of 59.3% while MLB49-89A

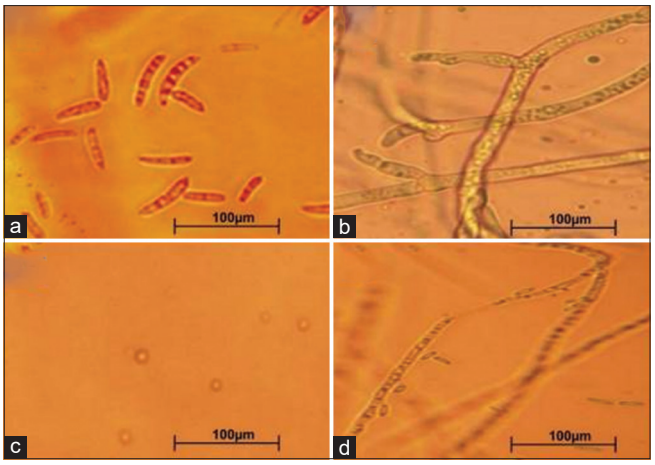

Figure 3: Microscopic structures of selected *Fusarium* spp. strains taken at x40 magnification. a) strain with spindle shaped macro conidia having 3 to 6 septa, b) strain with conidiophores, c) strain with spherical micro conidia and d) strain with septate hyphae

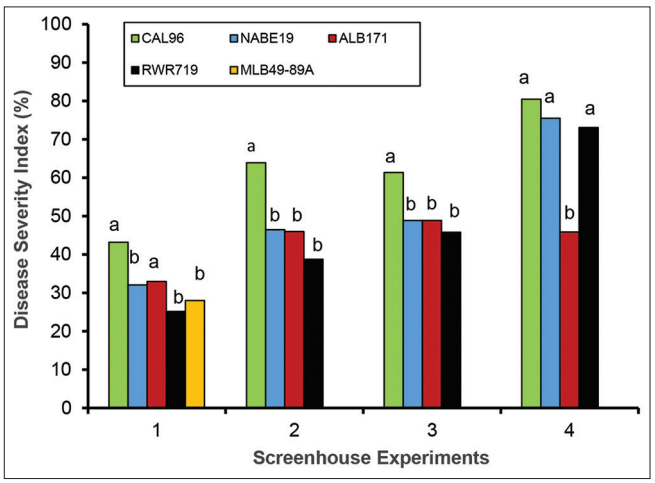

Figure 4: Average Disease Severity Index (DSI) for combined *Fusarium* spp. strains on five common bean varieties CAL96, ALB171, NABE15, RWR719 and MLB49-98A following screenhouse pathogenicity studies in four different experiments. For each experiment, different letters above bars indicate differences in DSI at $P \leq 0.05$ (Tukey's Studentized range test). MLB49-89A was screened only in experiment 1 because we did not have enough seed for the variety

(the FRR tolerant check) had the least DSI of 28%. However, the latter was only used in experiment 1, because there was an insufficient seed of the variety. Common bean varieties RWR719, ALB171 and NABE19 showed moderate DSI compared to CAL96.

DISCUSSIONS

Common bean root rots are a major constraint to bean production in Uganda. A previous study by Paparu *et al.* (2018) reported FRR as the second most important root rot disease of beans after Southern blight. Therefore, detailed knowledge of the distribution of FRR disease and characterization of the strains is important for the development of management strategies.

Prevalence and Incidence of Fusarium Root Rot

Results of surveys across the major common bean agroecology zones in Uganda revealed significant variations in the prevalence and incidence of FRR. The highest prevalence and incidence of FRR disease was in the KP zone followed by the LVC and SWH agroecology zones. A survey conducted on bean root rots in selected agroecology zones of Uganda in 2013 and 2014 reported the highest FRR prevalence and incidence in the SWH zone (Paparu *et al.*, 2018). In the studies of 2013 and 2014, the KP zone was not surveyed, making it difficult to compare the two studies. However, the observed spatial variations in the occurrence of FRR disease could be explained by (i) climatic factors, (ii) type of varieties grown and (iii) the disease management practices used by common bean farmers (Moya-Elozondo *et al.*, 2011; Trabelis *et al.*, 2017; Paparu *et al.*, 2018).

Climatic factors such as precipitation, relative humidity and temperature influence the temporal and spatial dynamics of microbial populations. The surveyed agroecology zones have distinct climatic factors, ranging from the hot and dry KP zone to the cool and wet SWH (Table 1). Negative correlations were observed between both prevalence and incidence and mean annual temperature and mean annual rainfall. In contrast, there was a very strong positive correlation between prevalence and mean annual relative humidity, and between incidence and mean annual relative humidity. Tusiime (2004) reported the occurrence of *Fusarium solani* f.sp *phaseoli* in the cool humid South Western highlands of Uganda. Moya-Elizondo *et al.* (2018) reported that the prevalence of root rot disease caused by *F. psuedograminearum* positively correlated with maximum summer temperatures while root rot caused by *F. culmorum* was more prevalent in cooler temperatures. The above findings indicate probable climatic pressures on *Fusarium* spp. distribution.

Common bean production is mostly done on a subsistence scale in Uganda and farmers usually preserve seed from previous harvests. In places like the KP and SWH zones, Calima-type varieties such as CAL96 from the Andean bean gene pool are predominant (Wilkus *et al.*, 2018). The findings of our study show that CAL96 was grown in more than 45% of gardens surveyed in the KP and SWH zones, yet earlier studies report it as being very susceptible to all root rots (Paparu *et al.*, 2020). This may therefore explain the high prevalence and incidence of FRR in KP and SWH. In our study, the prevalence and incidence of FRR were also influenced by the crop growth stage sampled. FRR was less common in young plants compared to older ones, most probably because some plants can survive the disease up to maturity by producing hypocotyl borne adventitious roots at the collar region after the death of the tap root, making the disease persist in older plants (Abawi & Corrales, 1990).

Morphology of *Fusarium* spp. Strains on PDA Media

Colony surface coloration included white, white/purple, white/pink, cream, cream/white, white/yellow, white/orange and white/green. Similar substrate colorations were observed. The colony textures included cottony aerial, cottony low and compact. We could not identify strains to species level using colony coloration or texture. Previous authors equally reported the inadequacy of morphological structures to identify *Fusarium* spp strains. For example, Igbal *et al.* (2010) reported purple coloration of mycelia in *Fusarium Mangiferae*. However, the same purple coloration of the mycelia was also reported for *Fusarium oxysporum* by Trabelsi *et al.* (2017). This confirms the difficulty of using colony morphology to identify *Fusarium* spp. strains.

We observed a wide range of growth rates (0.17-1.68 cm/day) for *Fusarium* spp. strains. Strains from the warmer NM agroecology zone had faster growth rates than those from the cooler EH zone. In previous studies, the highest growth rate of 0.53 cm/day was reported for *Fusarium* spp. obtained from common bean (Tusiime, 2004). In the current study, only 8 strains out of 196 had growth rates ≤0.53 cm/day. The variation in growth rates of strains observed in our study may indicate the diversity of *Fusarium* spp. causing bean root rot in Uganda's agroecology zones.

Morphological characterization could not differentiate *Fusarium* spp. strains due to the lack of distinct morphological features associated with different species. Lack of production of distinct morphological structures and similarities in shape and size of morphological structures such as the micro- and macroconidia may lead to errors in the identification of fungal pathogens. Therefore, there is a need to use more specific techniques for identifying *Fusarium* spp. strains.

Pathogenicity of *Fusarium* spp. Strains on Common Bean

All isolates tested were pathogenic on common bean. Among the varieties tested, CAL96 was the most susceptible and MLB49-89A the least susceptible. Paparu *et al.* (2020) also reported MLB49-89A as being resistant to *Fusarium* root rot and southern blight of common beans. Disease Severity Index varied among strains from different agroecology zones, but the 10 most virulent strains were from 5 out of the 6 agroecology zones sampled. Similar findings were reported by Kiprop *et al.* (2002) when they screened *F. udum* strains for virulence on cowpea (*Vigna unguiculata* L. Walp.) where the most virulent strains were from five agroecology zones out of eight surveyed. Despite the high prevalence and incidence of FRR in the KP zone, none of the 10 most virulent strains originated from there. On the contrary, 4 of the least virulent strains were from KP. This may imply that irrespective of the virulence of existing strains, there are conditions/factors within this agroecology that promote the development of FRR disease. Alternatively, one can argue that *Fusarium* strains from KP may require conditions like those of their origin to express high virulence.

CONCLUSION

Fusarium spp. causing root rots and wilts of common bean in Uganda are morphologically diverse. The fact that high FRR incidence and severity were observed in different agroecology zones with different temperatures and relative humidity

indicates the pathogen's adaptability to diverse climatic factors, likely resulting in the recently observed high incidences of FRR disease with varying symptoms in smallholder farms in different bean agroecologies in Uganda. The threat of FRR disease to common bean was confirmed in our study, because all varieties grown by the farmers are susceptible to the disease. Given that there is no resistance to FRR among bean varieties commonly grown by smallholder farmers in Uganda, our study offers novel resources for breeders willing to take the first step towards breeding for FRR resistance in common bean. Resources developed by the current study include; 1) Knowledge of the incidence and prevalence of FRR in the different bean agroecology zones of Uganda, and 2) *Fusarium* spp. strains with known virulence stored at NaCRRI. In the absence of resistance varieties, we advise farmers to manage FRR disease using effective and affordable methods such as fungicide seed treatment, inclusion of non-host plants in crop rotations, removal and destruction of infected plants that act as sources of inoculum, and use of inorganic and organic soil fertility amendments.

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