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# Comparative Population Genetics of *Exserohilum turcicum* in Smallholder Farms of Kenya and Uganda

Jabulile N. N. Mahlangu | Dave K. Berger | David L. Nsibo

Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

**Correspondence:** David L. Nsibo (david.nsibo@fabi.up.ac.za)

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## ABSTRACT

*Exserohilum turcicum*, the causal agent of northern leaf blight (NLB), poses a major threat to maize production in sub-Saharan Africa. Effective disease management depends on understanding the biology and population genetic structure of the pathogen, yet studies from Africa remain limited. Here, we investigated the genetic diversity and evolutionary forces shaping *E. turcicum* populations in Kenya and Uganda. A total of 494 strains were genotyped using 12 microsatellite markers. Gene diversity was relatively low in Kenya ($H_e = 0.45$) and Uganda ($H_e = 0.42$) compared with previous reports. Analysis of molecular variance revealed weak but significant differentiation between countries ($\Phi_{PT} = 0.178$, $p = 0.001$), with more variation occurring within countries (82%) than among them (18%). Cluster analyses showed high levels of gene flow within each country but limited admixture between countries, suggesting geographical barriers to dispersal. Grouping populations according to shared agroecological zones did not result in distinct genetic clustering, indicating that population structure was not driven by agroecological similarity. This interpretation was supported by a weak isolation-by-distance correlation ($R^2 = 0.077$), consistent with restricted long-distance gene flow. Most populations deviated from the expected 1:1 mating-type ratio, highlighting the importance of clonal reproduction in shaping population structure. Collectively, these findings suggest that *E. turcicum* populations are largely panmictic within countries but exhibit restricted cross-country movement. These results provide a valuable foundation for developing sustainable, regionally targeted strategies to manage NLB in East Africa.

## 1 | Introduction

Maize is one of the most important staple crops worldwide, with global production during the 2023/24 cropping season estimated at 1.23 billion tonnes (USDA 2024). Northern leaf blight (NLB) is the most prevalent foliar disease of maize globally, causing yield losses of up to 72%, particularly in highly susceptible maize cultivars under favourable conditions (Berger et al. 2020; Savary et al. 2019). In addition to maize, alternative hosts of NLB include sorghum (*Sorghum bicolor*), Sudan grass (*Sorghum drummondii*), Johnson grass (*Sorghum halepense*) and the wild maize progenitor teosinte (*Zea mays* subsp. *parviglumis*) (de Lange et al. 2014; Leonard and Suggs 1974; Nagaraja and Das 2016). NLB initially manifests as chlorotic spots, which mature into large, elliptical greyish lesions capable of coalescing and blighting entire leaves, thereby reducing the photosynthetic capability of the plant (Kotze et al. 2019; Turgay et al. 2021). Management strategies of NLB include crop rotation, deep tillage, the deployment of resistant cultivars and the application of chemical fungicides (Abadi et al. 1993; Hooda et al. 2017; Montemarani et al. 2018; Sartori et al. 2015, 2017).

Although NLB was first observed in Parma, Italy, it was formally documented in the United States in 1878 (Drechsler 1923). The disease has since been reported across all major maize-producing regions of the world, with frequent occurrences documented in North America, Europe, South America and East Asia (Hooda et al. 2017; Human et al. 2016). In Africa, the

earliest reports of NLB date back to 1924 in Uganda, 1926 in Kenya and 1931 in South Africa (Doidge and Bottomley 1931; Duke 1926; Emechebe 1975). Since these reports, NLB has been documented in all maize-producing countries in sub-Saharan Africa (Doidge and Bottomley 1931; Duke 1926; Emechebe 1975; Kloppers and Tweer 2009; Ramathani et al. 2011). *Exserohilum turcicum*, an ascomycete within the Dothideomycetes, is the causal pathogen of NLB in maize (Leonard and Suggs 1974). This pathogen is heterothallic in nature and sexual reproduction is governed by the *MAT1* locus, requiring the presence of two compatible partners, each carrying either *MAT1-1* or *MAT1-2* idiomorphs for successful recombination (Bihon et al. 2014; Turgeon and Yoder 2000). The teleomorphic stage of *E. turcicum* is extremely rare in nature and has been documented only once in a single study from Thailand, where it was isolated directly from infected maize debris and excised under laboratory conditions (Bunkoed et al. 2014; Fan et al. 2007). Due to the rarity of its sexual stage, inferences about sexual reproduction within *E. turcicum* populations rely on determining the mating-type distribution and frequency within populations and the assessment of the non-random association of alleles at different loci (Borchardt et al. 1998a, 1998b, 1998c; Human et al. 2016; Nieuwoudt et al. 2018). Sexual recombination has the potential to generate novel haplotypes with increased virulence and adaptability, thereby facilitating the breakdown of host resistance. In contrast, asexual reproduction via conidia maintains the local existence of the pathogen within a field and facilitates the rapid dispersal of beneficial alleles and haplotypes (McDonald and Linde 2002; Zhan 2016).

Population genetic studies of *E. turcicum* have provided crucial insights into its reproductive strategies, population differentiation, genetic diversity, population structure, gene flow and migration patterns (Human et al. 2016; Nieuwoudt et al. 2018; Weems and Bradley 2018). Previously, randomly amplified polymorphic DNA (Borchardt et al. 1998b, 1998c) and amplified fragment length polymorphic markers (Muiru et al. 2010) have been used to investigate the genetic architecture of *E. turcicum* populations infecting maize, with results consistently showing high levels of genetic diversity within this pathogen. Although these markers enable fast and cost-effective detection of DNA polymorphisms, they have low levels of polymorphism and are not easily reproducible, thereby making them unreliable for population genetic studies (Kumari and Thakur 2014; Skoneczny et al. 2015).

The development and application of microsatellite markers have substantially advanced our knowledge of the population dynamics of *E. turcicum* in recent years. These markers are highly polymorphic, widely distributed across eukaryotic genomes, inexpensive to develop and apply, and do not require high-quality or quantity of DNA for successful genotyping of fungal populations (Oliveira and Azevedo 2022; Singh 2015). Microsatellite markers have been used extensively to investigate the population structure of numerous economically important cereal pathogens (Jia et al. 2015; Liu et al. 2020; Mekonnen et al. 2020; Muller et al. 2016; Nsibo et al. 2019, 2021; Piotrowska et al. 2016). Specifically, they have been used to study *E. turcicum* populations in South Africa (Haasbroek et al. 2014; Human et al. 2016; Nieuwoudt et al. 2018), Turkey (Turgay et al. 2021) and China (Cui et al. 2024). Collectively, these studies confirm that *E. turcicum* exhibits high genetic diversity, largely attributed to a mixed reproductive lifestyle and high gene flow and migration, resulting in a predominantly panmictic population structure. Moreover, there is evidence of host specificity, as indicated by the limited or complete absence of shared haplotypes between *E. turcicum* populations isolated from maize and sorghum (Cui, Deng, et al. 2022; Nieuwoudt et al. 2018).

Importantly, all population genetic studies of *E. turcicum* conducted to date have been conducted either across different countries or between distinct host species. Despite Kenya and Uganda being among the earliest countries to report the existence of NLB in the region, there is limited information on the population genetics of *E. turcicum* in these two countries. Moreover, the population structure of *E. turcicum* between Kenya and Uganda, and other African countries, as well as the influence of geographic boundaries on its population differentiation and gene flow, remain largely unresolved. This knowledge gap has hindered efforts to understand the origin and migration patterns of this pathogen, thereby derailing concerted efforts to develop more effective management strategies and monitoring programmes for tracking pathogen dispersal and colonisation in new agroecological zones (AEZs) in Africa.

Evolutionary forces, such as migration, gene flow, mutation, reproduction and selection, may interact to collectively influence *E. turcicum*'s capacity to overcome host resistance and reduce its sensitivity to management practices, such as fungicide applications. Therefore, it is important to study these evolutionary forces at regional and global scales to elucidate their roles in shaping the population structure and persistence of *E. turcicum*. This study aimed to determine the genetic diversity of *E. turcicum* and elucidate the evolutionary forces shaping the genetic structure of the pathogen among smallholder farms in Kenya and Uganda, as well as between the two countries. Specifically, this study (i) determined the genetic diversity of *E. turcicum* in Kenya and compared it to that in Uganda, (ii) investigated the population structure and degree of genetic differentiation between *E. turcicum* populations in the two countries, and (iii) evaluated the role of sexual recombination within *E. turcicum* populations in Kenya and compared these dynamics with those observed in Uganda. This study has enhanced our understanding of the population biology of *E. turcicum* and lays a foundation for more accurate predictions of its future spread to enable the development of proactive and effective management strategies.

## 2 | Materials and Methods

### 2.1 | Sampling and Fungal Isolation

A total of 600 maize leaf samples exhibiting characteristic NLB symptoms (Figure 1A) were collected from smallholder maize fields in Kenya and Uganda (Table 1). The sampling strategy followed a diagonal transect sampling design, as described by Muller et al. (2016). All samples were grouped in accordance with AEZs. Samples from Kenya were grouped into theLower Highlands, Lower Midlands and Upper Midlands AEZs as defined by Jaetzold and Schmidt (1983). Likewise, samples collected from Uganda were initially assigned to Uganda's AEZs namely, Kyoga Plains, Highland Ranges, Lake Victoria Crescent,

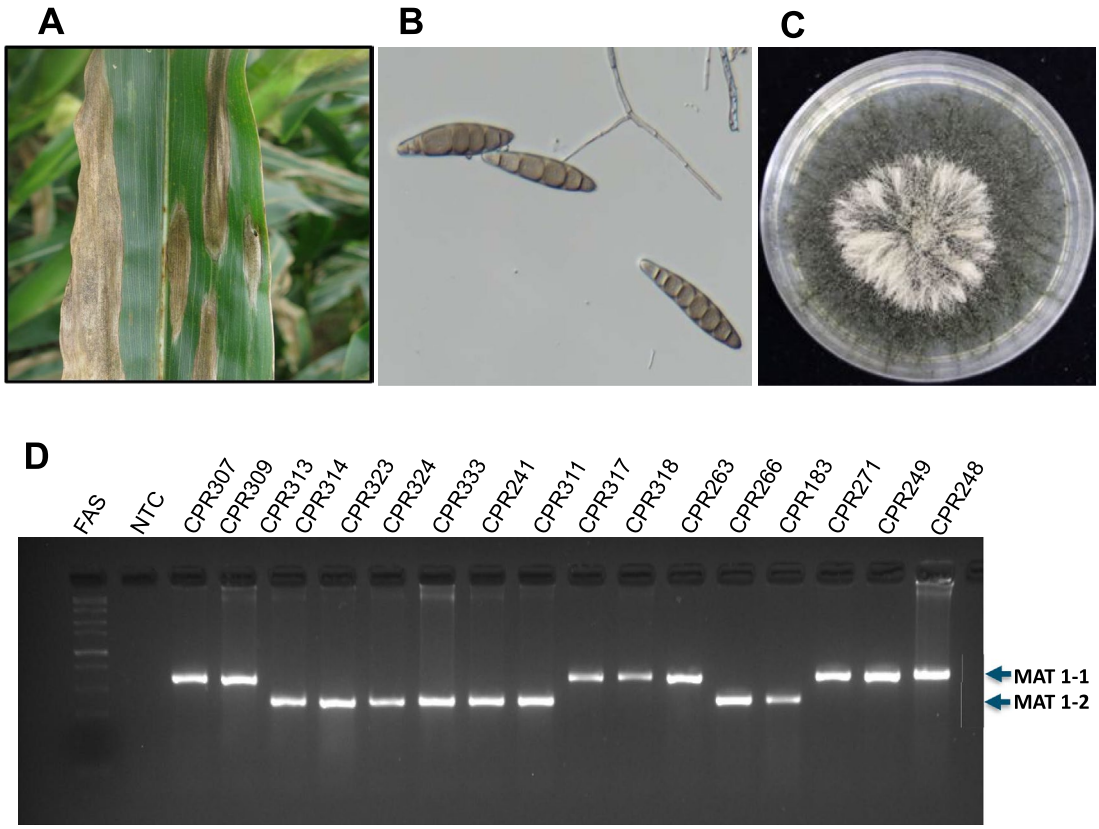


**FIGURE 1** | (A) Mature elliptical lesions of northern leaf blight. (B) *Exserohilum turcicum* conidia on potato dextrose agar. (C) Colony of *E. turcicum* from a single conidium on potato dextrose agar. (D) Amplification of the *MAT1* gene region of representative *E. turcicum* strains for species identification and mating type determination. The amplicon sizes of *MAT1-1* and *MAT1-2* are 608 and 393 bp, respectively. Lane 1: FAST DNA ladder (New England Biolabs), lane 2: Non-template control (NTC), lanes 3–18: *E. turcicum* strains collected from Uganda.

Eastern and Western Savannah Grasslands (Haneishi 2013; Masiga et al. 2021). For comparative analysis and uniform representation, all Uganda AEZs were subsequently harmonised to the closest Kenyan equivalents based on similarities in altitude, rainfall, temperature and major cropping systems (Figure 2, Table 1).

To induce sporulation of *E. turcicum*, 2 × 2 cm segments of symptomatic leaf tissue were surface sterilised with 2% (v/v) sodium hypochlorite for 2 min, followed by two rinses with sterile distilled water. Sterilised leaf pieces were then plated on sterile moistened filter paper and incubated at 25°C for 4 days. Single monoconidial spores (Figure 1B) characteristic of *E. turcicum* were isolated and transferred onto quarter-strength potato dextrose agar (PDA; 10 g potato dextrose agar with 12 g bacteriological agar; Merck) supplemented with 50 mg/mL cefotaxime (Austell Pharmaceuticals) and incubated until the mycelial growth stage (Craven and Fourie 2011). Subsequent series of culturing and subculturing yielded pure *E. turcicum* strain cultures (Figure 1C). A total of 618 strains were successfully preserved in 30% glycerol at −80°C for future analyses.

## 2.2 | DNA Extraction and PCR Amplification

Genomic DNA was extracted from 494 strains using a modified cetyltrimethylammonium bromide (CTAB) protocol following the methods of Doyle and Doyle (1987) and Schenk et al. (2023). Briefly, following the separation of nucleic acids into the aqueous phase and organic materials into the organic phase, RNase digestion (10 mg/mL) was performed for 30 min, followed by a 1:1 phenol-chloroform purification step. The DNA was then precipitated, eluted in double-distilled water and quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific).

To confirm that *E. turcicum* was the causal pathogen of NLB in the seven AEZs sampled across Kenya and Uganda, a mating-type diagnostic PCR assay was performed as described by Haasbroek et al. (2014). Specifically, *MAT1-1* and *MAT1-2* gene regions were amplified using species-specific mating-type primers: MAT 1–1F (5′-CTCGTCCTTGGAGAAGAATATC-3′), MAT 1–2F (5′-GCTCCTGGACCAAATAATACA-3′) and the common reverse primer MAT_CommonR (5′-AATGCGGAC ACGGAATAC-3′) in a multiplex PCR. The thermal cycling conditions consisted of an initial denaturation at 95°C for 2 min; 30 cycles at 95°C for 30 s, 61°C for 30 s and 72°C for 1 min; with a final extension at 72°C for 20 min (Haasbroek et al. 2014). Mating-type amplicons of each strain were visualised by gel electrophoresis, with amplicons of 608 bp for *MAT1-1* strains and 392 bp for *MAT1-2* strains (Figure 1D).

## 2.3 | Microsatellite Genotyping and Allele Scoring

Multiplex PCR amplification of 12 microsatellite markers (Table 2) was performed, followed by Genescan analysis for

**TABLE 1** | Geographical origin, coordinates, climate data, number of strains and date of collection of *Exserohilum turcicum* included in the study.

| Kenya equivalent zone | Uganda equivalent zone | District | Locality | Location | Rainfall (mm) | Temperature | No. of | Date of collection |
|---|---|---|---|---|---|---|---|---|
| Kenya_Lower Highlands[a] | Highland Ranges | Bomet | Sotiik | −0.6787, 35.1273 | 1500–2000 | Cool | 26 | Aug 2022 |
| | | Kericho | Bureti | −0.4752, 35.1726 | 950–3000 | Cool | 32 | Aug 2022 |
| | | Meru | Central Imenti | −0.1380, 37.6774 | 1500–1800 | Cool–moderate | 23 | Aug 2022 |
| | | Nanyuki | Umande | 0.0445, 37.1519 | 700–900 | Cool dry | 13 | Aug 2022 |
| | | Nyeri | Madhira | −0.4672, 37.0645 | 1500–2000 | Cool | 10 | Aug 2022 |
| | | Nyeri | Gitinga | −0.4672, 37.0645 | 950–3000 | Cool | 7 | Aug 2022 |
| | | Vihiga | Sabatia | 4.1021, 34.7230 | 950–3000 | Cool | 27 | Aug 2022 |
| Kenya_Lower Midlands[a] | Eastern Savannah Grassland | Embu | Mbeere | −0.4835, 37.4643 | 600–800 | Hot | 5 | Aug 2022 |
| | | Kisumu | Nyakach | −0.2644, 34.9695 | 700–900 | Hot | 22 | Aug 2022 |
| | Western Savannah Grassland | Siaya | Gem | 0.0954, 34.5276 | 1200–1500 | Warm | 25 | Aug 2022 |
| Kenya_Upper Midlands[a] | Kyoga Plains | Bungoma | Kanduyi | 0.5927, 34.6125 | 1600–1900 | Warm humid | 25 | Aug 2022 |
| | | Bungoma | Webuye East | 0.5850, 34.7999 | 500–2400 | Warm | 24 | Aug 2022 |
| | | Kakamega | Mumias East | 0.3354, 34.4975 | 1600–2000 | Warm humid | 41 | Aug 2022 |
| | | Kakamega | Lurambi | 0.2796, 34.7711 | 500–2400 | Warm | 35 | Aug 2022 |
| | | Kisumu | Maseno | −0.0044, 34.5966 | 1500–1800 | Warm humid | 32 | Aug 2022 |
| | | Muranga | Kandala | −0.9500, 37.1157 | 500–2400 | Cool–moderate | 29 | Aug 2022 |
| Uganda_Lower Highlands[b] | Highland Ranges | Kapchorwa | Siron Kisisin | 1.3311, 34.3508 | 500–2400 | Cool | 29 | Jul 2022 |
| | | Mbale | Mbale City | 1.0784, 34.1566 | 500–2400 | Cool–moderate | 15 | Jul 2022 |
| Uganda_Lower Midlands[b] | Western Savannah Grassland | Kiryandongo | Gwala | 2.2213, 32.2414 | 1000–1200 | Warm | 2 | Jul 2022 |
| | Eastern Savannah Grassland | Lira | Senior Quarters | 2.2440, 32.9054 | 900–1100 | Hot | 1 | Jul 2022 |
| | Western Savannah Grassland | Masindi | Nyambindo | 1.7828, 31.862 | 1300–1700 | Warm | 11 | Jul 2022 |
| Uganda_Upper Midlands[b] | Kyoga Plains | Pallisa | Pallisa | 1.1596, 33.7154 | 900–1200 | Hot | 9 | Jul 2022 |
| | | Iganga | Namasinge | 0.5872, 33.4650 | 1200–1500 | Warm | 10 | Jul 2022 |
| | | Iganga | Kinu | 0.8221, 33.4921 | 500–1500 | Warm | 10 | Jul 2022 |
| Uganda_Lake Victoria Basin[b] | Lake Victoria Crescent | Mukono | Mpoma | 0.4295, 32.7641 | 1400–1800 | Warm humid | 25 | Jul 2022 |

[a]Agroecological classifications, rainfall and temperature data derived from Jaetzold and Schmidt (1983).
[b]Agroecological classifications, rainfall and temperature data derived from Haneishi (2013) and Masiga et al. (2021).

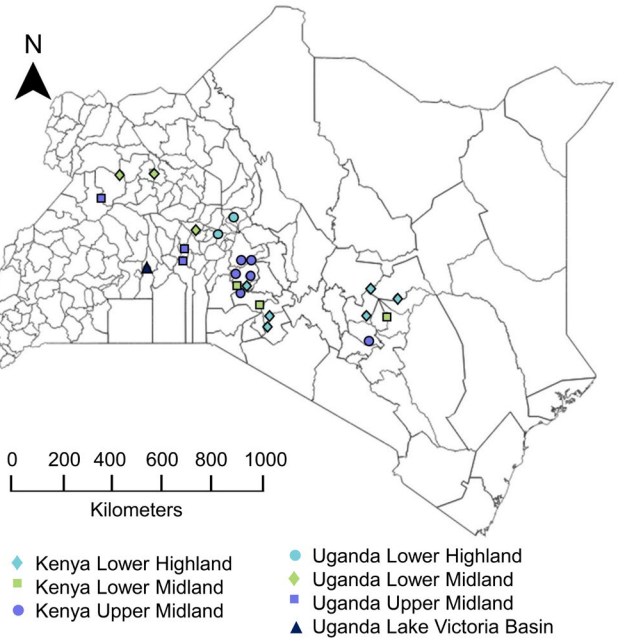


**FIGURE 2** | Collection sites of leaves infected with northern leaf blight from fields in Uganda and Kenya.

precise determination of fragment size. Three multiplex amplification reactions, hereafter referred to as SSR panels, were set up to amplify the 12 microsatellite markers based on the expected allelic size ranges and the fluorescent dye labels of the forward primers, as described by Haasbroek et al. (2014). The panels were configured as follows: SSR panel 1.1: SSR020, SSR021, SSR023 and SSR026; SSR panel 1.2: SSR002, SSR006 and SSR022; and SSR panel 2: SSR010, SSR024, SSR025, SSR027 and SSR031. Multiplex PCRs were performed in 20 μL volumes containing Platinum Multiplex master mix (Thermo Fisher Scientific), 100–150 ng of DNA and 2 μL of primer mix, consisting of equal volumes of each primer pair at optimised concentrations of 0.5–1.25 μM. The thermal cycling conditions were an initial denaturation at 95°C for 3 min; 30 cycles at 95°C for 30 s, 61°C for 15 s and 72°C for 1 min; with a final extension at 60°C for 45 min (Haasbroek et al. 2014). For fragment analysis, 1 μL of SSR panel 1.1 and 1 μL of SSR panel 1.2 amplicons were pooled together into one panel and diluted in 8 μL of distilled water. Both SSR panels 1 and 2 were independently submitted for capillary electrophoresis using an ABI PRISM 3500xl Genetic Analyser with an LIZ500 size standard (Applied Biosystems) to determine the fragment sizes for each microsatellite marker (Haasbroek et al. 2014). Alleles were scored using GeneMapper v. 6 software (Thermo Fisher Scientific).

## 2.4 | Gene and Genotypic Diversity

To validate that the 12 microsatellite markers provided sufficient power to resolve the population genetic structure, a haplotype accumulation curve was generated using the R package poppr v. 3.0.2 (Kamvar et al. 2014). This was to confirm that the 12 markers used in this study were adequate to allow the discovery of *E. turcicum* multilocus haplotypes (MLHs) and reliably discriminate between haplotypes within the studied populations. Although there was variability in the sample sizes, with Uganda Lower Midlands having the smallest population, bias in diversity estimates were mitigated by rarefying the allelic richness and related genetic diversity indices to the smallest population sample size. A non-clone-corrected dataset comprising 494 *E. turcicum* strains (representing all strains within each population) was used to determine the total number of alleles and private alleles across the 12 microsatellite markers. A clone-corrected dataset (retaining only one representative of each haplotype per population) was used to generate MLHs and calculate the genotypic and haplotype diversity indices.

Nei's unbiased gene diversity ($H_e$; Nei 1978), which is the measure of the probability that two randomly chosen alleles from any studied population are unique, was calculated using GenAlEx v. 6.501 (Peakall and Smouse 2012) according to the formula $H_e = \frac{n}{n-1}\left(1 - \sum p_i^2\right)$, where $p_i$ is the frequency of the $i$th allele and $n$ is the sample size (Peakall and Smouse 2012). The haplotype diversity of each population was determined using the Shannon–Wiener index of MLH diversity ($I$; Shannon 2001) and Simpson's index ($\lambda$; Simpson 1949). The Shannon–Wiener index of MLH diversity was calculated in GenAlEx v. 6.501 using the formula $I = -\sum p_i \ln(p_i)$, where $p_i$ is the frequency of the $i$th haplotype. The Simpson's index, which measures the probability that two haplotypes randomly selected from any population have the same alleles, was calculated based on the formula $\lambda = \Sigma\, p_i^2$, where $p_i$ is the proportion (frequency) of the $i$th haplotypes in the population, using the R package poppr v. 3.0.2 (Kamvar et al. 2014). The number of shared and private alleles, and allelic richness per locus, was determined for each population based on the rarefaction to the smallest population size of 14, using ADZE v. 1.0 software (Szpiech et al. 2008). The clonal fraction (CF), defined as the proportion of haplotypes arising through asexual reproduction, was calculated as CF = 1 − (number of unique haplotypes/total number of strains) and expressed as a percentage (Zhan et al. 2003).

## 2.5 | Statistical Analysis

Statistical differences among all populations in Kenya and those of Uganda (Kenya vs. Uganda) were assessed using the two-sample Student's *t*-tests implemented in Microsoft Excel assuming equal variances. All genetic indices calculated in this study were tested at country level to determine whether significant differences existed. The assumptions of the *t*-test were evaluated by visual inspection of the data distribution.

## 2.6 | Population Structure and Differentiation

Hierarchical analysis of molecular variation (AMOVA) was performed using GenAlEx v. 6.501 with 999 permutations to test for population differentiation under the null hypothesis of unrestricted gene flow within and among countries. The $\Phi_{PT}$ and standardised $\Phi_{PT}$ values were generated to estimate the degree of genetic differentiation (i) within and among haplotypes from AEZs within Kenya, and (ii) within and among haplotypes from AEZs within Uganda. Additionally, the degree of differentiation

**TABLE 2** | Primer description and size ranges for 12 primer pairs amplifying polymorphic microsatellite regions of *Exserohilum turcicum*, as described by Haasbroek et al. (2014).

| Panel | Locus | Sequence (5′–3′) | Motif repeat | GenBank accession number | Allelic range (bp) |
|---|---|---|---|---|---|
| 1.1 | SSR020 | GCGCGTTAATAGGGACTAGC | ATT | KJ439653 | 443–455 |
| | | CCTGCGAAGGCGATCTATTAC | | | |
| | SSR021 | GGTAAGTGCCGAAAGTGCC | AG | KJ439654 | 215–223 |
| | | TTCTGGATCCCACGGTTCG | | | |
| | SSR023 | CATATGCAGCGCTTGTCGG | GT | KJ439657 | 244–258 |
| | | AGAACTCATGGCCCTCGTG | | | |
| | SSR026 | GGACAAGTCCAGCGCAAAG | AAG | KJ439660 | 315–383 |
| | | ATGGTATCTGGTGCCCACG | | | |
| 1.2 | SSR002 | ATCATACTCGGGCGTCCAC | GCT | KJ439651 | 433–436 |
| | | TCAGCCTGCTCGACTGAAG | | | |
| | SSR006 | TGGGTGAGATCGAAGACGC | ACC | KJ439652 | 430–448 |
| | | TCCGGTCAAACTCAAGGGC | | | |
| | SSR022 | GGTGCAACCTTTCTCCGAC | AAT | KJ439656 | 324–343 |
| | | TGGCTCCAACTGGATTGGG | | | |
| 2 | SSR010 | TCTGTGCTGAGAAGCCCAC | GTT | KJ439653 | 330–333 |
| | | CAACCACGTGCATGATCCC | | | |
| | SSR024 | TGGCCACACTCTATGGCTG | AAG | KJ439658 | 387–493 |
| | | GAGCTTGACAAACGGCGAG | | | |
| | SSR025 | TACTCGCTAAGCACGTGGG | AC | KJ439659 | 273–291 |
| | | CCGTTTCCCAACTCGCATC | | | |
| | SSR027 | CAGCTTTGTCAAGGGCGTC | AC | KJ439661 | 191–205 |
| | | GTTCGCGACAGGTAATGGC | | | |
| | SSR031 | TGCCTCGTATCGTGCTACC | ACAT | KJ439663 | 289–431 |
| | | CTTGGATGTGCTCTGGAAACC | | | |

was determined within and between Kenya and Uganda and as a whole.

Bayesian clustering analysis based on the clone-corrected haplotypic microsatellite dataset was conducted using STRUCTURE v. 2.3.4 (Pritchard et al. 2002). The STRUCTURE software uses a Bayesian model-based clustering approach to assign haplotypes to populations, assuming $K$ genetic clusters/populations defined by allele frequencies (Pritchard et al. 2002). This analysis was performed using the admixture model with a burn-in period of 100,000 iterations followed by 1,000,000 Markov chain Monte Carlo iterations across 20 independent runs. The optimal number of clusters ($K$) was obtained using the $\Delta K$ method (Evanno et al. 2005), implemented in Clumpak (available at: http://clumpak.tau.ac.il) (Kopelman et al. 2015).

Discriminant analysis of principal components (DAPC), a non-parametric clustering method, was used to graphically visualise the genetic relationships among haplotypes (Jombart et al. 2010). The DAPC was performed using the find.clusters command of the R package adegenet v. 1.3 (Jombart 2008) in order to establish the genetically differentiated clusters across all studied *E. turcicum* populations.

## 2.7 | Mating-Type Analysis

Mating-type frequencies and distributions were assessed by amplifying *MAT* loci using a multiplex PCR assay (Haasbroek et al. 2014). $\chi^2$ goodness-of-fit tests were used to assess deviations from the expected 1:1 ratio of random mating in sexually reproducing populations, with statistical significance evaluated at $\alpha = 0.05$ (95% confidence interval). Two measures of linkage disequilibrium, namely the index of association ($I_A$) and the standardised index ($\bar{r}_d$), were calculated using the complete and clone-corrected datasets using Multilocus v. 1.3 (Agapow and Burt 2001). The observed measures were compared against

distributions generated from 1000 random permutations, with a significance threshold of 0.05. This was to identify deviations from random mating among loci, which is indicative of clonality and limited recombination.

### 2.8 | Gene Flow and Migration

To assess the statistical correlation between genetic distance and geographic distance, a Mantel test was performed in GenAlEx v. 6.501 using the clone-corrected dataset with 1000 permutations. To determine gene flow between populations from Kenya and Uganda, the number of migrants ($N_m$) was calculated in GenAlEx v. 6.501 using the formula $N_m = [(1/\Phi_{PT}) - 1]/2$, where $\Phi_{PT}$ represents the degree of population differentiation. The presence of shared haplotypes was investigated to assess the potential for long-distance haplotype dispersal within *E. turcicum* populations.

To further evaluate migration within and among regions in Kenya and Uganda, Migrate-N v. 3.6.11 (Beerli and Felsenstein 2001) was used. Migrate-N estimates the migration rates between populations based on the maximum likelihood and coalescence theory (Beerli 2006). The analysis assumes the Wright Fisher model, occurrence of random mating, no selection, non-overlapping generations, constant mutation rates and effective population sizes (Beerli and Felsenstein 2001). The mutation-scaled migration rate (M), representing the number of migrants per generation, was calculated for the following scenarios: (i) full migration between Kenya and Uganda, (ii) unidirectional movement of haplotypes from Kenya to Uganda, (iii) unidirectional movement of haplotypes from Uganda to Kenya and (iv) panmixia (assumed as a single large population with no structure). A Brownian motion approximation with a stepwise mutation model was implemented using five parallel MCMC chains, each with 10,000 steps, 50 increments with a burn-in of 50,000 steps. A static heating scheme with four different temperature levels (1.0, 1.5, 3.0 and 1,000,000) was used to improve convergence following Nsibo et al. (2021).

## 3 | Results

### 3.1 | Gene and Genotypic Diversity

Analysis of the 12 microsatellite markers revealed 120 alleles in seven populations ($n = 420$) of *E. turcicum* from Kenya and Uganda. Visual observation of the haplotype accumulation curve revealed that the haplotypic diversity levelled off at 11 markers (Figure S1). All markers were polymorphic, with the number of alleles per locus ranging from two (SSR010) to 32 (SSR024), with an overall average of 10 alleles across all loci (Table 3). The Simpson diversity index ($1 - D$) per locus varied from 0.02 (SSR010) to 0.89 (SSR024), with an average value of 0.47. The genetic evenness, which is the relative distribution of allele frequencies at a locus, ranged from 0.36 (SSR010) and 0.83 (SSR022) with an average of 0.62 (Table 3).

A total of 494 *E. turcicum* strains were obtained, representing 420 MLHs across seven populations and an average of 12 effective MLHs (eMLHs) between Kenya and Uganda was observed (Table 4). Populations from Kenya had a significantly higher frequency of private alleles (%Pa = 32%, $p = 0.022$) than those from Uganda (%Pa = 8%, $p = 0.022$). An average allelic richness (Ra) of $2.2 \pm 0.21$ for Kenya populations and $1.96 \pm 0.22$ for Uganda populations (Table 4) was observed. Furthermore, the percentage of polymorphic loci was generally high for all populations, ranging from 83% to 100% for Kenya and 92% to 100% for Uganda. The clonal fraction was generally higher in populations from Uganda, ranging from 12.9% to 20%, than in populations from Kenya, which ranged from 7.5% to 17.8% (Table 4).

**TABLE 3** | Summary statistics for the 12 microsatellite markers used to assess the genetic diversity of *Exserohilum turcicum* in Kenya and Uganda.

| Locus | No. of alleles | Simpson index ($1 - D$) | Nei's unbiased gene diversity ($H_e$) | Evenness |
|---|---|---|---|---|
| SSR002 | 9 | 0.63 | 0.63 | 0.74 |
| SSR006 | 8 | 0.59 | 0.59 | 0.75 |
| SSR010 | 2 | 0.02 | 0.02 | 0.36 |
| SSR020 | 3 | 0.17 | 0.18 | 0.52 |
| SSR021 | 4 | 0.37 | 0.38 | 0.67 |
| SSR022 | 6 | 0.60 | 0.60 | 0.83 |
| SSR023 | 4 | 0.44 | 0.44 | 0.74 |
| SSR024 | 32 | 0.89 | 0.90 | 0.69 |
| SSR025 | 7 | 0.15 | 0.15 | 0.38 |
| SSR026 | 22 | 0.63 | 0.63 | 0.44 |
| SSR027 | 6 | 0.48 | 0.48 | 0.71 |
| SSR031 | 17 | 0.61 | 0.62 | 0.59 |
| Average | 10.0 | 0.47 | 0.47 | 0.62 |

The highest genotypic diversity was recorded in Kenya Upper Midlands ($I = 0.931$, $\lambda = 0.993$), followed by Kenya Lower Highlands ($I = 0.910$, $\lambda = 0.99$) (Table 4). The lowest genotypic diversity was observed in Uganda Lower Midlands ($I = 0.754$, $\lambda = 0.917$). Overall, the genotypic diversity of all populations was significantly higher in Kenya ($I = 0.91$, $\lambda = 0.99$) than Uganda ($I = 0{,}77$, $\lambda = 0.95$) at $p < 0.05$. The average evenness index ($E_5$) was 1 for populations from both countries (Table 4).

Gene diversity across the seven populations ranged from 0.40 to 0.46, with Kenya Lower Midlands exhibiting the highest gene diversity ($H_e = 0.46$), followed by Kenya Upper Midlands ($H_e = 0.45$). In contrast, Uganda Lake Victoria Basin recorded the lowest level of gene diversity ($H_e = 0.40$; Table 3). Overall, Kenya exhibited a slightly higher average gene diversity ($H_e = 0.45$) than Uganda ($H_e = 0.42$); however, these differences were not statistically significant (Table 4).

**TABLE 4** | Indices of genetic diversity for 484 strains of *Exserohilum turcicum* collected from the six sampled maize-growing regions of Kenya and Uganda.

| Population | *N* | MLH | eMLH | Na | Ne | Pa | %Pa | Ra | %P | %CF | *I* | $\lambda$ | $E_5$ | $H_e$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kenya_ Lower Highlands | 144 | 125 | 12 | 6.75 | 2.34 | 14 | 13 | 2.01 ± 0.21 | 100 | 13.2 | 0.91 | 0.99 | 1.00 | 0.44 |
| Kenya_ Lower Midlands | 53 | 49 | 12 | 4.42 | 2.41 | 5 | 5 | 2.05 ± 0.21 | 83 | 7.5 | 0.88 | 0.98 | 1.00 | 0.46 |
| Kenya_ Upper Midlands | 185 | 152 | 12 | 7.25 | 2.65 | 15 | 14 | 2.02 ± 0.22 | 92 | 17.8 | 0.93 | 0.99 | 1.00 | 0.45 |
| Uganda_ Lower Highlands | 44 | 37 | 12 | 3.92 | 2.37 | 2 | 2 | 1.92 ± 0.23 | 92 | 15.9 | 0.77 | 0.97 | 1.00 | 0.42 |
| Uganda_ Lower Midlands | 14 | 12 | 12 | 3.08 | 2.28 | 2 | 2 | 2.04 ± 0.24 | 92 | 14.3 | 0.75 | 0.92 | 1.00 | 0.44 |
| Uganda_ Upper Midlands | 29 | 25 | 11.8 | 3.75 | 2.19 | 1 | 1 | 1.97 ± 0.20 | 100 | 13.8 | 0.79 | 0.96 | 0.98 | 0.43 |
| Uganda_ Lake Victoria Basin | 25 | 20 | 12 | 3.83 | 2.35 | 4 | 4 | 1.93 ± 0.24 | 92 | 20.0 | 0.76 | 0.95 | 1.00 | 0.40 |
| Kenya Populations | 382 | 326 | 12 | 6.14 | 2.47 | 34 | 32 | 2.20 ± 0.21 | 92 | 12.9 | 0.91 | 0.99 | 1.00 | 0.45 |
| Uganda Populations | 112 | 94 | 12 | 3.65 | 2.30 | 9 | 8 | 1.96 ± 0.22 | 94 | 16.0 | 0.77 | 0.95 | 0.99 | 0.42 |
| Kenya versus Uganda | ns | ns | ns | 0.022 | ns | 0.022 | 0.022 | ns | ns | ns | 0.0003 | 0.04 |  | ns |

*Note: N*, number of individuals observed; MLH, number of multilocus haplotypes observed; eMLH, the number of expected MLH at the smallest sample size ≥ 10 based on rarefaction; Na, number of alleles; Ne, number of effective alleles; Pa, number of private alleles; %P, percentage of polymorphic markers; %CF, clonal fraction, calculated as CF = 1 − (number of unique haplotypes/total number of strains) and expressed as a percentage (Zhan et al. 2003); *I*, Shannon–Wiener Index of MLH diversity (Shannon 2001); $\lambda$, Simpson's index (Simpson 1949); $E_5$, evenness (Grünwald et al. 2003; Ludwig and Reynolds 1988; Pielou 1969); $H_e$, Nei's unbiased gene diversity (Nei 1978).

### 3.2 | Mating-Type Distribution and Recombination

Among the 469 *E. turcicum* strains from Kenya and Uganda screened for mating types, both *MAT 1-1* (Uganda = 33, Kenya = 156) and *MAT 1-2* (Uganda = 73, Kenya = 207) idiomorphs were detected. However, their distributions deviated significantly from the expected 1:1 ratio of a randomly mating population, with a higher frequency of the *MAT 1-2* idiomorph observed overall (Table 5). Significant deviations from the 1:1 ratio were observed in populations from Kenya Upper Midlands, and all populations from Uganda except the Lake Victoria Basin population (*MAT1-1*/*MAT1-2* = 1.0; Table 5). Similarly, the $\chi^2$ analysis revealed a significant deviation from the expected 1:1 mating-type ratio for Kenya Upper Midlands, Uganda Lower Highlands, and for Uganda Lower and Upper Midlands (Table 5). Furthermore, the measures of linkage disequilibrium ($I_A$ and $r_d$) were significant in all populations (Table 5).

### 3.3 | Population Differentiation

Hierarchical analysis of molecular variance conducted among populations in Kenya revealed that the majority of genetic variability was within regions (99%), with only 1% attributable to differences among regions (Table 6). Overall, the populations in Kenya showed a significant lack of population differentiation, as indicated by low $\Phi_{PT}$ values ($\Phi_{PT} = 0.01$, $p = 0.001$) and high gene flow among populations indicated by high number of migrants ($N_m$) ranging from 51 to 90 (Table 6).

For the populations in Uganda, AMOVA revealed that 98%–100% of the genetic variation was within populations, with only 1%–2% among populations (Table 6). A panmictic pattern was observed between Uganda Lower Highlands and Upper Midlands ($\Phi_{PT} = -0.02$, $p = 0.928$, $N_m = 95$) and between

**TABLE 5** | The distribution of *MAT 1–1* and *MAT 1–2* idiomorphs and measures of linkage disequilibrium of 470 *Exserohilum turcicum* strains from six sampled maize-growing regions of Kenya and Uganda.

| Population | *N* | *MAT1-1* | *MAT1-2* | *MAT1-1/ MAT1-2* | $\chi^2$ (*p*) | PrC (*p*) | $I_A$ (*p*) | $\bar{r}_d$ (*p*) |
|---|---|---|---|---|---|---|---|---|
| Kenya_Lower Highland | 136 | 66 | 70 | 0.9 | 0.12 (0.732) | 0.24 (<0.001) | 0.72 (<0.001) | 0.07 (<0.001) |
| Kenya_Lower Midland | 53 | 27 | 26 | 1.0 | 0.02 (0.891) | 0.33 (<0.001) | 1.52 (<0.001) | 0.14 (<0.001) |
| Kenya_Upper Midland | 174 | 63 | 111 | 0.6 | 13.24 (<0.001) | 0.20 (<0.001) | 0.63 (<0.001) | 0.06 (<0.001) |
| Subtotal (Kenya population) | 363 | 156 | 207 | 0.8 | | 0.26 (<0.001) | 0.95 (<0.001) | 0.09 (<0.001) |
| Uganda_Lower Highland | 39 | 9 | 30 | 0.3 | 11.31 (<0.001) | 0.49 (<0.001) | 2.01 (<0.001) | 0.19 (<0.001) |
| Uganda_Lower Midland | 11 | 2 | 9 | 0.2 | 4.46 (0.035) | 0.79 (<0.001) | 3.25 (<0.001) | 0.31 (<0.001) |
| Uganda_Upper Midland | 30 | 9 | 21 | 0.4 | 4.80 (0.028) | 0.44 (<0.001) | 2.32 (<0.001) | 0.21 (<0.001) |
| Uganda_Lake Victoria Basin | 26 | 13 | 13 | 1.0 | 0.000 (1.000) | 0.76 (<0.001) | 3.25 (<0.001) | 0.30 (<0.001) |
| Subtotal (Uganda population) | 106 | 33 | 73 | 0.5 | | 0.617 (<0.001) | 2.71 (<0.001) | 0.25 (<0.001) |

*Note: N*, number of strains per region assayed for mating type (not clone-corrected). $\chi^2$ values to test the null hypothesis of a 1:1 distribution ratio with one degree of freedom (*p*-values in parentheses; $p > 0.05$ indicates populations where the null hypothesis was not rejected, indicating random mating). A non-clone-corrected dataset was used in this study. PrC, proportion of compatible pairs of loci generated in Multilocus v. 1.3 (Agapow and Burt 2001); $I_A$, index of association; $\bar{r}_d$: standardised index of association between microsatellite markers, calculated by 999 permutations using Multilocus v. 1.3. A clone-corrected microsatellite dataset was used for the calculations. The null hypothesis is no linkage disequilibrium (LD), $p > 0.05$ indicates populations that are not in LD, which could be due to random mating.

Uganda Lower Midlands and Lake Victoria Basin ($\Phi_{PT} = -0.01$, $p = 0.605$, $N_m = 96$). Pairwise comparisons of populations from Kenya and Uganda revealed a significant population differentiation, with $\Phi_{PT}$ values ranging from 0.17 to 0.20 ($p = 0.001$; Table 6), being supported by very few migrants ($N_m$ ranging from 2 to 3). Overall, AMOVA detected considerable population differentiation between Kenya and Uganda ($\Phi_{PT} = 0.1$; $p = 0.001$), with 10% accounting for the genetic variation between the two countries. This result was supported by a low estimated number of migrants between countries ($N_m = 5$).

### 3.4 | Population Structure

The $\Delta K$ plot revealed that the most probable number of genetic clusters was $K = 2$ (Figure 3A), with all regions within Uganda clustering into a single genetic cluster, and the regions from Kenya forming a distinct genetic cluster (Figure 3B). Despite this clear separation, admixture signatures were detected in all populations. As the number of clusters increased above two ($K = 3$ and $K = 4$), there was considerable admixture in all populations. DAPC showed limited population structure between countries (Figure 4A) and an overlap of haplotypes within countries (Figure 4B,C). Overall, gene flow between countries was limited, with the Kenya Upper Midlands and Uganda Upper Midlands being identified as the major sources of inter-regional migrants (Figure 4A).

### 3.5 | Migration Patterns of *E. turcicum* Populations in Kenya and Uganda

The Mantel test (Figure 5A) revealed a weak but significant positive correlation between the geographic and genetic distances among *E. turcicum* populations from Kenya and Uganda ($r = 0.068$, $p = 0.010$). Migration analysis assuming the full migration scenario was presented as a 7×7 migration matrix encompassing populations from Kenya Lower Highlands, Lower Midlands and Upper Midlands as well as Uganda Lower Highlands, Lower Midlands, Upper Midlands and Lake Victoria Basin. Kenya had the largest effective population sizes, ranging from 2.09 (Kenya Lower Midlands) to 4.46 (Kenya Upper Midlands) (Table 7). On the other hand, Uganda had the lowest effective population sizes, ranging from 0.91 (Uganda Lower Midlands) to 1.93 (Uganda Lower Highlands and Upper Midlands) (Table 7).

The estimated mutation-scaled migration rates ranged from 1.30 to 2.54 within Kenya and from 1.42 to 2.42 within Uganda. Inter-country migration rates were generally lower, ranging from 1.50 (0.52–2.42) (migration from Uganda Lake Victoria Basin to Kenya Upper Midlands) to 1.66 (0.56–2.64) (migration from Kenya Lower Highlands to Uganda Lower Highlands). The highest migration rates involved Kenya Upper Midlands (mean outgoing migration rate = 1.99) and Uganda Upper Midlands (mean outgoing migration rate = 2.06), making them the major contributors to regional gene flow (Figure 5, Table 7).

**TABLE 6** | Population differentiation and analysis of molecular variance (AMOVA) for 484 strains of *Exserohilum turcicum* from six sampled maize-growing regions of Kenya and Uganda.

| Comparison | Source | df | SS | MS | Estimated variance | % of the total variance | $\Phi_{PT}$ ($p$) | $N_m$ (haploid) |
|---|---|---|---|---|---|---|---|---|
| Kenya_Lower Highland and Kenya_Lower Midland | Between populations | 1 | 4 | 4 | 0.01 | 1 | 0.01 (0.125) | 90 |
| | Within populations | 172 | 460 | 3 | 2.68 | 99 | | |
| Kenya_Lower Highland and Kenya_Upper Midland | Between populations | 1 | 6 | 6 | 0.02 | 1 | 0.01 (0.008) | 60 |
| | Within populations | 275 | 730 | 3 | 2.65 | 99 | | |
| Kenya_Lower Highland and Uganda_Lower Highland | Between populations | 1 | 37 | 37 | 0.60 | 19 | 0.19 (0.001) | 2 |
| | Within populations | 160 | 416 | 3 | 2.60 | 81 | | |
| Kenya_Lower Highland and Uganda_Lower Midland | Between populations | 1 | 15 | 15 | 0.55 | 17 | 0.17 (0.001) | 2 |
| | Within populations | 135 | 356 | 3 | 2.64 | 83 | | |
| Kenya_Lower Highland and Uganda_Upper Midland | Between populations | 1 | 29 | 29 | 0.64 | 20 | 0.20 (0.001) | 2 |
| | Within populations | 148 | 389 | 3 | 2.63 | 80 | | |
| Kenya_Lower Highland and Uganda_Lake Victoria Basin | Between populations | 1 | 22 | 22 | 0.56 | 18 | 0.18 (0.001) | 2 |
| | Within populations | 143 | 372 | 3 | 2.60 | 82 | | |
| Kenya_Lower Midland and Kenya_Upper Midland | Between populations | 1 | 5 | 5 | 0.03 | 1 | 0.01 (0.032) | 51 |
| | Within populations | 199 | 537 | 3 | 2.70 | 99 | | |
| Kenya_Lower Midland and Uganda_Lower Highland | Between populations | 1 | 30 | 30 | 0.66 | 20 | 0.20 (0.001) | 2 |
| | Within populations | 84 | 223 | 3 | 2.66 | 80 | | |
| Kenya_Lower Midland and Uganda_Lower Midland | Between populations | 1 | 15 | 15 | 0.68 | 19 | 0.19 (0.001) | 2 |
| | Within populations | 58 | 164 | 3 | 2.83 | 81 | | |
| Kenya_Lower Midland and Uganda_Upper Midland | Between populations | 1 | 25 | 25 | 0.68 | 20 | 0.20 (0.001) | 2 |
| | Within populations | 72 | 196 | 3 | 2.72 | 80 | | |
| Kenya_Lower Midland and Uganda_Lake Victoria Basin | Between populations | 1 | 22 | 22 | 0.68 | 20 | 0.20 (0.001) | 2 |
| | Within populations | 67 | 179 | 3 | 2.67 | 80 | | |

(Continues)

**TABLE 6** | (Continued)

| Comparison | Source | df | SS | MS | Estimated variance | % of the total variance | $\Phi_{PT}$ ($p$) | $N_m$ (haploid) |
|---|---|---|---|---|---|---|---|---|
| Kenya_Upper Midland and Uganda_Lower Highland | Between populations | 1 | 35 | 35 | 0.55 | 17 | 0.17 (0.001) | 2 |
| | Within populations | 187 | 493 | 3 | 2.63 | 83 | | |
| Kenya_Upper Midland and Uganda_Lower Midland | Between populations | 1 | 13 | 13 | 0.47 | 15 | 0.15 (0.001) | 3 |
| | Within populations | 162 | 432 | 3 | 2.67 | 85 | | |
| Kenya_Upper Midland and Uganda_Upper Midland | Between populations | 1 | 28 | 28 | 0.60 | 18 | 0.18 (0.001) | 2 |
| | Within populations | 175 | 466 | 3 | 2.66 | 82 | | |
| Kenya_Upper Midland and Uganda_Lake Victoria Basin | Between populations | 1 | 21 | 21 | 0.52 | 16 | 0.16 (0.001) | 3 |
| | Within populations | 170 | 448 | 3 | 2.64 | 84 | | |
| Uganda_Lower Highland and Uganda_Lower Midland | Between populations | 1 | 4 | 4 | 0.05 | 2 | 0.02 (0.168) | 24 |
| | Within populations | 47 | 119 | 3 | 2.53 | 98 | | |
| Uganda_Lower Highland and Uganda_Upper Midland | Between populations | 1 | 1 | 1 | 0.00 | 0 | −0.02 (0.928) | 95 |
| | Within populations | 60 | 152 | 3 | 2.54 | 100 | | |
| Uganda_Lower Highland and Uganda_Lake Victoria Basin | Between populations | 1 | 3 | 3 | 0.01 | 1 | 0.01 (0.282) | 89 |
| | Within populations | 55 | 135 | 2 | 2.45 | 99 | | |
| Uganda_Lower Midland and Uganda_Upper Midland | Between populations | 1 | 3 | 3 | 0.03 | 1 | 0.01 (0.222) | 41 |
| | Within populations | 35 | 92 | 3 | 2.63 | 99 | | |
| Uganda_Lower Midland and Uganda_Lake Victoria Basin | Between populations | 1 | 2 | 2 | 0.00 | 0 | −0.01 (0.605) | 96 |
| | Within populations | 30 | 75 | 2 | 2.49 | 100 | | |
| Uganda_Upper Midland and Uganda_Lake Victoria Basin | Between populations | 1 | 3 | 3 | 0.03 | 1 | 0.01 (0.183) | 38 |
| | Within populations | 43 | 108 | 3 | 2.51 | 99 | | |
| Kenya and Uganda (overall) | Between countries | 1 | 87 | 87 | 0.58 | 18 | 0.18 (0.001) | 2 |
| | Within countries | 418 | 1108 | 3 | 2.65 | 82 | | |

*Note:* $\Phi_{PT}$, the measure of the genetic differentiation between countries (Peakall and Smouse 2012). All values are significant at $p < 0.05$. The null hypothesis states that there is no genetic differentiation between populations. $N_m$: absolute number of migrants per generation between different populations.
Abbreviations: df, degrees of freedom; MS, mean of squared observations; SS, sum of squared observations.

## 4 | Discussion

The population genetics of *E. turcicum* in Africa remains poorly characterised, with prior studies limited to Kenya and South Africa using RAPDs and microsatellite markers, respectively. This study represents the first use of microsatellite markers to investigate the population genetic diversity of *E. turcicum* populations north of South Africa. The findings of

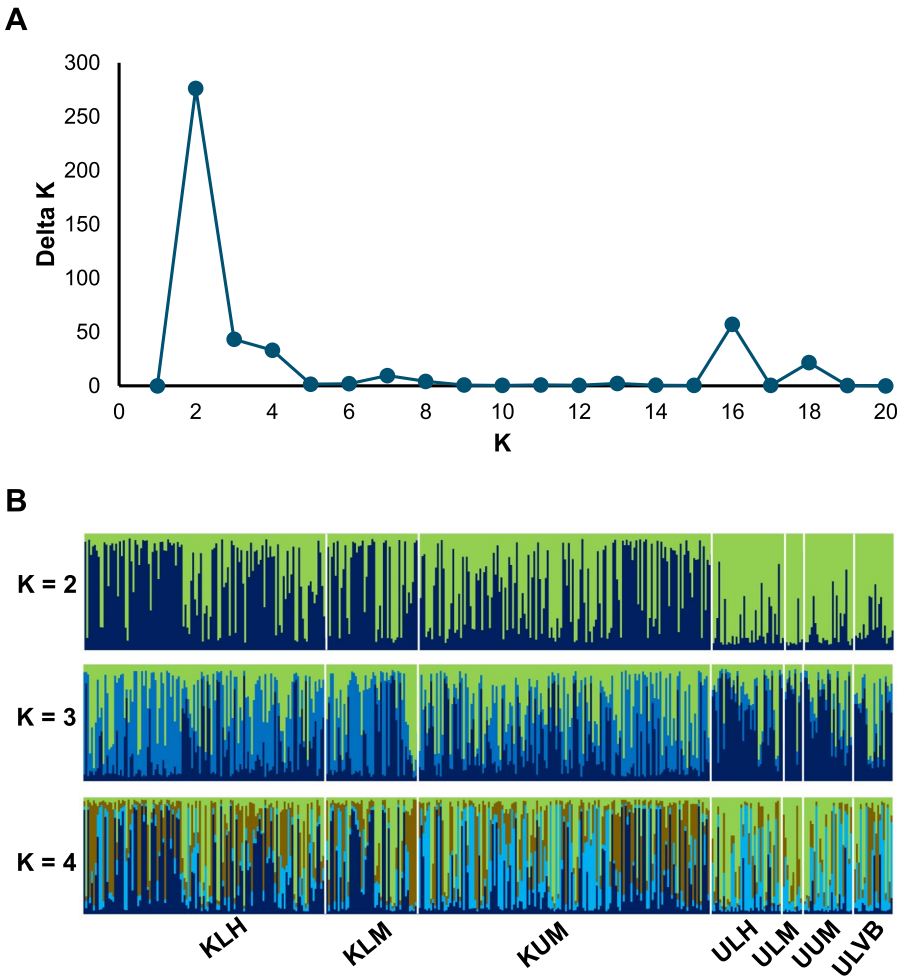


**FIGURE 3** | (A) Δ*K* plot implemented in CLUMPAK, representing the most likely number of genetic clusters based on the Evanno method (Evanno et al. 2005). (B) Population genetic structure of 420 *Exserohilum turcicum* strains from Kenya and Uganda implemented in STRUCTURE v. 2.3.4 (Pritchard et al. 2002). Each vertical bar represents an individual multilocus haplotype (MLH). KLH: KenyaLower Highlands, KLM: Kenya Lower Midlands, KUM: Kenya Upper Midlands, ULH: Uganda Lower Highlands, ULM, Uganda Lower Midlands; ULVB, Uganda Lake Victoria Basin; UUM, Uganda Upper Midlands.

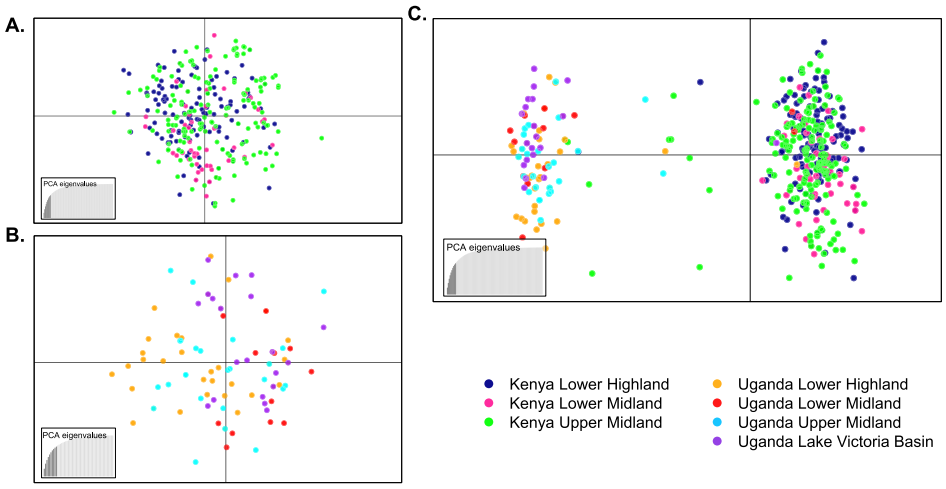


**FIGURE 4** | Discriminant analysis of principal components (DAPC) of (A) 420 *Exserohilum turcicum* strains in seven sampled regions in Kenya and Uganda; (B) 326 *E. turcicum* strains collected from Kenya; and (C) 94 *E. turcicum* strains collected from Uganda.

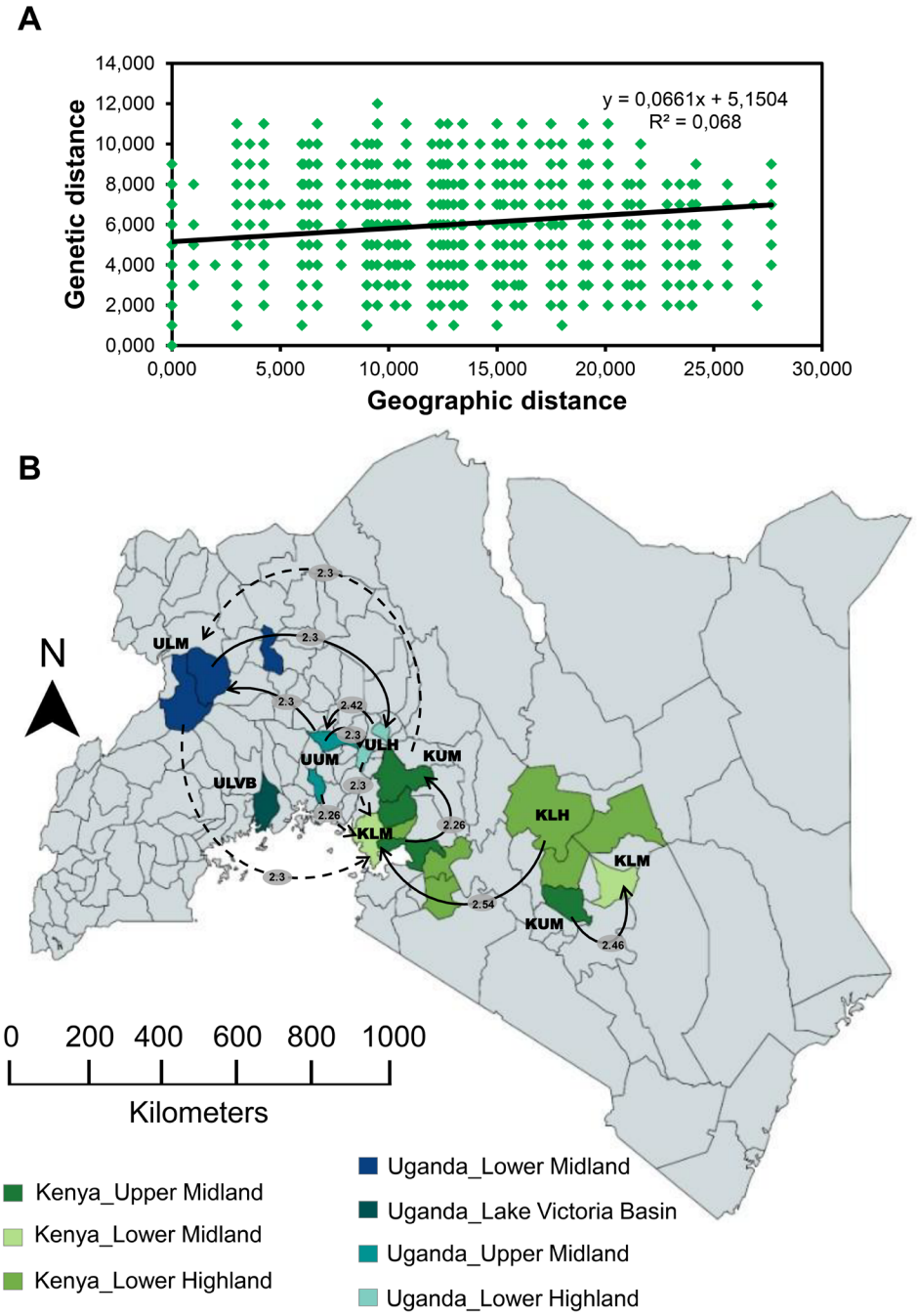


**FIGURE 5** | (A) Mantel test depicting correlation between Nei's genetic distance and geographic distance for *Exserohilum turcicum* populations in Uganda and Kenya implemented in GenAlEx v. 6.501 (Peakall and Smouse 2012). (B) Migration patterns among *E. turcicum* populations in Kenya and Uganda as implemented in Migrate-N v. 5.0.6 (Beerli 2006). Mutation-scaled migration rates are specified in grey ellipses (for migration routes for $m \geq 2.26$) based on the full migration scenario. Dashed arrows indicate migration routes between the two countries, and solid lines indicate migration routes within a country. KLH, Kenya Lower Highlands; KLM, Kenya Lower Midlands; KUM, Kenya Upper Midlands; ULH, Uganda Lower Highlands; ULM, Uganda Lower Midlands; ULVB, Uganda Lake Victoria Basin; UUM, Uganda Upper Midlands.

this study thus revealed that (i) *E. turcicum* exhibited lower levels of genetic diversity than previous population studies; (ii) there was limited occurrence of sexual reproduction, as indicated by the deviations from random mating expectations; (iii) *E. turcicum* populations were structured according to country, suggesting restricted movement of genotypes and limited gene flow between Kenya and Uganda; and (iv) there were signatures of directional migration of *E. turcicum* detected between Kenya and Uganda.

In this study, both gene and genotypic diversities of *E. turcicum* populations from Kenya and Uganda were relatively low when compared with previous population genetics studies that employed microsatellite markers (Human et al. 2016; Nieuwoudt et al. 2018). Notably, these results are consistent with recent studies from China (Cui et al. 2024; Cui, Zhao, et al. 2022) and Turkey (Turgay et al. 2021), which also reported low levels of genetic diversity in *E. turcicum* populations based on both microsatellite and single-nucleotide polymorphism (SNP) markers. It

**TABLE 7** | Population estimates of effective population size (θ) and pairwise mutation-scaled migration rates of six *Exserohilum turcicum* populations based on four scenarios.

| Source population | θ[a] | Sink population | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | KLH | KLM | KUM | ULH | ULM | UUM | ULVB |
| KLH | 4.19 | — | 2.54 [1.32–3.64] | 1.30 [0.44–2.00] | 1.66 [0.56–2.64] | 1.90 [0.80–2.92] | 1.98 [0.80–3.04] | 1.87 [0.60–3.04] |
| KLM | 2.09 | | — | 2.26 [1.20–3.20] | 1.90 [0.64–3.04] | 1.86 [0.64–2.96] | 1.82 [0.60–3.00] | 1.70 [0.60–3.10] |
| KUM | 4.46 | 1.78 [0.80–2.72] | 2.46 [1.12–3.68] | — | 1.82 [0.68–2.84] | 2.30 [1.16–3.32] | 1.90 [0.84–2.84] | 1.68 [0.84–2.64] |
| ULH | 0.93 | 1.53 [0.52–2.52] | 2.30 [1.04–3.48] | 1.78 [0.64–2.76] | — | 2.14 [1.04–3.12] | 2.42 [1.28–3.48] | 2.28 [1.28–3.26] |
| ULM | 0.91 | 1.70 [0.72–2.64] | 2.30 [1.12–3.36] | 1.70 [0.56–2.68] | 2.30 [1.16–3.32] | — | 1.42 [0.60–2.16] | 2.00 [0.60–2.78] |
| UUM | 1.93 | 1.70 [0.68–2.68] | 2.26 [0.96–3.44] | 1.58 [0.52–2.52] | 2.30 [1.16–3.36] | 2.30 [1.20–3.28] | — | 2.19 [0.60–3.10] |
| ULVB | 1.89 | 1.69 [0.68–2.78] | 2.11 [0.96–3.20] | 1.50 [0.52–2.42] | 2.25 [1.16–3.30] | 2.28 [1.20–3.18] | 2.02 [1.20–2.88] | — |

*Note:* Mode values of migration rate, M. 95% confidence intervals are shown in brackets.
Abbreviations: KLH, Kenya Lower Highlands; KLM, Kenya Lower Midlands; KUM, Kenya Upper Midlands; ULH, Uganda Lower Highlands; ULM, Uganda Lower Midlands; ULVB, Uganda Lake Victoria Basin; UUM, Uganda Upper Midlands.
[a]$\theta = 2N_e\mu$, where $N_e$ is the number of individuals in a population (Beerli 2006).

can be concluded that low genetic diversity observed this study is not unique to East African populations but a common pattern in several other maize-producing regions.

Low genetic diversity within pathogen populations has previously been attributed to reduced rates of sexual recombination, low mutation rates and small effective population sizes, all of which limit the generation and maintenance of allelic variation (Ellegren and Galtier 2016; Xu et al. 2019). In contrast, larger effective population sizes have the potential to shield populations against genetic drift, thereby reducing the probability of new mutations being fixed and contributing to the preservation of higher genetic diversity (Xu et al. 2019). This study has successfully established that there are high levels of clonality and low sexual recombination within *E. turcicum* populations in Kenya and Uganda. Notably, comprehensive measurements of effective population size and mutation rates have not yet been determined in *E. turcicum* populations. Therefore, future research is essential to establish the relationship between the observed low genetic diversity, effective population size and mutation rates at the genomic level of *E. turcicum*.

Despite the lower gene diversity observed in Kenya and Uganda, the populations exhibited a high genotypic diversity, as depicted by the large number of MLHs recovered relative to the total number of *E. turcicum* isolates studied. The low gene diversity reflects uneven allelic frequencies within populations rather than a lack of genotypic variation. This is common within populations of fungal plant pathogens that predominantly undergo clonal reproduction where mutation, migration and local adaptation (McDonald and Linde 2002; Milgroom 1996; Taylor et al. 1999) can generate novel, distinct multilocus genotypes. Similar results of low gene–high genotypic diversity were reported for *E. turcicum* populations from South Africa (Human et al. 2016; Nieuwoudt et al. 2018), indicating that clonal reproduction, limited recombination and geographical separation play a role in the structuring of *E. turcicum*. In addition, restricted migration and gene flow between populations has the potential to limit allelic exchange between populations while maintaining high genotypic richness locally (Grünwald et al. 2017; McDonald and Linde 2002; Milgroom 1996).

In this study, a significant linkage disequilibrium supported by unequal mating-type ratios was observed in most *E. turcicum* populations in Kenya and Uganda. The null hypothesis of random mating was only supported in a few populations, specifically Kenya Lower Highlands, Kenya Lower Midlands and Uganda Lake Victoria Basin, where both mating types occurred at an approximate 1:1 ratio. This even distribution of mating types corroborates the results of Borchardt et al. (1998b), who reported evidence of sexual recombination in Kenya, Mexico and southern China based on low levels of linkage disequilibrium and the occurrence of mating types at equal frequencies and distributions.

Further support for the role of sexual recombination in contributing to the genetic variation of *E. turcicum* has been well established in populations from China (Cui, Deng, et al. 2022; Dai et al. 2021; Ma et al. 2020), South Africa (Human et al. 2016; Nieuwoudt et al. 2018), Turkey (Turgay et al. 2021) and the United States (Ferguson and Carson 2004), even though a sexual

stage has rarely been observed in nature. The only confirmed observation in nature and laboratory excision of the sexual stage of *E. turcicum* has so far been in Thailand (Bunkoed et al. 2014). The presence of both mating types, even in populations where significant linkage disequilibrium was observed, suggests that sexual recombination may occur over time within these *E. turcicum* populations, albeit at low frequencies. However, asexual reproduction seems to play a dominant role in pathogen survival and spread and should be continuously monitored.

The low levels of sexual recombination observed in this study may be due to continuous maize cultivation in East Africa. In Kenya and Uganda, the presence of both monomodal and bimodal rainfall, characterised by long and short rain seasons, allows for year-round maize production (Palmer et al. 2023). Under these growth conditions, sexual reproduction is likely to occur only on maize debris at the end of the growing season, whereas asexual reproduction predominates during the active production season (Human et al. 2016). This pattern is not unique to *E. turcicum*, as similar dynamics have been observed in *Cercospora zeina* populations in eastern Africa, which exhibit low levels of sexual recombination due to continuous farming compared to populations from southern Africa, where a single growing season predominates (Nsibo et al. 2019). Therefore, further investigation is needed to establish the role of agricultural practices in shaping the genetic diversity and reproductive lifestyle of *E. turcicum* across different regions of Africa.

The findings of this study revealed partial population differentiation between countries, as evidenced by the low $\Phi_{PT}$ value and low estimated number of migrants between Kenya and Uganda. This supports previous findings in South Africa, where partial regional differentiation and high gene flow among *E. turcicum* populations have been observed (Human et al. 2016). Similarly, partial genetic differentiation, indicated by low fixation indices and high genetic variation within populations, has been reported in *E. turcicum* populations in China (Cui, Deng, et al. 2022; Ma et al. 2020). This observed panmictic gene flow within each country highlights the role of short- and long-distance dispersal of *E. turcicum* spores. *E. turcicum* is an airborne pathogen with the potential for long-distance dispersal. For instance, in South Africa, shared haplotypes were detected across provinces separated by up to 700 km, illustrating the extensive long-distance migratory ability of *E. turcicum* (Human et al. 2016). We hypothesise that the interplay between each country's geographical landscape coupled with anthropogenic activities of each country may partially contribute to the observed partial differentiation.

Uganda's landscape is characterised by a relatively flat central plateau encircled by a mountain range (Scott 1998). This flat interior landscape facilitates easier and widespread gene flow within the country, as confirmed by the AMOVA results, thus supporting the formation of a large panmictic *E. turcicum* population. In contrast, Kenya's geographical landscape comprises flat coastal plains that transition into the flat central highland plateau bisected by the Great Rift Valley (Bennun and Njoroge 2000; Scott 1998). Each region studied within Kenya is separated by a physical geographic barrier including Mount Kenya and the Great Rift Valley. Despite these geographical barriers, there is partial differentiation which suggests that airborne spore dispersal, potentially facilitated by prevailing winds and anthropogenic activities between regions, overcomes these internal geographic boundaries. This is further supported by the high rates of migrants observed in Kenya.

The study revealed clear clustering by country, with limited overlap in haplotypes between the populations of Kenya and Uganda. The two countries are separated by significant geographic features, including the Mount Elgon range (4320 m) and Lake Victoria, which are hydrologically connected via the River Nzoia, which originates from Mount Elgon. We hypothesised that these natural barriers substantially restrict gene and genotypic flow of *E. turcicum* populations, thereby contributing to the observed population structure. This hypothesis is supported by the existence of partial population differentiation between *E. turcicum* populations collected from AEZs in western Kenya and eastern Uganda, as well as by the weak but significant positive correlation detected in the Mantel test.

Similar patterns of restricted gene flow due to geographic barriers have previously been reported between *E. turcicum* populations from Austria and Switzerland, where the Alps mountain range acts as a major barrier (Borchardt et al. 1998c). Similarly, limited gene flow was reported for *Puccinia striiformis* populations between Pakistan and China, attributed to the Himalayas mountain range (Awais et al. 2023). More broadly, geographic barriers, such as mountains, rivers and oceans, have been shown to influence gene and genotype flow in numerous fungal populations, including *Aspergillus fumigatus*, *P. striiformis, P. striiformis* f. sp. *tritici* and *Phytophthora cinnamomi* (Brown and Hovmøller 2002; Hovmøller et al. 2023; Liang et al. 2021; Zhou et al. 2023).

Despite the shared AEZs (Lower Highlands, Lower Midlands and Upper Midlands) between Kenya and Uganda, the findings in this study illustrated that clustering of *E. turcicum* populations was primarily driven by country of origin, coupled with limited levels of admixture between countries. This was confirmed by the STRUCTURE and DAPC results. These results indicated that geographical separation and regional connectivity, in addition to other evolutionary factors including differential modes of reproduction, mutation and restricted gene and genotype flow (McDonald and Linde 2002), contribute the most to the population structure of *E. turcicum* rather than shared climatic and agronomic conditions between locations. The observed population structure in this study is also potentially driven by differences in seed systems, maize germplasm deployment in each country, farming practices and country-specific NLB management strategies (CIMMYT 2025; Mastenbroek et al. 2021; Vernooy et al. 2022). These differences could reinforce country-specific *E. turcicum* population structure rather than patterns driven by AEZs.

Furthermore, migration analysis revealed evidence of directional migration of *E. turcicum* between Kenya and Uganda. Comparable low migration rate values have been reported for *Pyrenophora tritici-repentis* populations across Europe, Asia, Australia, North America and South America, which may be attributed to the large geographic distances between continents (Gurung et al. 2013). In contrast, high migration rates

attributed to anthropogenic activity have been documented for *C. zeina* populations in Kenya and Uganda, with Uganda acting as the primary donor of migrants to Kenya (Nsibo et al. 2021).

These collective findings suggest that effective geographic barriers can substantially limit gene and genotype flow, restricting the long-distance dispersal of haplotypes. Despite the high airborne dispersal potential of *E. turcicum*, Mount Elgon and Lake Victoria appear to act as significant physical barriers that restrict the natural movement of *E. turcicum* spores between Kenya and Uganda. Therefore, the limited migration of *E. turcicum* populations observed between Kenya and Uganda is probably influenced by anthropogenic activities rather than natural wind-driven dispersal. Anthropogenic activities such as trade and informal seed exchange have been implicated in long-distance pathogen dispersal across Africa, including dispersal of *C. zeina* (Nsibo et al. 2021). Potential pathways for the movement of *E. turcicum* could include formal and informal maize trade routes between Kenya and Uganda, involving infected plant material such as seeds, husks or cobs, as well as inadvertent transfer of spores via clothing and equipment of researchers and farm workers.

Although *E. turcicum* has been reported as a seedborne pathogen (Rossi and Reis 2014), the evidence remains inconclusive given the very low incidences observed, and whether the pathogen is seed-transmitted has yet to be definitively determined. Consequently, a more comprehensive sampling from additional maize-growing regions in Kenya and Uganda is needed to fully determine potential historical and contemporary migration events and clarify the role of anthropogenic pathways in shaping regional *E. turcicum* population dynamics.

The limitation of migration analysis using Migrate-N is the assumption that random mating occurs among populations. However, in our study the mating-type ratios and linkage disequilibrium tests indicated a deviation from random mating and confirmed the role of clonal reproduction as a major evolutionally factor that drives *E. turcicum* population structure. The violation of this assumption therefore implied cautionary and relative interpretation of the results. Nevertheless, Migrate-N remains a very useful tool for detecting migration patterns, gene flow and the connectivity of populations even when all model assumptions are not strictly met (Beerli et al. 2019), and especially when used complementary to other analyses such as AMOVA and STRUCTURE.

This study represents the first application of microsatellite markers to assess the population diversity and structure of *E. turcicum* in Kenya and Uganda. Overall, the findings of this study demonstrated that *E. turcicum* populations have low genetic diversity in both countries, with limited gene flow between them. While both mating types were recorded in the studied populations, which can facilitate the emergence of more virulent genotypes, clonality appears to dominate. The level of restricted migration of *E. turcicum* detected between Kenya and Uganda probably results from anthropogenic activities, given that there is limited wind dispersal of genotypes, and the existence of geographic barriers between the two countries, such as the Mount Elgon range and Lake Victoria. Studies of this nature are critical for informing the effective management of NLB, as they elucidate the major evolutionary factors driving genetic diversity. These findings underscore the need to sample extensively within a field and among fields from all major maize-growing areas in Kenya, Uganda and the rest of East Africa to capture additional populations that might better explain the migratory patterns of *E. turcicum*. Further studies should consider the influence of cultivar genotypes on the genetic diversity of *E. turcicum*. Given the economic importance of maize in the region, it is important to implement stringent and regionally tailored management strategies that will limit the spread of *E. turcicum* within the region. Ultimately, this study serves as a foundation for ongoing surveillance and monitoring efforts of *E. turcicum* spread and severity in sub-Saharan Africa and contributes to the global understanding of the population genetics and evolutionary potential of this important maize pathogen.

### Author Contributions

**Jabulile N. N. Mahlangu:** formal analysis; investigation; resources; data curation; writing – original draft; visualisation. **Dave K. Berger:** conceptualization; interpretation; writing – review and editing; supervision; funding acquisition. **David L. Nsibo:** conceptualization; formal analysis; interpretation; writing – review and editing; visualisation; supervision; project administration; funding acquisition.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Supporting Information**

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Haplotype accumulation curve for 12 microsatellite loci in six *Exserohilum turcicum* populations. The number of haplotypes identified plotted against the number of loci analysed was calculated with 1000 permutations.