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ORIGINAL ARTICLE

Genetic diversity of *Colletotrichum kahawae* populations in Burundi

N. Niko[1,2] · L. Blondin[3] · N. Motisi[3] · C. Cilas[3] · C. Neema[2]

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Abstract
Microsatellite markers were used to assess genetic diversity of *Colletotrichum kahawae* isolates from Burundi. Sampling was carried out through all coffee growing areas in Burundi. The indices of genetic diversity such as the number of different alleles (Na), the number of effective alleles (Ne) revealed low genetic diversity with an average of 1.900 and 1.196 respectively. Higher than expected heterozygosity and significant linkage disequilibrium are consistent with clonal reproduction. The results showed a weak genetic diversity between the populations from different region and the low value of Fst indicated that the *C. kahawae* populations of Burundi were weakly differentiated. Our results confirm the clonality of *C. kahawae* and its low dispersion ability. The weak differentiation between the populations and the occurrence of one clonal lineage in Burundi are consistent with previous study suggesting that the East African populations of *C. kahawae* are the most recently populations derived from the ancestral Angolan population.

Keywords *C. kahawae* · *Microsatellite markers* · Coffee berry disease · Genetic diversity

Introduction

Colletotrichum kahawae is the pathogen that causes coffee berry disease (CBD). It was first reported in 1922 in arabica coffee plantations around Mount Elgon in western Kenya (Rayner 1952; Nutman and Roberts 1960a). CBD is a disease still limited to the African continent in Arabica coffee producing countries. The damages caused by this fungus to the coffee production are very important. It caused losses of up to 75% of production and the abandonment of coffee cultivation in several districts in western Kenya where it first appeared (McDonald 1926; Nutman and Roberts 1960b). Currently, the disease is reported to cause up to 80% yield loss, but on susceptible cultivars in an environment favorable to the pathogen, 100% loss is recorded (Bekele Etana 2018). Several CBD control measures, including spraying with systemic fungicides were developed but did not last due to the development of resistance in the pathogen over the years. (Prabhakaran Nair 2010; Bekele Etana 2018). In addition, the cost of fungicides exceeds the financial capacity of farmers. Thus, the implementation of other strategies such as the development of resistant varieties would be the most beneficial means of CBD management for small coffee growers.

An epidemiological study of CBD carried out in coffee farms in Burundi from 2019 to 2021 revealed that the incidence of CBD varied according to agroecological zones and coffee cropping systems. Temperature and relative humidity were responsible for the observed differences in CBD incidence. In addition, the incidence of CBD was higher in coffee trees in full sun than in coffee trees grown in shade systems. The analysis of the potential of different species of shade plants to reduce the dispersion of the inoculum showed that certain species of shade plants such as banana and ficus are potentially interesting for limiting the spread of CBD (Niko. N, unpublished results). Therefore, they can be used as one of the CBD management strategies. Knowledge of the genetic diversity of *C. kahawae* populations will help to understand how *C. kahawae* populations will evolve in response to the different strategies adopted for CBD management.

The study of the population structure of *C. kahawae* carried out with techniques based on vegetative compatibility

✉ C. Neema
claire.neema@supagro.fr

[1] Institut des Sciences Agronomiques du Burundi (ISABU), Avenue de la Cathédrale, Bujumbura BP 795, Burundi

[2] Institut Agro Montpellier, UMR PHIM, Montpellier F-34398, France

[3] CIRAD, UMR PHIM, Montpellier F-34398, France

groups (VGC) and RAPD molecular markers showed their variability according to origin (Manga 1999). The populations of Cameroon are very different from those of East Africa. Moreover, within the populations of East Africa, there is a significant difference between the population of Kenya and the populations of Burundi, Malawi and Tanzania (Manga 1999). Knowledge of the genetic variation of plant pathogen populations is essential for the development of a sustainable disease control system (Milgroom and Peever 2003). This information can be obtained using highly informative genetic markers such as microsatellites due to their high level of polymorphism and ubiquity in eukaryotic genomes (Feldman et al. 1999; Moniruzzaman et al. 2016). In this study, microsatellite markers specific to *C. kahawae* were used to study the genetic diversity of populations from Burundi.

Materials and methods

Colletotrichum kahawae sampling

Sampling of *Colletotrichum kahawae* populations from Burundi, was carried out through all coffee growing areas in Burundi. The samples collected consisted of green berries showing symptoms of CBD. All the samples were collected in bag envelopes identified by the GPS coordinates, the municipality and the Province of origin and were transported within 24 h to the Crop Protection Laboratory of ISABU (The National Institute of Agronomic Sciences of Burundi) where they were kept at 4 °C while waiting for the isolation of the pathogen (Bersisa 2018; Alemu et al. 2020).

Isolation and purification of *Colletotrichum kahawae*

Infected coffee berries were disinfected with 90% alcohol to remove all external contamination. A fragment of infected internal tissue located in the growth front of the symptomatic lesion was cut out and placed in Petri dishes containing the PDA culture medium supplemented with two antibiotics, streptomycin and penicillin at a concentration of 50 mg/liter. The inoculated dishes were incubated at 20 °C for 7 days. At the end of the incubation period, the homogeneous colonies which have the morphological and cultural characteristics of *Colletotrichum kahawae* (mycelial colonies of dark greenish gray color in the center which declines to white or grayish-white starting from outside) according to the previously descriptions of the different authors were selected and sub-cultured on fresh PDA culture media for purification (Hindorf 1970; Vossen et al. 1976).

Morphological and cultural characterization

A mycelial disc (4 mm) was taken from the growth front of the colonies of isolates cultures and placed on fresh PDA culture medium. For each isolate, three dishes were subcultured and the new cultures were divided into three batches. The first batch was incubated at 20 °C, the second at 25 °C and the third at 30 °C. Colonies growth were monitored and measured on two perpendicular diameter every 24 h for 14 days. Growth rate was calculated as the 14-day average of mean daily growth. Two replicates were performed for each isolate. The results of this manipulation facilitated the subsequent purification of other isolates. A pair of dishes for each isolate was therefore subcultured and a part of them was incubated at room temperature (20–25 °C) and another part at 30 °C. Colonies that grew normally at room temperature but were inhibited at 30 °C were selected for single-spore cultures.

Single spore culture

Cloning was performed with colonies maintained in culture for 14 days to promote sporulation. 1 ml of sterile distilled water was added to the Petri dishes containing the 14-day-old colonies of *Colletotrichum kahawae*. With a sterile inoculating loop, the surface of the culture was lightly scraped to produce a suspension of conidia. This suspension was then spread on the PDA culture medium without antibiotics and the dishes were incubated at 20 °C for 24 h. The germinated spores were taken individually under binoculars and transferred to the fresh PDA culture medium. The single spore strains of *Colletotrichum kahawae* were multiplied and stored under vacuum in two copies at −20 °C. One copy is stored at CIRAD Montpellier in France, another copy is stored at ISABU in Burundi.

DNA extraction

The cultures derived from a single spore from the original isolates of *Colletotrichum kahawae* were multiplied on a liquid culture medium (1/2PDB and 1/2v8) to obtain the mycelium needed for DNA extraction. Mycelial explants approximately 4–5 mm in diameter were taken from areas of active sporulation near the growth edge of a fresh 7-day-old culture. They were transferred to a multi-well plate containing 6 ml/well of medium, i.e. one isolate per well. The plates were covered and cultures were incubated at 25 °C for 7 days. The DNA extraction was performed by adapting the modified CTAB method for phytopathogenic fungi (Gontia-Mishra et al. 2014).

PCR amplification

The mycelium of each isolate was recovered after incubation and dried on paper towel, then transferred to a previously prepared 2 ml tube. 1 ml of digestion solution was added to each reaction tube and vortexed to suspend the mycelium. The whole was incubated for 30 min at room temperature to allow digestion of the cell walls and then centrifuged for 5 min. The supernatant was discarded and the rest was suspended with 1 ml of lysis buffer (CTAB+ Na2SO3 at 1% + proteinase K at 1 mg/ml + RNase at 0.2 mg/ml). The suspension was incubated for 1 h at 65 °C and then centrifuged at 13,000 rpm for 5 min. The supernatant was transferred to 2 ml reaction tube and 1 ml of CIAA was added and mixed gently by inversion many times before centrifuged them for 10 min. Approximately 800 µl of the supernatant above the whitish layer is collected in a 1.5 ml tube. A volume of isopropanol cooled to −20 °C equivalent to 2/3 of the collected volume was added to each tube, carefully mixed by inversion and incubated at −20 °C for 1 hour to precipitate the DNA. The DNA pellets were recovered by centrifugation at 12,000 rpm for 20 min and were drained on towel paper. 500 µl of 70% ethanol were added to wash DNA pellets and were let stand for 10 min to dissolve all proteins. The supernatant was removed with micropipette and the DNA pellets were dried completely (for approximately 15 min). DNA pellets were then taken up in 50 µl distilled water while waiting further use. The NanoDrop 2000 Spectrophotometer (Thermo Scientific) was used to estimate the genomic DNA quality and concentration. PCR mixtures were conducted in 25 µl volume containing 5 µl of the buffer 5X; 1 µl of dNTPs (10 mM each), 1 µl of primer forward (10 µM), 1 µl of primer reverse (10 µM), and 0.25 µl of Taq polymerase (5 U/µl). When the mixtures were ready, 23 µl were distributed in each well of microplate and 2 µl of 20 ng/µl of DNA was added. The amplification was carried out in a programmable thermocycler (Thermocycleur Biometra TOne 96) with an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min and extension for 1 min at 72 °C. The final step consisted of extension for 10 min at 72 °C. The PCR products were checked on 1.5% agarose gel using a DNA molecular weight marker "1kb Promega". The migration was carried out at 100 V for 25 min and the nucleic acid bands were visualized under UV light using an ethidium bromide solution.

Species identification

Colletotrichum kahawae species was verified by Sanger sequencing PCR products and Blast sequences analysis (BLAST http://blast.ncbi.nlm.nih.gov/Blast.cgi). The polymerase chain reaction (PCR) was performed with universal ITS primers 5F/4R using the kit "Go taq" (Promega). Additional identification was performed with primers of the Apn2/MAT locus which improve the molecular systematics of *C. gloeosporioides* complex (Silva et al. 2012a, b). The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT).

Primers design and diversity analysis of *Colletotrichum kahawae* in Burundi

Twelve polymorphic SSR markers developed by Byesse D. (unpublished results) on a collection of African *C. kahawae* isolates were used to analyze the genetic diversity of *C. kahawae* isolates from Burundi. These markers were selected in an enriched library according to Malausa et al. (2011). The repeating motifs of all polymorphic SSR markers consist of dinucleotides (69%) and trinucleotides (31%) and allow the detection of a total of 33 alleles (Table 1). (GenBank OR061020-OR061031)

Data analysis

The indices and statistics of genetic diversity were determined with the GenAlEx 6.5 software (Peakall and Smouse 2006). These parameters include the allele frequencies, number of different alleles (Na), effective number of alleles (Ne), number of private alleles (Np), Shannon's Information Index of diversity (I) observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F), percentage of polymorphic loci (%P), genetic differentiation (Fst), gene flow (Nm) and Nei's genetic distance (NeiD). The Analysis of Molecular Variance (AMOVA) including the calculation of the coefficient of genetic differentiation between populations (PhiPT), was performed with GenAlEx software to partition genetic variability into different components and examine the differentiation within and between populations (Marulanda et al. 2014; Pagnotta 2018; Sokolova et al. 2022).

Results

Identification of *Colletotrichum kahawae* isolates in Burundi

Ninety-five isolates from different coffee growing areas of Burundi were selected after purification based on morphological features of colonies, mycelial growth rate and ITS sequences. The mycelium of the colonies has a dark greenish gray color that degrades to grayish-white from the

Table 1 Characteristics of 13 polymorphic microsatellites markers used in this study for genetic diversity analysis of *Colletotrichum kahawae*

Locus	Primer sequences	Annealing temperature	Product size	Repeat sequence	Allelic frequency	Number of alleles
CBD03	F: TCCGTCCCCAAAGTCCTC R: GTGCCAAGACCGCAGAAA	61 °C	212 pb	$(CA)_{13}$	0.00	2
CBD05	F: CGCCAACTAACCATCTTGTG R: CGAATCGTGTTGAGAGGTGA	61 °C	236 pb	$(CA)_{15}$	0.04	2
CBD08	F: CGAGTTGAGACGAGGCAAAC R: CTAAAGCCGTGGTGGACAAA	61 °C	193 pb	$(CA)_{18}$	0.22	3
CBD09	F: ATTGCCAAGCTCCGATTTAC R: CGAGTATCCCCTTTTCCCTT	61 °C	295 pb	$(GT)_{15}$	0.03	2
CBD11	F: CGCTCAGGGAGAGAAGGAG R: GTTGGGTAGGATGGGATGG	62 °C	214 pb	$(CA)_{12}$	0.03	3
CBD13	F: TACCTCACCTTACCCTCCCC R: TGGAATTGGTGCCGTTACTT	61 °C	172 pb	$(CA)_{11}$	0.19	3
CBD16	F: GGAGCCAACTGCATCAGAA R: TGCTGGTAGGGGTAAAGGG	61 °C	243 pb	$(CA)_{17}$	0.10	2
CBD21	F: CACCATCTTTGCTCTCTCCC R: CACGAGTTGCTGATGACGA	63 °C	226 pb	$(CA)_{16}$	0.26	3
CBD22	F: CCTTCTTGGTGTGTCTTGACTG R: TTGGGAAGAACTGGTGGTGT	61 °C	196 pb	$(CA)_{17}$	0.21	4
CBD34	F: ATTGCATTGCGAATAACGAC R: TTATTACCATTGTCGTCGCC	60 °C	97 pb	$(ACG)_5$	0.09	2
CBD53	F: GCTGCTGTTCCACCACATAA R: AATACAACCGGCGATACCAC	60 °C	243 pb	$(CGT)_7$	0.34	2
CBD54	F: GAGGTCTTCAAAGCCTCGTG R: GTCCCTTTCGTCTGTGTCGT	60 °C	248 pb	$(ACG)_7$	0.13	3
CBD55	F: TTTGATGAGGACAACGACGA R: AGGCTGGCACAGCTTTGTAT	60 °C	252 pb	$(ACG)_4$	0.03	2
Mean					**0.13**	**2.54**

center to the edge of the colony. Mycelial growth rate was slower and ranged between 4.5 to 7 mm per day at 20 °C with an average of 6.1 and between 5 and 10 mm per day at 25 °C with an average of 6.4. At 30 °C, the growth of the colonies was inhibited from the 3rd day. Statistically, the growth rates at 20 °C and 25 °C were not significantly different ($p = 0.125$). To confirm that the strains studied were from *C. kahawae* lineage, a subset of 20 strains covering the observed diversity were tested by sequencing PCR products amplified with primers of the Apn2/MAT locus. The results showed that all the strains tested matched at 100% with the sequences of *C. kahawae* species confirming their taxonomic assignment. The phylogenetic tree was built with these sequences and those of related species in the data bank (Supplementary file S1). The 20 strains studied are grouped in one cluster with strains of *C. kahawae* from data bank. These results showed clearly the differentiation of *C. kahawae* isolates from Burundi from it closely related species.

For genetic diversity analysis, the 95 isolates were assigned into four populations according to their geographical zones of origin (Table 2).

Table 2 Origin of *Colletotrichum kahawae* isolates from Burundi collected in 2020

Population	Origin (Province)	Geographic location	Altitude (m)	Number of isolates
BUKAPOP	Bubanza	Northwest	1453–1610	4
	Kayanza	North	1770–1947	14
MARUPOP	Makamba	South	1558–1914	12
	Rutana	Southeast	1533–1795	12
MMGPOP	Muramvya	Midwest	1712–1723	2
	Mwaro	Midwest	1686–1747	9
	Gitega	Center	1636–1684	3
MUNGPOP	Muyinga	Northeast	1539–1814	17
	Ngozi	North	1576–1819	22
Total				**95**

SSRs polymorphism and genetic diversity of *Colletotrichum kahawae* isolates

The genetic diversity of *C. kahawae* populations from Burundi was analyzed using 12 microsatellite markers identified previously and only 5 of them revealed polymorphism

with a total of 13 alleles. The microsatellites CBD08, CBD16 and CBD22 were monomorphic in the population (MMGPOP) and polymorphic in all others. The locus CBD03 was polymorphic for two populations (MUNGPOP and MARUPOP) and the locus CBD09 was polymorphic in MARUPOP only (Table 3). A total of 10 haplotypes were identified, among them one haplotype was dominant in all populations and represent 67% of the isolates.

The results of genetic diversity analysis indicated that the expected heterozygosity (He) ranged from 0.101 to 0.237 with an average of 0.134 while the observed heterozygosity (Ho) was null. The indices of genetic diversity such as the number of different alleles (Na), the number of effective alleles (Ne) revealed low genetic diversity with an average of 1.900 and 1.196 respectively. These results confirm the clonality of *C. kahawae* in Burundi.

Table 3 Genetic diversity of *Colletotrichum kahawae* populations from Burundi

SSR markers	Alleles	Allele frequency per population			
		BUKAPOP	MUNGPOP	MMG-POP	MARU-POP
CBD08	203	0.000	0.026	0.000	0.043
	212	0.059	0.395	0.000	0.174
	214	0.941	0.579	1.000	0.783
CBD09	311	0.000	0.000	0.000	0.130
	313	1.000	1.000	1.000	0.870
CBD03	235	1.000	0.868	1.000	0.826
	237	0.000	0.132	0.000	0.174
CBD16	257	0.111	0.026	0.000	0.043
	259	0.833	0.947	1.000	0.957
	261	0.056	0.026	0.000	0.000
CBD22	209	0.056	0.026	0.000	0.043
	219	0.944	0.921	1.000	0.870
	220	0.000	0.053	0.000	0.087

Population genetic diversity

The highest genetic diversity was found for the population MARUPOP with an average value of Ne = 1.329. The ranking was the same with the Shannon diversity index (I) with an average across all loci ranged from 0.000 to 0.426 (Table 4). One locus (CBD09) revealed a private allele (Np) for the MARUPOP population. The averages of population genetics statistics indicate significant low to moderate genetic differentiation (Fst = 0.0578), and a significant potential gene flow (Nm = 4.807) between populations. The range of genetic differentiation index (Fst), gene flow (Nm) and Nei's genetic distance between every two populations was from 0.026 to 0.092, from 2.457 to 9.271 and from 0.005 to 0.037, respectively. The results of calculation of genetic distance revealed that the Nei's pairwise genetic distances between the populations varied from 0.005 to 0.037. The longest genetic distance was found between MUNGPOP and MMGPOP, while the lowest genetic distance was found between BUKAPOP and MMGPOP (Table 5). The results of the analysis of molecular variance (AMOVA) show highest levels of variation within populations (93%). The low value of PhiPT (0.071) was also in accordance with the low genetic differentiation among populations. In addition, a mean and significant gene flow (Nm) of 3.59 was observed between populations (Table 6).

Table 4 Genetic diversity indices over 13 SSR loci for *C. kahawae* populations of Burundi

Population	Na	Ne	Np	I	Ho	He	uHe	F	%P
BUKAPOP	1.800	1.130	0	0.199	0.000	0.101	0.104	1.000	60.00%
MUNGPOP	2.400	1.323	0	0.348	0.000	0.197	0.200	1.000	80.00%
MMGPOP	1.000	1.000	0	0.000	0.000	0.000	0.000		0.00%
MARUPOP	2.400	1.329	0.2	0.426	0.000	0.237	0.243	1.000	100.00%
Mean	***1.900***	***1.196***	***0.05***	***0.243***	***0.000***	***0.134***	***0.137***	***1.000***	**60.00%**

Na number of different alleles, *Ne* number of effective alleles, *Np* number of private alleles, *I* Shannon's Information Index, *Ho* Observed heterozygosity, *He* Expected heterozygosity, *uHe* Unbiased expected heterozygosity, *F* Fixation index, *%P* percentage of polymorphic loci, Mean correspond to the mean of the different indices

Table 5 Pairwise genetic distance among populations of *C. kahawae* from Burundi

Pop1	Pop2	Fst	Nm	NeiD	Nei I	Nei uD	Nei uI
BUKAPOP	MUNGPOP	0.067	3.456	0.035	0.966	0.032	0.969
BUKAPOP	MMGPOP	0.043	5.601	0.005	0.995	0.003	0.997
MUNGPOP	MMGPOP	0.092	2.457	0.037	0.963	0.036	0.965
BUKAPOP	MARUPOP	0.051	4.645	0.017	0.983	0.012	0.988
MUNGPOP	MARUPOP	0.026	9.271	0.017	0.983	0.012	0.988
MMGPOP	MARUPOP	0.068	3.414	0.014	0.986	0.011	0.989
Mean		**0.0578**	**4.807**	**0.021**	**0.979**	**0.018**	**0.983**

Fst F-statistics, *Nm* Gene flow, *NeiD* Nei Genetic distance, *Nei uD* Nei unbiased genetic distance, *Nei I* Nei genetic identity, *Nei uI* Nei unbiased genetic identity

Table 6 Analysis of molecular variance (AMOVA) within and between *C. kahawae* populations from Burundi, based on 13 SSR loci

Source of variation	Df	SS	MS	Est. Var.	%P	PhiPT	*P*-value	Nm
Among pops	3	14.069	4.690	0.134	7	0.071	0.001	3.259
Within pops	89	155.006	1.742	1.742	93			
Total	92	169.075	6.431	1.875	100			

Df Degrees of freedom, *SS* Sum of squares, *MS* mean squares, *Est. Var.* Estimate Variance, *%P* Percentage of variation, *PhiPT* Genetic differentiation, *Nm* Average of gene flow

Discussion

In this study, ninety-five isolates of *C. kahawae* were collected from different coffee growing regions of Burundi, after which morphological characterization and genetic variation analysis were performed. Morphologically, the color of the colonies of the different isolates varied between white gray, light gray and dark gray. The color variation was also observed on the mycelium according to the age of the colonies. This ranged from dark greenish gray in the center of the colony to greyish white at the edge of the colony. These findings were comparable to results reported by other authors on previously performed studies (Hordofa 2019; Alemu et al. 2020). Alemu et al. (2021) in the study of pathogenic variability among *Colletotrichum kahawae* Waller & Bridge population from major coffee growing regions of Ethiopia reported that the growth rate of the mycelium of *C. kahawae* ranged between 4.27 mm/day and 5.63 mm/day. Cabral et al. (2020) found their part that the CBD-causing isolates grew more slowly than non-CBD-causing isolates. The results of our study on the rate of mycelial growth were in the same order as those reported in previous studies. Overall, *C. kahawae* strains have a whitish mycelial color during the first 6 days of incubation, then colony colors change to light gray, then gray and dark gray. The morphological characteristics as well as the growth rate of the mycelium allowed us to attribute the isolates to the species *Colletotrichum kahawae*. The rapid growth of isolates in the temperature range between 20 and 25 °C could contribute to the aggressiveness of *C. kahawae* isolates in arabica coffee. Investigating the relationship between morphological characteristics and pathogenicity of *C. kahawae* isolates Hordofa (2019) stated that faster mycelial growth rate and high sporulation capacity would contribute to the aggressiveness of *C. kahawae* isolates in *C. arabica*. On our side, we observed a faster mycelial growth of the colonies for certain isolates at 20 and 25 °C (10 mm/day), which could be related to the virulence of the isolates.

Previous studies on the characterization of genetic diversity of *C. kahawae* populations have used techniques other than microsatellite markers. Bersisa (2018), used morphological (colony radial growth, colony color, colony texture) and cultural features to identify and characterize *C. kahawae* isolates from Southeastern Ethiopia. To assess CBD resistance in arabica coffee collections from Gidame, western Ethiopia, Hordofa (2019) also identified and characterized isolates of *C. kahawae* using morpho-cultural characteristics of *C. kahawae*. Alemu et al. (2021) on their side have used pathogenicity and morpho-cultural features to determine the variation among *Colletotrichum* isolates associated with coffee berry disease in Ethiopia. Molecular markers such as microsatellites were used for several groups of fungal pathogens (Sharma et al. 2014) and certain species of *Colletotrichum*. Marulanda et al. (2014) used microsatellites to assess the genetic diversity and population structure of *Colletotrichum* spp. causal agent of anthracnose in Andean blackberry. Moges et al. (2016) developed and used SSRs for genetic diversity and population structure of *Colletotrichum gloeosporioides* from citrus in Ethiopia and found them with a low level of genetic diversity. Ciampi et al. (2011) tested cross-amplification in different *Colletotrichum* species and found certain primer pairs specific to *C. acutatum* which allowed him to separate *C. gloeosporioides* and *C. acutatum*, two closely related species that can cohabit on the same citrus, causing distinct diseases. Estimation of genetic diversity based on microsatellite markers also revealed high diversity among *C. truncatum* isolates from India (Sharma et al. 2014). In this study, 12 polymorphic SSRs were used to analyze genetic diversity of *C. kahawae* isolates in Burundi. The size of genetic diversity can be estimated by genetic diversity parameters, such as the effective number of alleles and Shannon's information index (Lu et al. 2017; Nie et al. 2022). The SSR markers developed by Moges et al. (2016) for assaying the genetic diversity of *C. gloeosporioides* isolates from citrus revealed a higher level of genetic diversity between 163 Ethiopian Citrus *C. gloeosporioides* isolates with an average number of effective alleles (Ne) of 1.54. The average number of effective alleles (Ne) was 1.606 in the study of genetic diversity of *C. gloeosporioides* species complex associated with Citrus in Tunisia (Bahri et al. 2019). In this study, the average number of effective alleles in four populations of *C. kahawae* was 1.196, which is lower than what was found for other *Colletotrichum* species. In addition, the mean value of Shannon's information index (I) was 0.243, indicating low genetic diversity of *C. kahawae* population of Burundi. Analysis of molecular variance (AMOVA) showed that genetic variation originated mostly within population (93%) and less between populations (7%). The results of this study show that Nei's genetic distances are low with an average of 0.021 and are consistent with Shannon's information

index (0.243). The low level of Fst in this study indicated that the *C. kahawae* populations of Burundi were weakly differentiated. The high frequency of one haplotype in all the regions suggest the occurrence of one clonal lineage in Burundi. These results confirm the clonality of *C. kahawae* and its low dispersion ability as previously shown with SNPs markers designed on *C. gloeosporioides* species by Vieira et al. (2018). The weak differentiation between the populations and the occurrence of one clonal lineage in Burundi are consistent with the results of Silva et al. (2012a, b) suggesting that the East African populations of *C. kahawae* are the most recently populations derived from the ancestral Angolan population.

These results on genetic diversity of *C. kahawae* populations of Burundi provide information which should be used for developing integrated strategies for coffee berry disease management.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s42161-024-01807-9.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. The GenBank accession numbers of the 12 SSRs sequences are GenBank OR061020-OR061031.

Declarations

Ethical approval The submitted work is original and have not been published elsewhere in any form or language.

Financial interests The authors declare they have no financial interests.

Conflict of interest The authors declare that they have no conflict of interest.

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