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Plant parasitic nematodes occurrence and genetic diversity of banana cultivars grown in Tanzania

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Abstract

The genetic diversity of bananas (*Musa* spp.), one of the most economically important crops in Tanzania, is underestimated with scarce reports available. In addition, cultivation of banana is severely constrained by plant parasitic nematodes (PPN). We assessed genetic diversity and population structure of 159 banana varieties from four agro-ecological zones (Northern, Southern highlands, Lake and Zanzibar islands) of Tanzania using 20 SSR markers analysed with UPGMA method. We also, assessed the PPN occurrence through isolation and counting of PPN from each banana genotype where leaf samples were collected. SSR primer pairs were polymorphic; and generated 63 distinct reproducible bands. The polymorphic information content values of each SSR marker ranged from 0.50 to 0.75 indicating high level of genetic diversity among banana varieties. The 159 banana varieties were grouped into two clusters: A and B, based on UPGMA cluster and population structure analysis. A total of 128 independent and 31 overlapping genotypes were identified. Higher kinship among genotypes was observed in cluster A compared with cluster B. Some of the clusters in A and B genotypes showed high genetic distance. The most prevalent and abundant nematode species was *Pratylenchus goodeyi* with a mean count of 63%, followed by *Radopholus similis* (31%) and *P. coffeae* (6%). The results from this study provides a foundation for understanding genetic variability of bananas existing in Tanzania and PPN occurrence that will be valuable information for breeding disease and pest-resistant bananas with high yield traits.

Keywords: Characterisation; Microsatellites; *Musa* spp.; Nematodes; Polymorphism; Varieties.
Abbreviations: SSR_simple sequence repeat; AFLP_amplified fragment length polymorphism; SNP_single nucleotide polymorphisms; RFLP_restriction fragment length polymorphism; ANOVA_Analysis of variance; RCF_relative centrifugal force; PIC_ Polymorphic information content; PPN_Plant parasitic nematodes; CTAB_Cetyl Trimethyl Ammonium Bromide; EDTA_Ethylene Diamine Tetra-acetic Acid; TAE_Tris-HCl Acetate EDTA; SSR_Simple Sequence Repeat; UPGMA_Unweighted Pair-group Method with Arithmetic Means; NTSYS_Numerical Taxonomy system.

Introduction

Banana (*Musa* spp.) is an important food and cash crop worldwide; and is the 4thmost important crop in developing countries after rice, wheat and maize. Tanzania is the second largest banana producer in East Africa after Uganda; and the seventh largest producer in the world (FAO, 2015). The crop is ranked the 3rd most important crop in Tanzania after cassava and maize (Kilimo Trust, 2012). The total area under banana production in Tanzania is ~290,000 ha, with a total production of 2.5 million tons of fruit per annum (FAO, 2015). The crop is mainly produced in almost all agroecological zones except the Central zone (Kilimo Trust, 2012). Banana is mainly produced by small-scale farmers in Tanzania and is a major contributor to food security and income for smallholder farmers. In Tanzania, green banana is generally used as a source of carbohydrate while ripe banana is used as a fruit that provide vitamins (A, C, B_6, folic acid and biotin) and minerals (potassium, calcium, magnesium, manganese, phosphorus, chloride, iodine, zinc and iron) (UNCST, 2007). Almost all parts of banana plant are used as feed for livestock while ripe banana is also used in brewing local drinks that are very famous in several cultural ceremonies in various communities such as weddings and funerals. Recently, banana production declined and has resulted to food insecurity, lost income for many banana farmers and decreased potential for bananas to contribute to the world market economy and urban markets (FAO, 2015). The decrease in banana yield is partially attributed to poor production practices and little information on the genetic resources available for crop improvement via breeding. Many varieties are susceptible to major diseases and pests, primarily plant parasitic nematodes (PPN). The development of improved banana varieties in Tanzania is

currently challenged by lack of information on existing banana genotypes and the associated pest and diseases. Knowledge on genetic resources that can be leveraged to breed high-yielding banana varieties which are resistant to biotic and abiotic stresses is crucial.

The characterization of crops based on DNA markers is a standard method for correct and quick identification of similar or closely-related cultivars. DNA markers, including RAPD (Jain et al., 2007; Onyango et al., 2010), AFLP (Wong et al., 2002; Noyer et al., 2005), RFLP (Gawel et al., 1992; Ning et al., 2007), SNP (Adesoye et al., 2012) and microsatellites (Lagoda et al., 1998; Creste et al., 2003) have been used successfully to characterize banana. Genetic diversity of banana based on SSR markers has been reported in several researches (Crouch et al., 1998; Hippolyte et al., 2010; Christelová et al., 2011; Amorim et al., 2012; Christelová et al., 2016). SSR markers have been widely used for genetic analysis and cultivar identification because of their abundance, co-dominance inheritance, high rate of polymorphism, reproducibility and ease of assay by PCR (Xie et al., 2011). Genetic similarity or difference among taxa can be measured through diversity analysis which provides necessary information for crop conservation and varietal development (Romero et al., 2009). Morphological characters have been used to classify genome constitution and ploidy levels in banana (Pillay et al., 2001). However, morphological characterization alone is insufficient because plants are large and have long life cycles; thus, molecular marker methods are more widely used for germplasm characterization.

In Tanzania, there is limited information on genetic diversity and population structure of banana varieties. There are only few reports on morphological diversity of banana; these are based on the work of Karamura and Mgenzi (2004). Similarly, there is hardly any reports on the diversity and occurrence of plant parasitic nematodes affecting banana production in Tanzania. The present study therefore is intended to assess genetic diversity and population structure of banana grown in Tanzania using SSR markers; and establish the occurrence of PPNs in banana varieties. This work will provide a foundation on the genetic resources available in Tanzanian banana germplasm as well as provide baseline information on the PPN occurrence in banana genotypes. This information is very useful for molecular breeding programs and PPN management of banana production.

Results

PIC analysis of SSR primer pairs

The genetic diversity analysis of 159 banana samples using 20 SSR markers revealed 63 polymorphic alleles. Polymorphic information content (PIC) values for the SSR which was used in this study varied from 0.50 to 0.75. SSR markers mMaCIR102, mMaCIR109 were highly effective in discriminating the most cultivars (Fig 2). In addition, 7 SSR markers; mMaCIR122, mMaCIR154, mMaCIR156, mMaCIR157, mMaCIR219, mMaCIR277and mMaCIR297 yielded the highest number of alleles per locus. PIC value was used to detect polymorphism within a population with respect to number of detectable alleles and the distribution of their frequency whereby all 20 SSR markers recorded values equal to or greater than 50% (Table 2).

Cluster analysis and genetic structure

All banana genotypes were grouped into two major clusters based on Jaccard's similarity index with the range of 0 to 6.0 distances (Fig 3). In cluster A, we obtained 24 overlapping and 80 independent/non-overlapping genotypes which makes a total of 104 genotypes in cluster A, while cluster B comprised of 55 genotypes: 7 overlapping and 48 non-overlapping (Fig 3; Table 3). This makes a total of 128 independent and 31 overlapping genotypes. The overlapping varieties in both clusters were divided into 11 groups (Table 3), whereby each group contained similar genotypes with different names from different geographic origin.

In addition, cluster A contained many varieties which were introduced from other countries compared with those in cluster B with many local varieties. Most of the introduced varieties in cluster A are from Kagera and Mbeya regions (Supplementary Table 1). Cluster A contains many overlapping varieties (24) compared to cluster B with seven (7) varieties (Fig 3; Table 3). Varieties in cluster A sub clusters *XII* and *XIII* are both triploid bananas of the genome AAA and AAB respectively as described by Perrier et al. (2009) and they are closely related to varieties in sub cluster *XIV* which are the AA edible diploids. The structure analysis found evidence of two distinct clusters, that supported the UPGMA cluster analysis (Fig 4).

Existence and occurrence of PPN in 4 agro-ecological zones surveyed

The PPN were generally found in all four-major banana growing zones surveyed. Based on the mean nematode counts, *Pratylenchus goodeyi* were the most numerous with a mean count of 496.16 (63%), followed by *Radopholus similis* (31%) and *P. coffeae*, (6%).

Analysis of variance (ANOVA) revealed a statistically significant difference between means of all nematode species, $F(2,474) = 27$, $P<0.05$). Similar results were obtained when nematode populations were analyzed based on banana varieties in clusters; $F(2,222) = 16$, at $P<0.05$ in cluster A and $F(2,249) = 11$, at $P<0.05$ in cluster B (Supplementary Table 2).The post- hoc Tukey HSD test carried out to make a pairwise comparison between mean of the different nematode species in both clusters, revealed that *P. goodeyi* differed significantly ($P<0.01$) with other nematode species (*R. similis* and *P. coffeae*) in cluster A (Fig 5) while in cluster B, *P. goodeyi* also differed significantly with *P. coffeae*. However, there was no significant difference between means of *R. similis* with either *P. goodeyi* or *P. coffeae*. Other nematode species, including *Meloidogyne* spp. and *Helicotylenchus multicinctus,* were also identified but their populations were very low (mean count <1%) hence, excluded from this study.

We also found that, banana varieties 'Gurutu/Zambia' from Kyela, Mbeya; 'Ndyali' from Ileje, Mbeya and 'Mshale' from Namtumbo, Ruvuma had the highest mean counts of *P. goodeyi*, *R. similis* and *P. coffeae,* respectively. Thus, banana varieties with the highest mean counts of *P. goodeyi*, *R.*

Table 1. The 20-simple sequence repeat (SSR) primers used in this study, including the estimated amplicon sizes and source references.

	Forward primer	Reverse primer	Marker	size (bp)	Reference
1	TGTTGGATTGGCTTCATC	CTTCGTTCAATGGTCTCCT	mMaCIR102	220	Hippolyte *et al* 2010
2	CCTCTTCTCCCTGTGTTG	CGGTTTAACATACCTATTCTTG	mMaCIR103	179	Lagoda *et al* 1998
3	CATCCACTTGCTTTTCCA	CTTCACGGCTTCCACA	mMaCIR105	264	Lagoda *et al* 1998
4	ACGCATGGTAAAGTGGAA	ACATTCAAATCACGTTGCT	mMaCIR108	111	Lagoda *et al* 1998
5	ACTCTAGTTCCAGAATAACTCCA	CAATCTTCATTAGCCAGTTGT	mMaCIR109	204	Hippolyte *et al* 2010
6	GTTCGGCTGGAGGTAGTT	AAGAACACGAAGGCAGG	mMaCIR112	330	Hippolyte *et al* 2010
7	GCAAGCCAAAGGGAA	ACCAACAAAGAATGGTGTAA	mMaCIR114	222	Hippolyte *et al* 2010
8	CGGTGACACTGGAAGGT	CAACTGAAGAACTGCCACTAA	mMaCIR122	204	Hippolyte *et al* 2010
9	CTAACCTTTGATTCTGTTTG	GTCCCTGATACACCATTC	mMaCIR129	214	Hippolyte *et al* 2010
10	CGTATTCTACATCTGCTTCTTT	GCAGTGATTAGGTGATGATTT	mMaCIR137	223	Hippolyte *et al* 2010
11	CATTCAGCATGGAAACCT	CTTCCTCAAACTGCTCCTC	mMaCIR154	311	Hippolyte *et al* 2010
12	CTTTCTGAAGGAAATTCTGAC	AGTGCAGCCCAATGAA	mMaCIR156	210	Hippolyte *et al* 2010
13	TGGTATTATTTCATAGCCCTTC	ATGGTATTGTTGGATGGTGT	mMaCIR157	272	Hippolyte *et al* 2010
14	GGGAGGGCAGAGGAA	GCCGAACTTGGTAATGTG	mMaCIR189	259	Hippolyte *et al* 2010
15	GAATCGCCTTAGTCTCACC	TCATGTGCTCCCATCTTT	mMaCIR195	285	Hippolyte *et al* 2010
16	GGGTAAGCTCAAGATGGAA	CAGACGCTAAACGACACC	mMaCIR219	320	Hippolyte *et al* 2010
17	AATGGATTGGGCATCAG	GGAGGGAGGAGGGTTT	mMaCIR247	178	Hippolyte *et al* 2010
18	ACGATAGGATTATTGGCTGT	GGCTCTTAATTTGACAAGAA	mMaCIR277	212	Hippolyte *et al* 2010
19	GGGTCCCTGTTGGCT	TTGCAGATTAGGGTGGG	mMaCIR280	221	Hippolyte *et al* 2010
20	GAACTCGGATTGTTCCTTT	AGGCTGATGGTAGCGAG	mMaCIR297	173	Hippolyte *et al* 2010

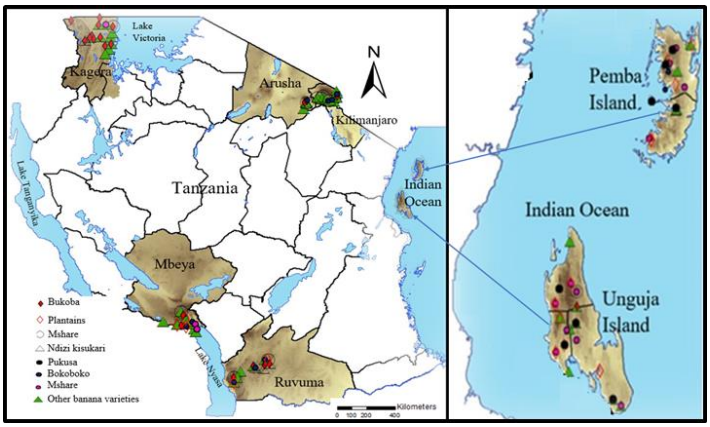

Fig 1. Map of Tanzania, showing the regions where banana plant samples were collected. The sampled areas are shaded with brown colour and different banana names collected are shown and given different symbols on key. Zanzibar islands are isolated from the main map and clearly drawn on the right side of the map.

Table 2. Polymorphisms detected by 20 SSR markers across 159 banana varieties.

Primer Name	Motif	Amplified genotypes	Number of polymorphic bands	Allelic patterns	Polymorphic Information Content value (PIC)
mMaCIR102	(AG)10, (TG)5	87	2	3	0.547
mMaCIR103	(CT)14,	85	3	4	0.663
mMaCIR105	(CA)8, (CT)15	109	3	4	0.593
mMaCIR108	(CA)7, (CA)4,	91	3	4	0.58
mMaCIR109	(CA)13,	76	2	3	0.531
mMaCIR112	(CA)5, (CA)15	103	2	3	0.528
mMaCIR114	(AC)7, (CT)28	86	3	4	0.536
mMaCIR122	(GT)8,	113	4	5	0.524
mMaCIR129	(CA)6,	91	3	4	0.66
mMaCIR137	(TC)12,	88	3	4	0.665
mMaCIR154	(CT)17	77	4	5	0.594
mMaCIR156	(TG)23	75	4	5	0.549
mMaCIR157	(CA)9, (TA)7	73	4	5	0.631
mMaCIR189	(CT)3,(CT)16	99	2	3	0.649
mMaCIR195	(GA)11, (GA)6	104	3	4	0.563
mMaCIR219	(GA)18, (AC)1	111	4	5	0.707
mMaCIR247	(GT)10,	106	3	4	0.698
mMaCIR277	(TG)12	88	4	5	0.756
mMaCIR280	(TC)7, (AC)7	91	3	4	0.708
mMaCIR297	(TC)9, (AC)13, (CA)9	78	4	5	0.508

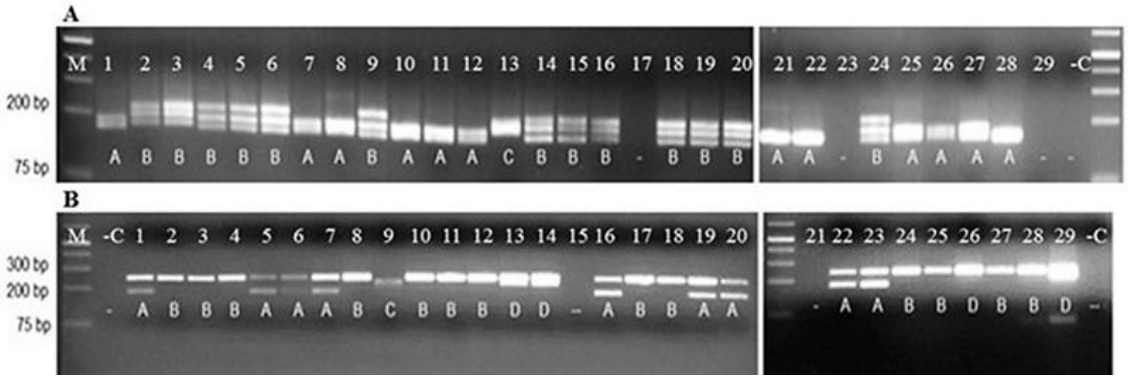

Fig 2. Agarose gels showing amplification profiles of primer pairs mMaCIR102 (A) and MaCIR129 (B). "M" = 1 kb plus DNA ladder. Letter "-C" represents a non-template negative control. The numbers 1 to 29 are the representative samples that correspond to banana varieties as listed in Supplementary Table 1. Capital letters A–D represent different types of banding patterns amplified by SSR markers in each representative DNA sample.

Table 3. Overlapping banana varieties in cluster A and B as established by using SSR loci.

Cluster	Group	Variety code	Variety name	Region
A	1	BT93, BT79	Uganda	Mbeya
	2	BT7, BT61, BT8, BT85, BT127, BT108	Bukoba	Ruvuma, Zanzibar, Mbeya, Pemba, Arusha
		BT109	Enjubo	Kagera
	3	BT13, BT66	Gros-Michel	Kagera
		BT137, BT153	Jamaica	Kilimanjaro, Arusha
	4	BT25, BT73, BT69	Kijakazi	Zanzibar, Pemba
	5	BT51, BT54	Matoke	Kilimanjaro
	6	BT36, BT57	Nchoncho	Kagera
	7	BT143, BT148	Nshansha	Kagera
	8	BT37, BT65	FHIA 17	Kagera, Mbeya
B	9	BT15, BT34	Mzuzu	Ruvuma
	10	BT53, BT101, BT144	Toki	Mbeya
	11	BT106, BT114	Mzuzu, Mzuzu mwekundu	Pemba

similis and *P. coffeae* were all from the Southern Highlands (Ruvuma and Mbeya regions), whereas varieties containing the least PPN mean count (mostly the introduced varieties; FHIA and Gros-Michel) were from the Lake Zone (Kagera region).

Discussion

In this study, SSR markers were successfully used to study genetic diversity of 159 banana varieties collected from 4 agro-ecological zones in Tanzania. The SSR markers used were highly polymorphic, denoting high genetic diversity both among and within banana varieties. The number of alleles per locus in each banana variety and the number of alleles per locus generated by each marker were variable. The variation ranged from 2 to 4 alleles per locus. These results were contrary to the results obtained in a previous study using SSR markers (Christelová et al., 2011) which found a higher number of alleles per locus (267 varieties), ranging between 8 and 24 per locus and a mean value of 14 alleles per locus in 65 banana genotypes.

Another study (Amorim et al., 2012) obtained low average number of alleles per locus (5.73) for 22 *Musa* genotypes obtained from the *Musa* germplasm (www.cnpmf.embrapa.br). Thus, the number of alleles among banana varieties obtained in this study is different from the other findings (Crouch et al., 1998; Hippolyte et al., 2010; Christelová et al., 2016). We considered the variations were due to different genotypes assessed in this study or the SSR data scoring method of presence/absence of DNA bands which may exaggerate or underestimate the number of alleles.

Moreover, the PIC of 20 SSR markers which were used in this study ranged from 0.5 to 0.75 with an average of 0.60. PIC values of 0.5 to 1 indicates high degree of genetic diversity whereas PIC values <0.5 indicates narrow genetic diversity. The SSR markers which were used in this study generated a total of 63 polymorphic alleles which were assessed across 159 banana plant samples. Other studies have been conducted worldwide using SSR markers to characterize *Musa* spp. These studies included a study by Creste et al. (2003) that generated 67 alleles assessed within 35 Brazilian *Musa* accessions using 11 SSRs. In addition, a study by Oriero et al. (2006) reported 23 alleles using 9 SSRs in 40 banana accessions in Nigeria. The different number of alleles among banana varieties collected in this study might have been

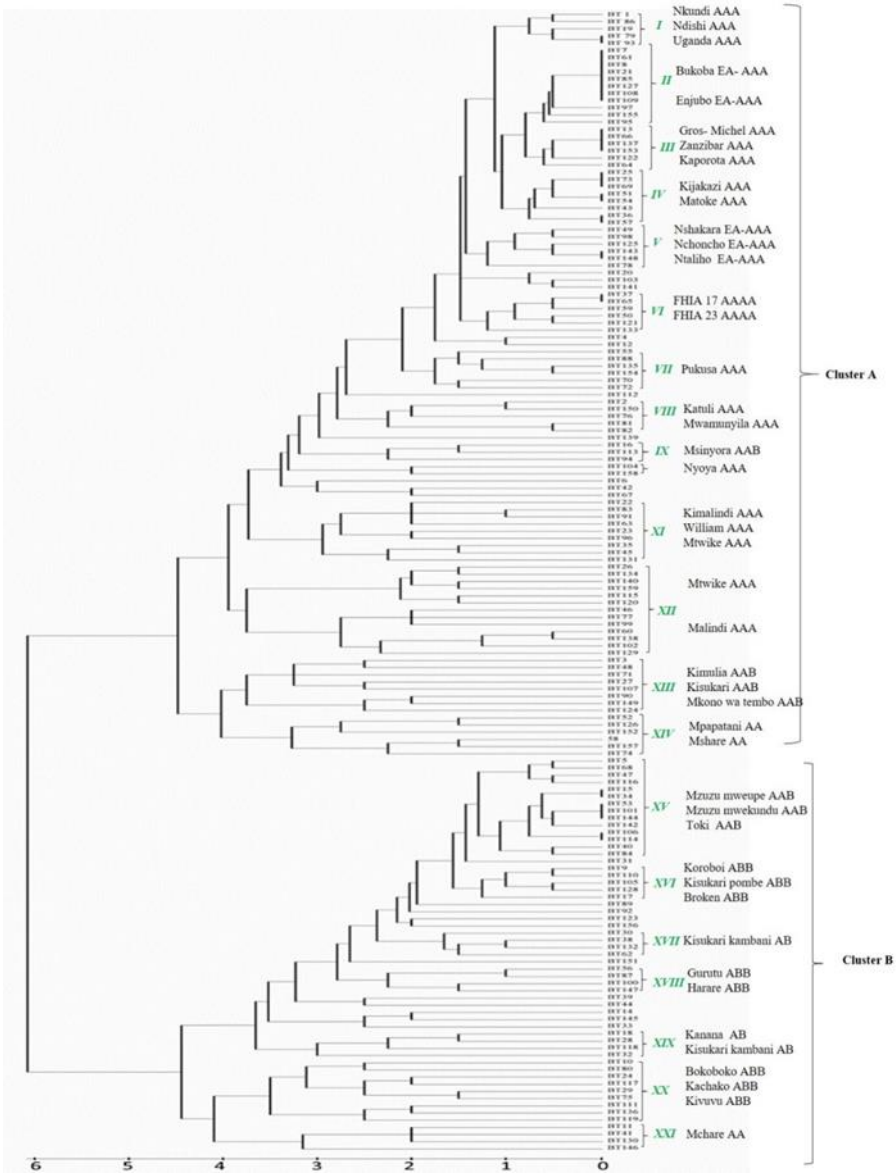

Fig 3. Dendrogram showing results of the UPGMA analysis on SSR markers with clusters A and B, which resulted in a total number of 139 unique varieties out of 159 banana samples characterized. Details of banana varieties (1–159) are provided in Supplementary Table 1.

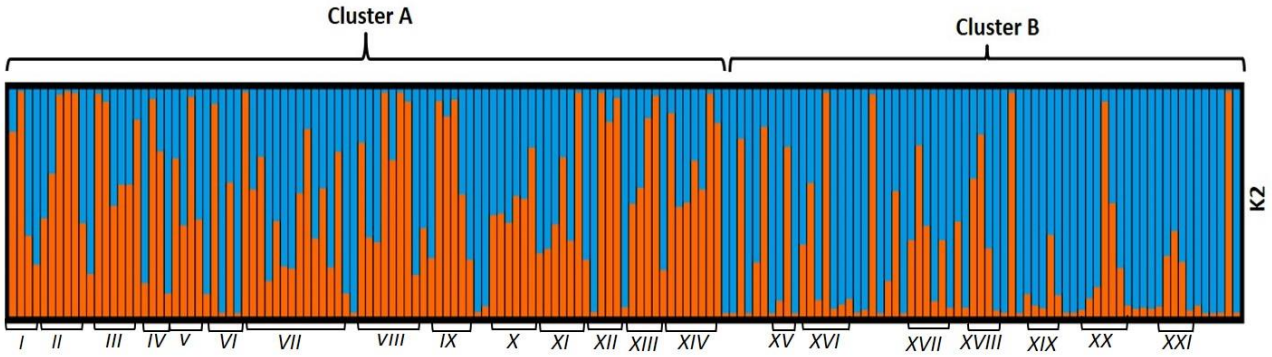

Fig 4. Genetic structure of the 159 banana varieties as inferred by STRUCTURE based on 19 SSR data set.

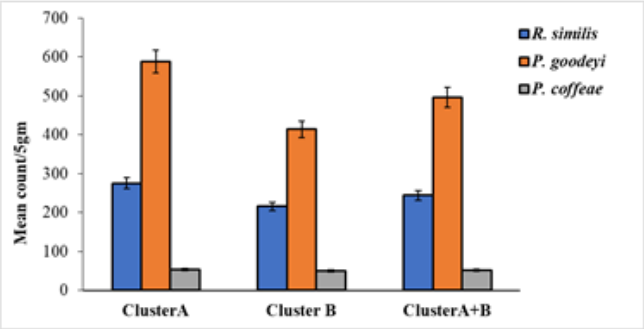

Fig 5. Mean count of nematode species in the roots of different banana varieties collected from major banana growing areas in Tanzania. Banana varieties are grouped into two distinct genetic clusters (cluster A and B). Details of the banana varieties are provided in Table 1. The error bars represent a 5% error.

caused by either the presence of duplicated alleles or duplicated chromosomal regions which are common in different *Musa* L. genomes; or might have been caused by the utilization of the large number of accessions from diverse varieties accessed in our study. The phylogenetic analysis revealed two major clusters: A and B, with a total of 128 independent and 31 overlapping genotypes. Higher kinship among genotypes was observed in cluster A with higher number of overlapping varieties (24) than in cluster B with (7). This indicates the presence of some common alleles within banana populations in Tanzania, which may have been resulted from lack of a well-organized system of varieties exchange across regions.

However, all banana genotypes in cluster A and B were grouped in sub clusters *I* to *XXI* and some other genotypes were grouped independently, indicating the presence of unique alleles and low allelic frequencies within Tanzanian banana germplasm. Banana genotypes that were grouped independently in cluster A may be crossed with other distantly related genotypes in cluster B to increase chances of obtaining banana varieties with disease resistance and high-yield traits.

The presence of more sub-clusters in cluster A (*I* to *XIV*) indicated that banana genotypes in cluster A were evolving more quickly and more distantly related. Based on a coefficient scale, some of genotypes in cluster A and B appeared to have the longest horizontal branches including genotypes in sub clusters *XIII* and *XIV* (cluster A) and *XIX*, *XX* and *XXI* (cluster B). This may be related to mutations which have been occurring over a period that may be resulted from banana domestication processes, adoption of new farming practices, or change in climatic conditions (Hippolyte et al., 2010).

In addition, our findings indicated that, there are many East African highland banana varieties (EA-AAA genome) and the introduced varieties (AAA and AAAA genome) in cluster A compared with those in cluster B. Most of these varieties were collected from Kagera and Mbeya regions. This confirms that there is a constant movement of banana planting material in these regions (which are along Tanzanian borders) compared to other regions where the samples were collected. The UPGMA cluster analysis of 159 banana varieties could not cluster the genotypes according to their genome composition or geographical origin. Different genomes in a cluster suggested the possibility of these banana varieties to have been originated from either a common ancestor or mutation related to the degree of crop planting, reproduction and/or damage by pest (Creste et al., 2003). Furthermore, our results indicate that some genotypes from different sub clusters in cluster A & B did not show any similarity from each other although they were from the same cluster, which implies the high degree of polymorphism.

On the other hand, we found higher PPN mean counts in most of banana genotypes from cluster A than cluster B. The presence of higher mean counts in most of cluster A genotypes might have been resulted from the susceptibility of many genotypes to the PPN in cluster A (particularly local varieties) compared with those in cluster B. Furthermore, banana varieties with the highest mean count of nematodes were from the Southern highlands zone (Mbeya and Ruvuma). This suggests the possibility of having many local and susceptible banana genotypes in Southern highland zone, poor management of banana field or high movements of planting materials from one area to another. Varieties with the least mean count of nematodes were from the Lake zone. This might have been due to many introduced varieties in the Lake zone which are tolerant to PPN; climatic conditions that does not favor nematodes reproduction; or proper management of pest and diseases among different banana fields.

High number of nematodes observed in all major banana growing areas in Tanzania indicates that most of banana varieties are highly susceptible to the burrowing and root lesion nematodes. These results are consistent with other studies (Rajab et al., 1999; Price, 2006; Luambano et al., 2019), that reported highly abundant specimens of the burrowing and the root lesion nematodes in banana growing areas in Tanzania. In addition, Coyne et al. (2009) also suggested higher susceptibility of the East African highland bananas that are the dominant varieties in East African countries, particularly in Tanzania as per this study. *P. goodeyi* had the highest mean count of all nematodes in both cluster A and B. This finding suggests that *P. goodeyi* is continuously spreading to various regions regardless of climate conditions and/or altitude (Coyne et al., 2018).

Genetic diversity of bananas existing in Tanzania established in this study and occurrence of major PPN is very useful for banana breeding programs as these programs mostly target on development of banana varieties with superior traits (Crouch at al., 1998). The SSR markers used in this study are highly informative and are proved to be a reliable technique for genetic variation studies as the clustering done by these SSR and structure analysis provided similar banana results.

Materials and Methods

Study sites

Field surveys were conducted in the major banana-growing areas of Tanzania (Fig 1), mainly in the Lake Zone (Kagera region), Southern Highlands (Mbeya and Ruvuma regions), Northern Zone (Arusha and Kilimanjaro) and the Islands of Zanzibar (Pemba and Unguja).

Sample collection

By using random-sampling procedure, banana leaves and root samples were collected from 53 fields across Tanzania (Fig 1). GPS (Garmin, Nandanam, India) was used to record all coordinates and altitude of each collection field for reference. Farmers provided local names of the banana varieties; and samples were collected from fields approximately 10–15 km apart. A single young banana leaf was sampled from each of three randomly selected banana plants in each field for DNA work and banana root samples were collected from the same plant (Speijer and De Waele, 2001) for nematode isolation and characterization. Leaf samples were kept in coffee filter bags and stored in silica gel. Root samples were kept in labelled plastic bags and stored in cool boxes to ensure nematode survival before transporting them to the TARI-Kibaha Laboratory where they were stored at 10 ºC before use.

Isolation of genomic DNA from banana leaves

Genomic DNA was extracted from banana leaves following a modified cetyl trimethyl ammonium bromide (CTAB) protocol (Xu et al., 2009), where 0.03 g of dried banana leaves from each variety were measured and placed in 1.5 mL microcentrifuge tubes containing metal beads. The leaves were ground in a geno-grinder (Spex Sample Prep 2010) for 40 sec. Pre-warmed extraction buffer (2% CTAB) of 700 µL mixed with 10% mercaptoethanol was dispensed in each tube. The mixture was incubated at 65 °C for 30 minutes and left on the bench at room temperature for 10 minutes. Equal volume (700 µL) of phenol:chloroform: isoamyl (25:24:1 ratio) was added and mixed by inverting the tubes for 10 min and centrifuged for 10 minutes at maximum speed of 20,000 x g relative centrifugal force (RCF) (MIKRO 220R, Hettich, Vlotho, Germany). Approximately 550 µL supernatant was pipetted to the clean microcentrifuge tubes and 450 µL of cold isopropanol was added. The tubes were incubated in ice for 20 minutes; then centrifuged for 10 minutes at 20,000 x g RCF. The DNA pellets were washed in 700 µL ethanol before being air dried for 45 minutes. Pellets were re-suspended by using sterile distilled water supplemented with 2 µL of 10 mM RNase A enzyme to degrade any remaining single-stranded RNA. DNA concentration and purity were determined by the optical density method using a Nano Drop Spectrophotometer ND 2000 (Thermal Scientific, Cambridge, MA, USA). Genomic DNA samples were then normalized to final concentrations of 25 ng/µL for use in genotyping.

SSR markers and PCR amplification

PCR of 20 SSR markers which are highly distributed in the banana genome was carried out by using 20 primer pairs (Table 1) designed and used by (Lagoda et al., 1998; Hippolyte et al., 2010). PCR was conducted in a 20 µL volume containing 50 ng/µL of genomic DNA, 5 υ/µL of one Taq polymerase enzyme, 10 mM dNTPs, 10× standard buffer and 10 µM of each primer. PCR conditions were as follows: initial denaturation at 94 °C for 3 minutes, followed by 30 cycles at 94 °C for 30 seconds, annealing for 30 seconds at temperature of respective primer pair, extension at 72 °C for 2 minutes and final extension at 72 °C for 10 min. All PCR reactions were carried out in Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were separated on 2% agarose gels, stained with ethidium bromide (0.5 µL/10 mL of gel) on TAE buffer (Tris-Acetate-EDTA) of pH 8.0, buffer at a constant 200 volts for 45 minutes. The gels were visualized by the UV Imaging Systems (Thermal Science, Upland, CA, USA) and fragment sizes were estimated by using 1 Kb Plus molecular size ladder (Thermo Fisher Scientific, Rochester, NY, USA).

Extraction and counting of nematodes from banana roots

Banana roots were washed with running water to remove adhering soils then rinsed with distilled water. The roots were chopped into several pieces of 1 cm and approximately 5 g were added in 100mls distilled water; then macerated ina kitchen blender for 10 seconds. Nematodes were extracted following the modified Baerman's techniques (Hooper et al., 2005) whereby all macerated materials were poured onto a two layers of napkin tissue paper positioned on a plastic sieve that was placed on a plastic plate. Some amount of water was added in a plastic plate to a level of touching the bottom of the plastic sieve to allow movement of nematodes from the macerated materials to the plastic plate. The whole setup was covered with a plastic plate to increase darkness and incubated on the bench at 28°C for 24 hours. Thereafter, the plastic sieve and tissues were removed and water that was collected on the plastic plate from the setup was poured into a clean beaker and was left for 30 minutes to settle. The top water was carefully decanted and 20 mls of the concentrate containing nematodes were left for nematode isolation and counting. Two millilitres of the concentrate were pipetted to the counting slide for identification and counting under a compound microscope (Leica, DM 2500, Leica Microsystems CMS GmbH, Wetzler, Germany) at 20× magnification. On a compound microscope, nematodes were firstly identified based on their distinguishing features to separate them to the genus and species level. PPN identification was done following common morphological characters normally used for their identification including the entire PPN body shape, number of lip annuli, shape of stylet and stylet knobs, shape and position of the reproductive organs including vulva and ovary (females)/testis and spicule (males), position of oesophageal gland and tail shape as described by Hunt et al. (2005), Siddiqi (2000) and Roy et al. (2018). The counting of nematodes was repeated three times for each sample. The mean count was used for further calculations and number of nematodes was calculated to represent nematodes in 100 gm of roots (Hooper et al., 2005).

Data analysis

Amplified DNA bands were scored for each SSR marker based on the presence or absence of bands, generating a binary data matrix for each SSR marker. Jaccard's similarity coefficient values were calculated and dendrograms based on similarity coefficient values were generated by using unweighted pair-group method with arithmetic means (UPGMA) method using NTSYS 2.0 software. Polymorphic information content (PIC) value of SSR markers was calculated using the formula explained by (Anderson et al., 1993).

The genetic structure analysis of the varieties was estimated by using the Bayesian clustering method implemented in STRUCTURE 2.3.4 software (Pritchard et al., 2000). This approach estimated the optimal number of genetic clusters (K) and calculated the membership proportion of varieties. The analyses were based on the admixture ancestral model for a range of K values from 1 to 10. We performed 10 runs for each K and removed those with extreme values of L(K) that were tagged as outliners according to Evanno et al. (2005). Each run was implemented with a burn-in period of 10,000 steps followed by 100,000 Monte Carlo Markov Chain iterations. The optimal number of K clusters was estimated with the ad hoc parameter (ΔK) of Evanno et al. (2005) in Structure Harvester (Earl and vonHoldt, 2012). We estimated the optimal alignment for the 10 replicates in CLUMPP (Jakobsson and Rosenberg, 2007).

Data on PPN in banana genotypes were subjected to analysis of variance (ANOVA) in a GenStat statistical package (14th edition, VSN International Ltd, Hemel Hempstead, UK). The Post-hoc Tukey's Honestly Significant Difference (HSD) test was used in pairwise comparison to analyse differences between means. Treatment means were compared by using least significance differences (LSD) at $p<0.05$ and $p<0.01$.

Conclusion

This paper reports important information that was necessary but missing on banana production in Tanzania. Genetic diversity and assessment of PPN affecting bananas cultivated in Tanzania is crucial information for accelerating breeding programs through crossing of distantly related banana genotypes to obtain a durable resistant crop that is highly productive. Future studies should focus on the development of saturated genetic linkage maps which is important to enable marker-assisted selection and improve crop selection efficiency based on tolerance or resistance to pests. Further studies should also focus on understanding the interaction of PPN with different banana genotypes and identify genes that are responsible for conferring tolerance or susceptibility to PPN.

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