Genetic diversity among Tanzanian banana (*Musa* spp.) varieties assessed by simple sequence repeat (SSR) markers

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General information

Banana

- Scientific name ‘’**Musa species**’’
- Botanical family ‘’**Musaceae**’’
- Banana – used as food and cash crop, feed livestock, ornamental, cultural practices and brewing
- Tanzania - a second banana producer in EA
- Banana - a third crop cultivated in Tanzania

Simple sequence repeat (SSR) markers

SSR markers have been widely used for genetic analysis and cultivar identification because of their;-

- Abundance
- Co-dominance inheritance
- high polymorphism
- Reproducibility, and
- Easy of assay by PCR

Genetic variation and relationships

Knowledge of genetic variation and relationships between accessions or genotypes is important;-

- To understand the genetic variability available and its potential use in breeding programs
- To estimate any possible loss of genetic diversity
- To offer evidence of the evolutionary forces shaping the genotypic diversities, and
- To choose genotypes to be given priority for conservation

Problem statement

- Banana and plantains (*Musa* spp.) are one of the most economically important fruit crops in Tanzania
- However, there is limited information on genetic and genomic resources that may be important in supporting the development of either:
- farmer-preferred (yield potential and large seed),
- breeders preferred (environments stress and pests and diseases resistance)
- and/or consumer preference traits (fruit quality)

- In addition, names and accurate identities of banana are not clearly known hence, limiting sustainable farming
- Characterization of crops based on DNA markers is the measures for correct and quick identification of similar or closely-related cultivars
- Hence this study….

Aim

- This study aimed at assessing genetic diversity of banana genotypes using SSR markers
  - For accurate identification of varieties
  - Banana molecular breeding programme initiation
  - disease management.

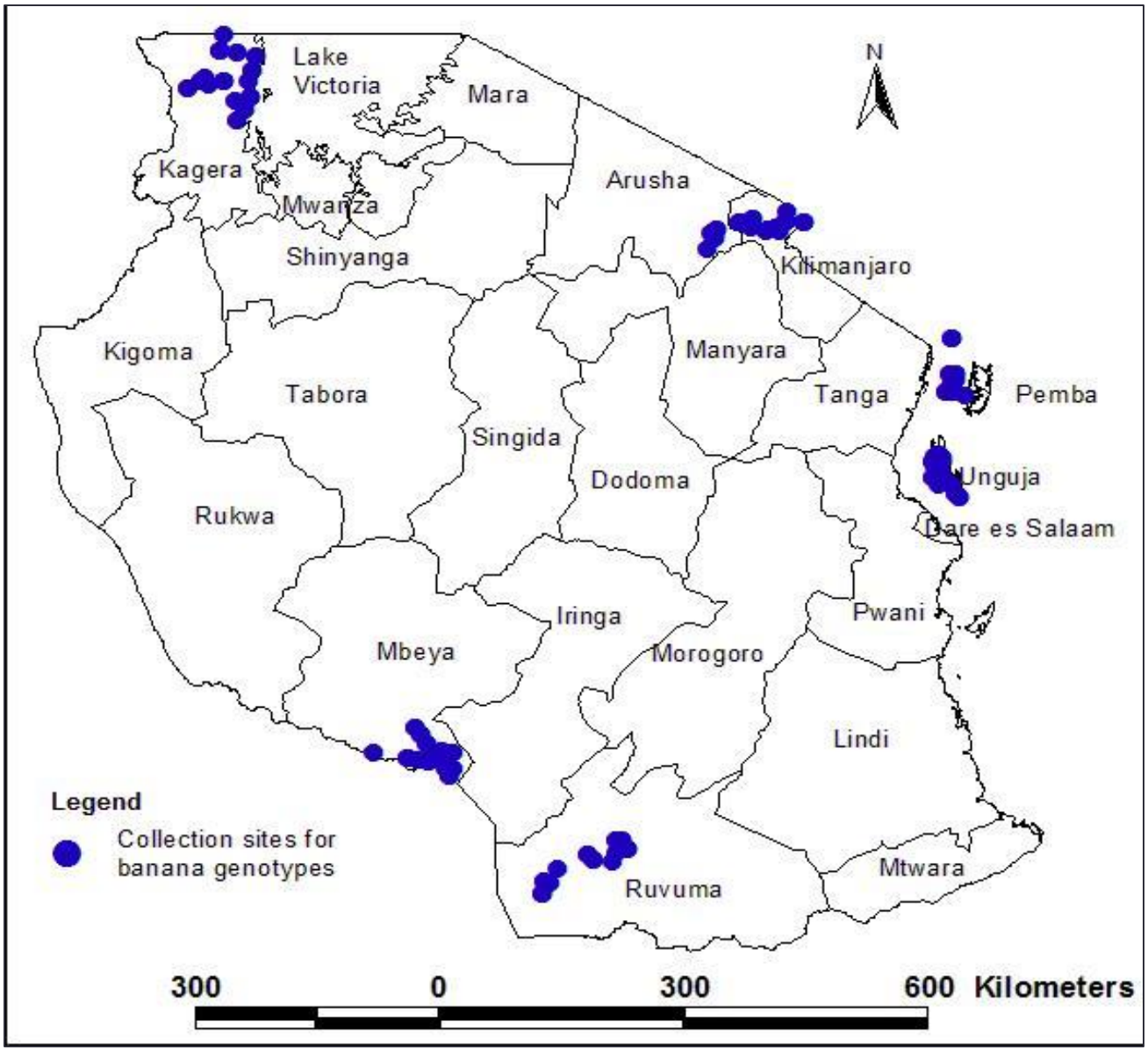
Material and method

Plant materials

- A total of 159 banana varieties used in THIS study were collected from major banana growing areas of Tanzania

Genomic DNA extraction

- Plant genomic DNA isolation was done using modified CTAB method


Lake
Victoria
Mara
N
Kagera
Arusha
Mwanza
Shinyanga
Kilimanjaro
Kigoma
Manyara
Tabora
Tanga
Pemba
Singida
Dodoma
Unguja
Rukwa
Dare es Salaam
Iringa
Pwani
Mbeya
Morogoro
Lindi
Legend
Collection sites for
banana genotypes
Mtwara
Ruvuma
300
0
300
600 Kilometers

SSR-PCR amplification

- Amplification of SSR markers were carried out

- A total of 20-polymorphic banana SSR primers were used in this study

Data analysis

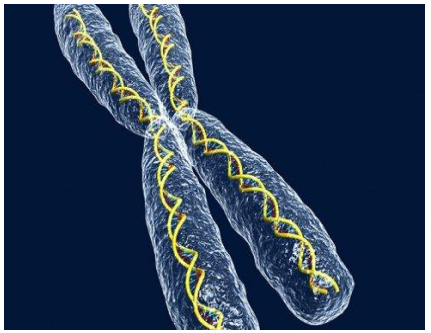
- The band profiles were scored only for distinct and reproducible bands as present (1) or absent (0)
- Binary matrix data was imported into
- Binary cluster analysis software NTSYS pc 2.0ver

Polymorphic information content (PIC)

- The PIC value of SSR markers was calculated using the following formula

$$PIC = 1 - \sum_{i=1} P_i^2 - \sum_{i=1} \sum_{j=i+1} P_i^2 P_j^2$$

- Where *Pi* and *Pj* are the population frequency of the *i*th and *j*th allele (Dalamu *et al.*, 2012)



Results and discussion

Genetic diversity based on PIC values

- Twenty SSR primer pairs were polymorphic and generated a total of 63 distinct reproducible bands

- The number of polymorphic bands detected with each primer pair ranged from 2 to 4 with an average of 3.15 per primer pair

- The polymorphic information content values of each primer pair ranged from 0.50 to 0.75 with an average of 0.60

Tab 1. Polymorphism detected by 20 markers in 159 banana genotypes

Primer Name	Motif	Amplified genotypes	Number of polymorphic bands	Allelic patterns	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

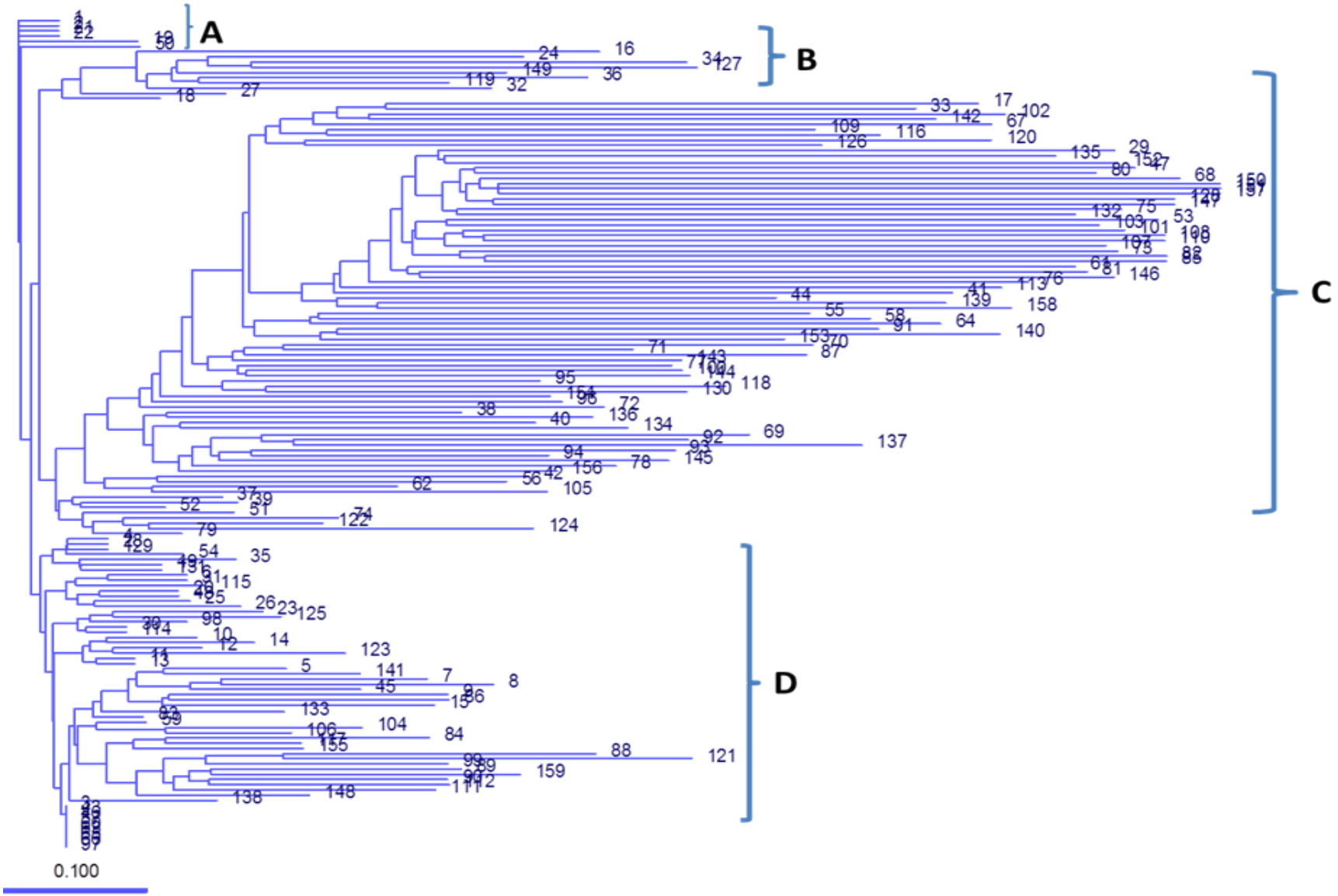
mMaCIR129	(CA)6,	91	3	4	0.66
mMaCIR137	(TC)12,	88	3	4	0.665
mMaCIR154	(CT)17	77	4	5	0.594
mMaCIR156	(TG)23	75	4	5	0.549
mMaCIR157	(CA)9,(TA)7	73	4	5	0.631
mMaCIR189	(CT)3,(CT)16	99	2	3	0.649
mMaCIR195	(GA)11,(GA)6	104	3	4	0.563
mMaCIR219	(GA)18,(AC)1	111	4	5	0.707
mMaCIR247	(GT)10,	106	3	4	0.698
mMaCIR277	(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
	Average	91.55	3.15	4.15	0.6095

Genetic diversity based on Dendrogram and Principal Coordinate Analysis (Fig 2 and Fig 3)

- The UPGMA cluster analysis separated the 159-banana cultivars into four major groups
- **Cluster A** is composed of fewest genotypes (1, 19, 21, 22 and 50) - All from Kagera except genotype no. 1 from Pemba
- **Cluster B** contains 9 genotypes: 3-Kag; 3-Mby; 1-Ruv; 1 ZNZ North and 1 Pemba
- **Cluster C:** The largest- with 77 genotypes
- **Cluster D:** 54 genotypes

- The results revealed a high genetic diversity among the 159-banana cultivars

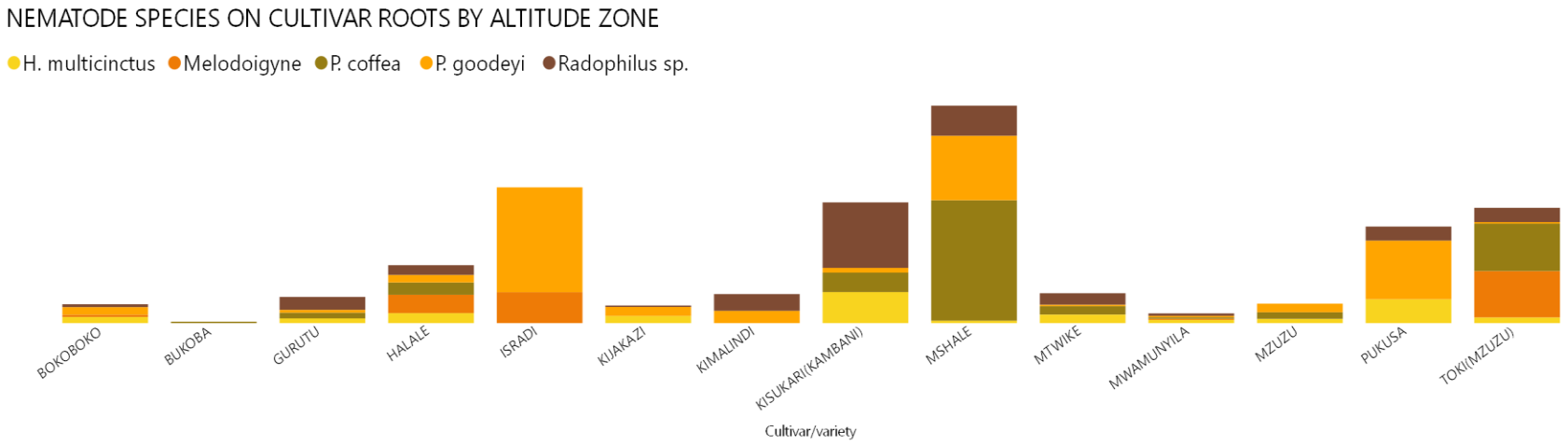
Fig 2. Dendrogram of banana varieties based on 20-SSR primer pairs



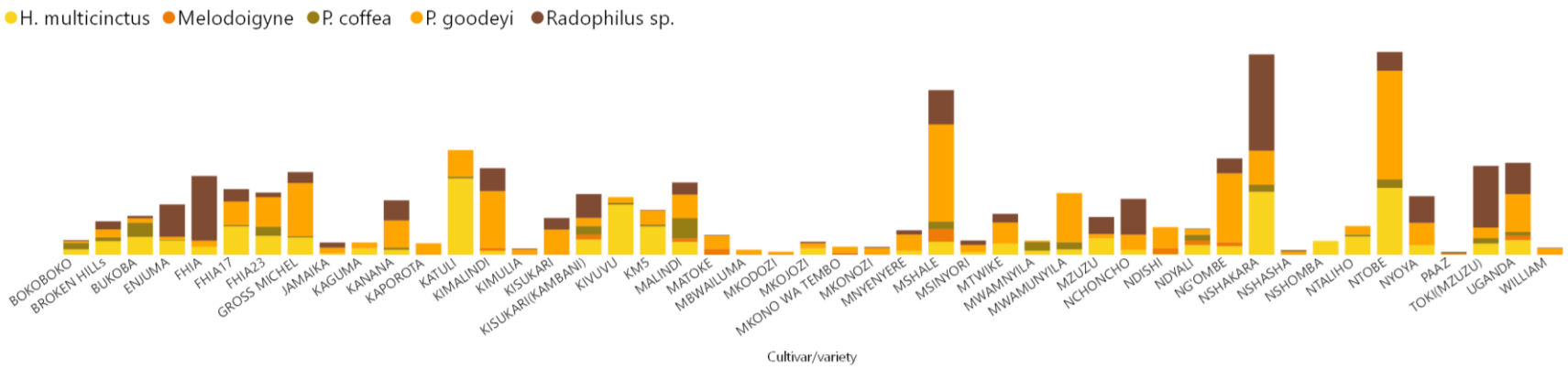
Tab 2. Closely related banana genotype with different names recorded from different locations

cluster A	Variety Name	Region	District
1	Ali Hassan Mwinyi	Pemba	Wete
19	Gross-Michel	Kagera	Bukoba
22	Gross-Michel	Kagera	Muleba
24	FHIA 17	Mbeya	Kyela
50	FHIA 17	Mbeya	Kyela
cluster D			
6	Bokoboko	Pemba	Wete
32	Kaguma	Kagera	Misenyi
46	Kisukari	Pemba	Micheweni
57	Kanana	Kagera	Muleba
60	Koroboi	Pemba	Wete
63	Malindi	Ruvuma	Namtumbo
65	Malindi	Kilimanjaro	Hai
66	Malindi	Arusha	Arumeru
97	Mtwike	Zanzibar	Unguja

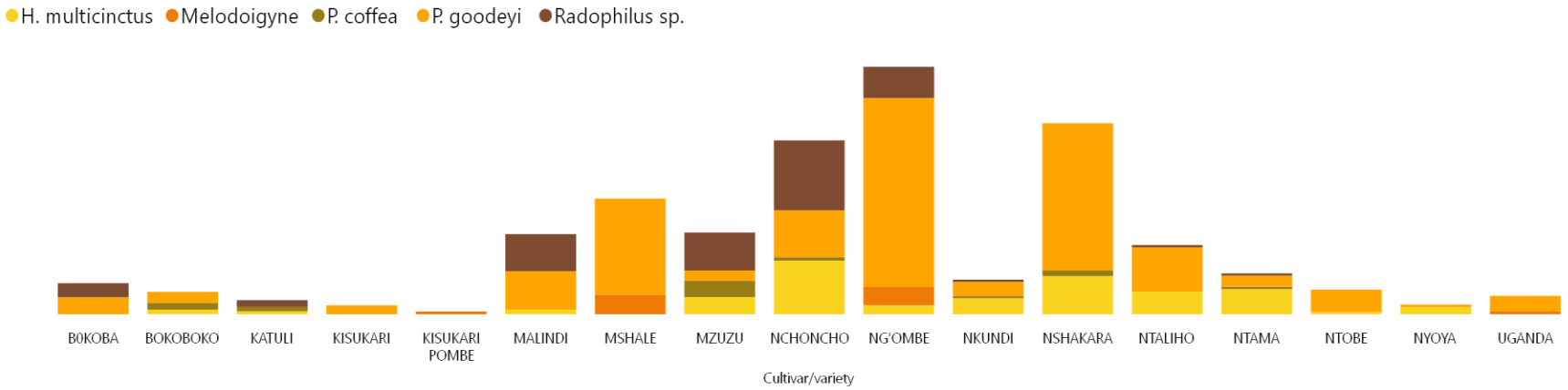
Nematode found on Banana cultivars from 501-1000 masl



Nematodes extracted from banana cultivars from 1001-1500 masl



Nematodes extracted from banana cultivars from 1501-2000 masl



Conclusions

- In this study, 20 sequences of the primers were highly suitable for studying genetic diversity of Tanzanian banana varieties
- The 20-SSR markers also identified possible duplicates in banana varieties collected
- High genetic diversity was demonstrated among 159-banana varieties cluster in four distinct groups

Conclusion cont.…

- The information that is important to know banana varieties susceptible to nematodes and those which are tolerant

- Some varieties are improved but found susceptible to other nematodes

Recommendation

- To initiate systematic breeding programs based on this developed genomic banana information

- Develop further study for the distinct traits in the formed clusters of banana and incorporate in breeding programs

- Construction of genetic linkage maps
- ✓DNA markers linked to the agronomic characteristics, fruit characteristics and other traits of interest for sustainable banana breeding in Tanzania.

Acknowledgement

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