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Full Length Research Paper

Genetic diversity analysis of Kenyan taro [*Colocasia esculenta* (L.) Schott] accessions using SSR markers

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Six SSR primer pairs were used to characterize 25 taro genotypes of Kenya. A total of 30 polymorphic alleles were generated. The number of alleles per locus ranged from 1 to 6 alleles, with an average of 3.0425 alleles across 18 loci obtained in the study. The polymorphic information content values ranged from 0.1875 to 0.5731 in all 18 loci with an average of 0.4120. Genetic diversity ranged from 0.25 to 0.6218. Genetic richness ranged between 1.5 and 4.67. The frequency of most common allele at each locus ranged from 51.21% to 75%. The pair wise genetic dissimilarity co-efficient indicated that the highest genetic distance was obtained between the Rift Valley and Nyanza taro germplasm populations (0.794). The closest allele similarity was between Western and Nyanza (83.1%) taro populations while the widest dissimilarity was between Rift Valley and Nyanza populations (45.2%). Being grouped into a distant cluster KK12 could be exploited as probable parental for the development of variant taro varieties. The SSR markers are comprehensive source for the identification of genetically distant taro accessions as well as in the replica sorting of the phenotypically close germplasm.

Key words*:* Cocoyam, genetic diversity, alleles, molecular variation, *Colocasia esculenta.*

INTRODUCTION

Taro [*Colocasia esculenta* (L.) Schott] is a member of the Araceace family that is a staple food for many people in developing countries in Africa, Asia and the Pacific (Agueguia et al., 1992). Taro is thought to have originated from the Indo-Malaysian region, perhaps in eastern India and Bangladesh or from Tropical America where it has been cultivated since pre-Columbian times (Asemota et al., 1996). It then spread eastwards into southern Asia and the pacific islands and westwards to Egypt and the eastern mediterranean. Finally, it spread to southwards and westwards into East Africa and West Africa (Bachman, 1994). Therefore taro is an introduced crop in east Africa (Ki-zerbo, 1990).

The morphology of taro is quite variant. The cultivated species of taro may be distinguished into two main groups - the “eddoes” types and the “dasheen” types (Valerio, 1988; Ki-zerbo, 1990; Onwueme, 1994; IPGRI, 1999). The eddoes types have side tubers (cormels) that may be 5 – 20 in number and become as big as the mother corm, while the cormels are usually absent in the dasheen types and it’s the mother corm which is the main storage organ (IPGRI, 1999). The corm and cormel which are the major economic parts have a nutritional value comparable to potato while the young leaves and petioles which are occasionally used for food contains about 23% protein on a dry weight basis (Wang, 1983). It is also a rich source of calcium, phosphorus, iron, vitamin C, thiamine, riboflavin and niacin, which are important constituents of human diet (Onwueme, 1999; Ndon et al., 2003).

Where it is grown in Africa, taro corms have a high economic value in urban markets. Taro production provides employment to a large number of people within the Lake Victoria basin and the crop maintains ground cover in the fields (Serem et al., 2008; Talwana et al., 2009). While a lot of taro is produced and consumed on a

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subsistence basis, quite a considerable amount is produced as a cash crop. Also surpluses from the subsistence production manage to find their way to markets, thereby playing a role in poverty alleviation. However, there is very limited local research on taro in Kenya that the actual contribution of taro to food security and economy is underestimated, and its profile on the national research and conservation agenda is miserably low. It is not surprising, therefore, that the average taro yields in Africa remain very low for the majority of smallholder producers with annual yield rarely exceeding one ton per hectare in East Africa (Serem et al., 2008; Talwana et al., 2009) compared to the African and world average of 5.9 and 6.6 tons/ha, respectively (FAO, 2008). Some of the main underlying causes for this paltry performance in East Africa include lack of certified seed, cultivation technology and effective crop management strategies (Serem et al., 2008). It is possible that the status of taro in Kenya can be improved to levels held by other important tubers like potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*). However, this will require development of technologies that would guarantee clean certified seed of desirable germplasm. This has created the need to understand how taro biodiversity and population biology impacts on crop growth and development in Africa. It is therefore important to develop appropriate molecular markers and assess the genetic diversity of East Africa taro cocoyam genotypes.

Cocoyam characterization has been undertaken using morphological descriptors (Karikari, 1973; Doku, 1983) and a combination of morphological characters and isozyme markers and total proteins (Aguegia, 1994; Offei et al., 2002). Recent advances in molecular genetics have provided techniques that allow researchers to study relationships among organisms at the molecular level. Molecular data are useful in cases where morphological characters alone are insufficient for the delineation of clear taxonomic groups. These markers, although valuable taro varietal group identification, reveal limited levels of inter and intra-varietal polymorphism. The similarity of common names and lack of obvious phenotypic variation among many taro accessions has often led scientists to suspect a high degree of genetic relatedness. This underscores the importance of the estimation of the genetic diversity within germplasm collections (Green and Pedersen, 1996).

Random amplified polymorphic DNA (RAPD) and SSRs (single satellite repeat sequences) techniques have proven to be useful methods for fingerprinting accessions of taro (Mace, 2000). RAPD markers, however, have limitations including questionable reproducibility of some bands, a requirement for stringent standardization of reaction conditions, co-migration of different amplification products, and dominance inheritance (Bachman, 1994). Despite these caveats, the RAPD method offers the highest potential for generating large numbers of markers with the greatest ease under limited resource conditions (Schnell et al., 1999). Microsatellites are DNA sequences composed of a tandem repetition of a simple short sequence, occurring in the genome of many higher organisms. Multiple allelic length variants can be identified at most microsatellite loci. Microsatellite markers are highly polymorphic, co-dominant loci and are abundant in most of species genomes. However the high of alleles per locus causes some bias in diversity estimates due to increased heterozygosity levels. The high mutation rates also means that microsatellites suffer from homoplasy problems (Schlotterer et al., 1998) and may also increase within-population component of variation.

Simple sequence repeats (SSR), have been employed to study taro resources. Sixteen microsatellites have already been developed for *C. esculenta* (Mace and Godwin, 2002), and seven of them were used in the SSR analysis for the taro germplasm resources in the Pacific Island region (Mace et al., 2006). In China, 11 novel microsatellite markers were isolated and developed from taro germplasm from China. However, the microsatellite markers were not transferable across genera to *Xanthosoma* (Singh et al., 2008). This study used SSRs to establish the population biology and diversity that exists amongst taro accessions in Kenya. This provided the opportunity to estimate the level and distribution of genetic variation within the populations of taro cocoyam used as food sources in East Africa.

MATERIALS AND METHODS

Plant material

Taro vegetative samples were collected from different regions in Kenya. Twenty five accessions of the Kenyan taro germplasm were collected from Western, Central, Nyanza and Rift valley region parts of Kenya (Table 1).

DNA extraction from taro germplasm

The taro DNA was extracted from the youngest leaves. The young leaf tissue was considerably chosen because it yielded DNA that was most suitable for polymerase chain reactions. The taro vegetative samples were used for the extraction of genomic DNA according to the CTAB protocol modified and optimized by Sharma et al. (2008). Young leaf tissue (500 mg) of fresh leaf material was collected and washed in distilled water and rinsed with 80% ethanol. This involved the use of liquid nitrogen flash freezing followed by grinding the frozen tissue with a mortar and pestle. Extraction buffers EBA and EBB were used in the extraction. 300 µl EBA, 900 µl EBB, and 100 µl SDS were added, vortexed and incubated at 65°C for 10 min. The tube was placed on ice and 410 µl cold potassium acetate added. Then mixed by inversion and

Table 1. Description of the Kenyan taro germplasm collections used in the study.

S/No	Accession Number	Accession name	Origin	Latitude	Longitude
1	KCT/GHT/31	Kigoi31	Central-Kenya	00.416666°	036.66666°
2	KCT/KGI/32	Kigirigasha32	Central-Kenya	00.416666°	036.66666°
3	KCT/NGC/33	Ngirigacha33	Central-Kenya	00.416666°	036.66666°
4	KWK/LKW/13	Lukuyw13	Western Kenya	0.28135°	034.75140°
5	KWK/ISW/14	Ishwa 14	Western Kenya	0.28135°	034.75140°
6	KWK/SHT/12	Shitao 12	Western Kenya	00.28273°	034.75186°
7	KWK/KAK/15	Kakamega T15	Western Kenya	0.28135°	034.75140°
8	KWK/KAK/16	Kakamega T16	Western Kenya	0.28135°	034.75140°
9	KWK/KAK/17	Kakamega T17	Western Kenya	0.28135°	034.75140°
10	KWK/BSA/42	Amak Tar72	Western Kenya	00.33333°	034.48333°
11	KMM/ELU/73	Eluhya73	Western Kenya	00.33333°	034.48333°
12	KMM/ENG/75	Mumias T75	Western Kenya	00.33333°	034.48333°
13	KMM/END/74	Enduma74	Western Kenya	00.33333°	034.48333°
14	KMM/MMU/78	Mumias T78	Western Kenya	00.33333°	034.48333°
15	KMM/MMU/79	Mumias T79	Western Kenya	00.33333°	034.48333°
16	KRT/KTL/61	Kiminini61	Rift Valley Kenya	00.89356°	034.92582°
17	KNY/SYA/51	Siaya51	Nyanza Kenya	00.0623°	034.28781°
18	KNY/KIS/81	Kisii T81	Nyanza Kenya	00.67831°	034.77197°
19	KNY/KIS/82	Kisii T 82	Nyanza Kenya	00.67831°	034.77197°
20	KNY/NYA/52	Kisumu NZ52	Nyanza Kenya	00.09170°	034.76196°
21	KNY/LVT/21	Lake VictoriaT21	Nyanza Kenya	00.75578°	034.43835°
22	KNY/LVT/22	Lake Victoria T22	Nyanza Kenya	00.75578°	034.43835°
23	KWK/BSA/41	Amagoro Busia41	Western Kenya	00.460769°	034.11146°
24	KWK/KAK/12	Kakamega T12	Western Kenya	0.28135°	034.75140°
25	KWK/LVT/23	Lake Victoria23	Nyanza Kenya	00.75578°	034.43835°

Table 2. Profiles of microsatellites loci (SSR markers) used taro germplasm collections.

SSR Primers	Allele Size Repeat Motif	Primer Sequence Forward Primer: (5´→3´) ; Reverse primer: (3´→5´)
Xuqtem55	(CAC)5	5´→3´: CTTTTGTGACATTTGTGGAGC 3´→5: CAATAATGGTGGTGGAAGTGG
Xuqtem73	(CT)15	5´→3´: ATGCCAATGGAGGATGGCAG 3´→5: CGTCTAGCTTAGGACAACATGC
Xuqtem84	(CT)18	5´→3´: AGGACAAAATAGCATCAGCAC 3´→5: CCCATTGGAGAGATAGAGAGC
Xuqtem88	(CAT)9	5´→3´: CACACATACCCACATACACG 3´→5: CCAGGCTCTAATGATGATGATG
Xuqtem91	(TG)6(GA)4	5´→3´: GTCCAGTGTAGAGAAAAACCG 3´→5: CACAACCAAACATACGGAAAC
Xuqtem97	(CA)8	5´→3´: GTAATCTATTCAACCCCCTTC 3´→5: TCAACCTTCTCCATCAGTCC
Xuqtem110	(TGA)6(TGGA)4	5´→3´: AGCCACGACACTCAACTATC 3´→5: GCCCAGTATATCTTGCATCTCC

Source: Mace and Godwin (2002).

the micro centrifuge, and then placed back on ice for 3 min. It was then centrifuged at 13,200 rpm for 15 min. The supernatant was transferred to a new 1.5 ml micro centrifuge tube, 540 µl of ice cold absolute isopropanol added, and incubated in ice for 20 minutes. It was then centrifuged at 10,200 rpm for 10 min. The supernatant was discarded and the pellet was washed once in 500 µl 70% ethanol and the dried. The dry pellet was suspended in 600 µl of TE and 60 µl 3M sodium acetate (pH 5.2) added, together with 360 µl ice cold absolute isopropanol. It was then incubated on ice for 20 min. The last three steps were repeated twice. The DNA was then stored at 4°C awaiting further analysis. The integrity of all 50 DNA samples were checked on 0.8% agarose gel stained with and run in a 5 x Tris Borate EDTA buffer at 80 V for 30 min. The concentration and purity of all DNA samples were determined by using XNanoDrop® ND1000 (Thermo Scientific) at A260 nm and A280 nm. Thereafter all DNA samples were normalized to 50 ng/µl working concentrations.

Polymerase Chain Reaction (PCR) with SSR markers

Seven highly polymorphic SSR markers (Table 2) which

have been reported as widely distributed in taro population genome were used in genotyping all the DNA samples. PCR was carried out in a Gene Amp®PCR system 9700 thermal cycler (Applied Biosystems, UK). Each 10 µl of a PCR reaction mix contained 50 ng/µl of each DNA sample, 1 µl X buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0), 0.25 mM dNTPs, 2.5 mM $MgCl_2$; 0.1µl of each of forward and reverse primers and 0.25 µl Taq polymerase. PCR conditions were: Initial denaturation at 95°C for 5 min. followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55 - 59°C for 1 min., extension at 72°C for 2 min. and final extension at 72°C for 10 min. 2 µl PCR amplification product was loaded on 2% a stained agarose gel electrophoresis and submerged in 0.5 X TBE buffer, run for one hour at 70 V. The bands on the gel were visualized and photographed under the ultra violet trans illuminator. PCR amplification bands/alleles were separated on polyacrylamide gel electrophoresis system. Presence and absence of DNA bands/alleles was recorded.

Data collection and analysis

Banding patterns were observed at each locus and recorded as presence or absence on a matrix. The bands molecular data was scored in a binary form which was initially configured as an input file and analyzed with POPGENE 1.31 (Yeh and Yang, 1999) and GENSTAT 5. Data generated from gene mapper based on alleles were analyzed using Power Marker V3.25 and GenAlEx softwares for genetic diversity parameters. The parameters included number of alleles per locus, major allele, major allele frequency, observed heterozygosity, percentage polymorphic loci, and gene diversity index (He-expected heterozygosity) as per Liu and Muse (2005).

Data analysis

Shannon's Diversity Index was used to calculate the diversity for the qualitative characters that indicated polymorphism in various traits using a formula by (Shannon and Weaver, 1963; Bisht, Mahajan and Patel, 1998).

Shannon Diversity Index (H) formula:

$$SDI_i = -\sum_{J=1}^{d_i} P_{ij} \log P_{ij}$$

Where

SDI= Shannon's Diversity Index for the I^{th} qualitative character; d_i being the character state for ith character, and the proportion of accessions for j^{th} character state of ith character .P_i = the proportion of the character state of accessions i.

Nei's Estimates technique and Cluster Analysis were conducted. The index proposed by Nei and Li, (1979) was used to calculate genetic similarities (Sij) between taro accessions (i) and (j) as;

Sij=2N/ (Ni+Nj)

Where Nj = the number of bands (alleles) in common between cultivars i and j, Ni and Nj are the number of alleles for cultivars i and j, respectively. The binary matrix data file created was then configured as an input file for data analysis. Nei's (1978) gene diversity index (*H*), number of polymorphic loci (*N*), percent of polymorphic loci (*P*), observed number of alleles (*A*) and genetic distance (*D*) for each population was derived using NYTS-pc 2.1 and GenAlEx 6.4 software assuming Hardy-Weinberg equilibrium. Correlation distance for the two taro accessions was calculated using binary Euclidean distance. A hierarchical cluster dendrogram based on Nei's (1978) genetic distance using UPGMA method modified from neighbor procedure of Phylip Version 3.5 between the tarogen populations. The similarity matrix was subjected to cluster analysis by unweighted pair-group method with arithmetic averages (UPGMA). The analysis for allelic patterns for locally co-dominant alleles across taro populations were analyzed to reflect various behavior of private alleles found among the populations and the formula were as follows:

Na (Freq >= 5%) = No of different alleles with a Frequency >= 5%
Ne=No of effective alleles= 1/(Sum pi^2)
I= Shannon's Information Index=-1* Sum (pi * Ln (pi))
Ho= Observed heterozygosity=No. of Hets/N
He= Expected heterozygosity= 1 - Sum pi^2
UHe=Unbiased Expected heterozygosity= [2N/(2N-1)] * He
No. Private Alleles = No. of Alleles Unique to a Single Population
No. LComm Alleles (<=25%) = No. of locally common alleles (Freq. >= 5%) Found in 25% or Fewer Populations
No. LComm Alleles (<=50%) = No. of locally common alleles (Freq. >= 5%) Found in 50% or Fewer population

Principal Co-ordinate Analysis (PCoA) was performed for the different taro populations used in the study to reveal the level of clustering per population. This was done according to variance/covariance method to determine the variable that could be used to assess the common patterns of variation among groups and subgroups of taro germplasm accessions. A table of eigen vectors and values was generated containing 25 germplasm collections used using NTSYS-pc version 2.1.

The data matrix was subjected to analysis of molecular variance (AMOVA) to partition the genetic variation into within and among the populations' components using GenAlEx software. Based on individual product profiles, a

Table 3. Cluster analysis of the total number of Alleles mapped from Microsatellite SSR Markers in Kenyan germplasm collections.

Locus	Chromosome	Western	Central	Rift Valley	Nyanza
		Number of Alleles (N)			
Xuqtem55	1H	3	3	2	4
Xuqtem73	2H	3	2	1	2
Xuqtem84	3H	5	2	1	4
Xuqtem88	4H	6	2	2	2
Xuqtem91	5H	5	2	2	4
Xuqtem97	6H	4	3	1	6
	Mean	4.67	2.33	1.5	3.67
	Total*	28	14	9	22

*N=73, Mean = 18.25 alleles.

Table 4. Analysis of the genetic characteristics of taro populations mapped from microsatellite SSR Markers in Kenyan taro collections.

Characteristic	Western	Central	Rift Valley	Nyanza	Mean
	Germplasm populations				
Genetic richness	4.67	2.33	1.5	3.67	3.025
Genetic diversity (*He*)	0.6218	0.479	0.25	0.5432	0.4753
Allele frequency	0.5121	0.639	0.7500	0.5789	0.6200
PIC Value	0.5731	0.398	0.1875	0.4895	0.4120

Euclidean distance matrix was generated and analyzed with the ARLEQUIN ver.3.01 software package (Excoffier et al., 2005). Genetic variation was subsequently partitioned within and between populations according to an analysis of molecular variance and significance values assigned to variance components based on random permutation (99 times) of individuals assuming no genetic structure.

RESULTS

All SSR primers revealed heterozygosity at loci by producing one to six bands that were visible on 2% agarose except Xuqtem110 primer that produced some false peaks and background noises which were difficult to score hence no optimizations were made and the primer was excluded from data analysis. A total of 28 alleles were produced. Across the populations, the total number of alleles generated by the 6 SSR markers ranged between 9 (Rift Valley accessions) to 28 (Western). The number of alleles produced ranged between 1 and 6. On average, the Western accessions of taro were most polymorphic followed by those of Nyanza, Central and Rift Valley (Table 3).

Generally, germplasm variability was high in germplasm of Western Kenya followed by those of Nyanza and Central regions. The Rift Valley accessions had the least diversity. Variations were observed in the genetic richness; genetic diversity, allele frequency and PIC value (Table 4). Genetic richness of the germplasm varied greatly and ranged from 1.5 to 4.67 (Table 4). The average genetic richness amongst the populations was 3.025. Western Kenyan accessions of taro had the highest genetic richness (4.67) followed by Nyanza (3.67) and Rift Valley recorded the least (1.5). The average genetic diversity was 0.4753, with the highest genetic diversity in the accessions of western Kenya (0.6218) and lowest in Rift Valley (0.25). However, the allele frequency was highest in Rift valley accessions (0.75) and lowest in those of western Kenya (0.5121). PIC values ranged between 0.1875 (Rift Valley) and 0.5731 (Western) with the average PIC value of 0.4120. In contrast, allele frequency was highest in Rift valley taro populations (0.75) and least in Western (0.5731). The most informative SSR markers with the highest PIC values were Xuqtem 88 (72.38%) and Xuqtem 97 (70.91%) (data not shown).

Generally, the expected and observed genetic diversity levels were different. The expected and observed genetic diversity levels generated by the SSR markers were significantly different for all primers except Xuqtem97 (Table 5). The observed (X^2 = 26.9733) and expected (X^2 = 30.578) genetic diversity generated by SSR primer Xuqtem97 was not significantly different (p = 0.01).

Population genetic structure analysis

Principle Coordinate Analysis (PCoA) of taro germplasm collections

A table of Eigen value by axis and sample Eigen was

Table 5. Analysis of genetic diversity test of significance X^2 (Chi-Square test) among SSR Markers for Kenyan taro collections

Marker	X^2 value (Observed)	df	X^2 Critical value (Expected)	p-value
Xuqtem55	19.4246*	6	16.812	0.0000
Xuqtem73	23.9531*	3	11.345	0.0100
Xuqtem84	47.0663*	10	23.209	0.0000
Xuqtem88	45.7963*	15	30.578	0.0000
Xuqtem91	26.1936*	15	30.578	0.0020
Xuqtem97	26.9733	15	30.578	0.0110
Total	31.5679	11	24.725	0.0038

*Significant at 0.01 level of significance.

Table 6. Percentage of genetic variation expressed from Eigen values using SSR markers among Kenyan taro populations.

Axis	Eigen value*	Percent	Cumulative percentage
1	5.525	22.04	22.04
2	3.966	15.82	37.86
3	3.058	12.20	50.06
4	2.514	10.02	60.08
5	2.006	8.00	68.08
6	1.733	6.91	74.99

*Eigen values from a reduced correlation matrix of observed relationships in the original binary data matrix and the individual proportion of variation they explain

Table 7. Summary of allelic patterns for locally co-dominant alleles across taro populations.

Population	Na	Na frequency >=5%	Ne	I	No. of Private Alleles	No. LComm Alleles (<=25%)	No. LComm Alleles (<=50%)	He	UHe
Central Kenya	2.33	2.33	2.06	0.75	0.00	0.00	0.50	0.49	0.59
Western Kenya	4.33	3.67	2.97	1.20	0.00	0.33	1.33	0.64	0.67
Rift Valley, Kenya	1.5	1.5	1.5	0.35	0.00	0.00	0.50	0.25	0.56
Nyanza, Kenya	4.0	4.0	2.51	1.04	0.00	0.50	1.50	0.56	0.60
Mean values	3.04	2.875	2.26	0.835	0.00	.2075	.9575	0.485	0.605

Key: Na (Freq >= 5%) = No of different alleles with a Frequency >= 5%; Ne=No of effective alleles= 1/(Sum pi^2); I= Shannon's Information Index=-1* Sum [pi * Ln (pi)]; Ho= Observed heterozygosity=No. of Hets/N; He= Expected heterozygosity= 1 - Sum pi^2; UHe=Unbiased Expected heterozygosity=[2N/(2N-1)] * He; No. Private Alleles = No. of Alleles Unique to a Single Population; No. LComm Alleles (<=25%) = No. of locally common alleles (Freq. >= 5%) Found in 25% or Fewer Populations; No. LComm Alleles (<=50%) = No. of locally common alleles (Freq. >= 5%) found in 50% or Fewer Population

generated (Table 6) for the 25 genotypes. The first three components of the axis had a cumulative per cent of 50.06% of the variation in the observed genetic relationships and reflecting species separation across the accessions. The first two axes (Eigen values 5.525 and 3.966 respectively) account for 50.06% of the diversity or variations in the observed relationships and reflect Kenya taro cultivars separation within the population as shown on Table 7 while PITCs Eigen values were 5.662 and 4.789 for the first two axis.

The second axis corresponds to variation within and between the taro populations. As per PCoA, the first and second principal coordinates were 22.04% and 15.2% respectively, accounting for over 37.86%. Principal co-ordinate analysis (PCoA) was performed within the different taro populations used in the study to reveal the level of clustering per population (Figure 1).The PCoA reflected a successful clustering analysis, with the accessions separating out as per each taro population. Majority of taro accessions from western Kenya show

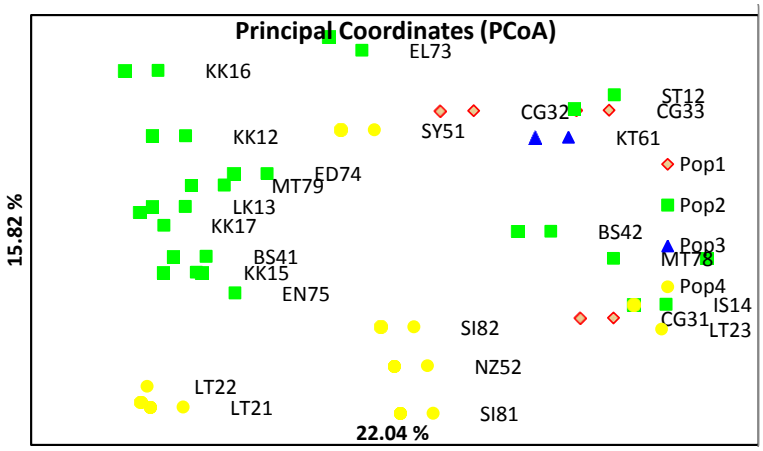

Figure 1. Distribution of Kenya taro populations on the first and second coordinates of Principal co-ordinates analysis (PCoA) performed with Microsatellite SSR markers. The taro accessions are represented according to their geographical regions of Kenya based on Jaccards' similarity coefficients.
Key: Pop1: Population from Central Kenya -CG31, CG32 and CG33; **Pop2**: Population from Western Kenya-LK13, IS14, ST12, KK15, KK16, KK17, BS42, EL73, EN75, ED74, MT78, MT79, BS41, KK12; **Pop3:** Population from Rift valley Kenya (KT61); **Pop4:** Population from Nyanza Kenya -SY51, SI81, SI82, NZ52, LT21,LT22, LT23.

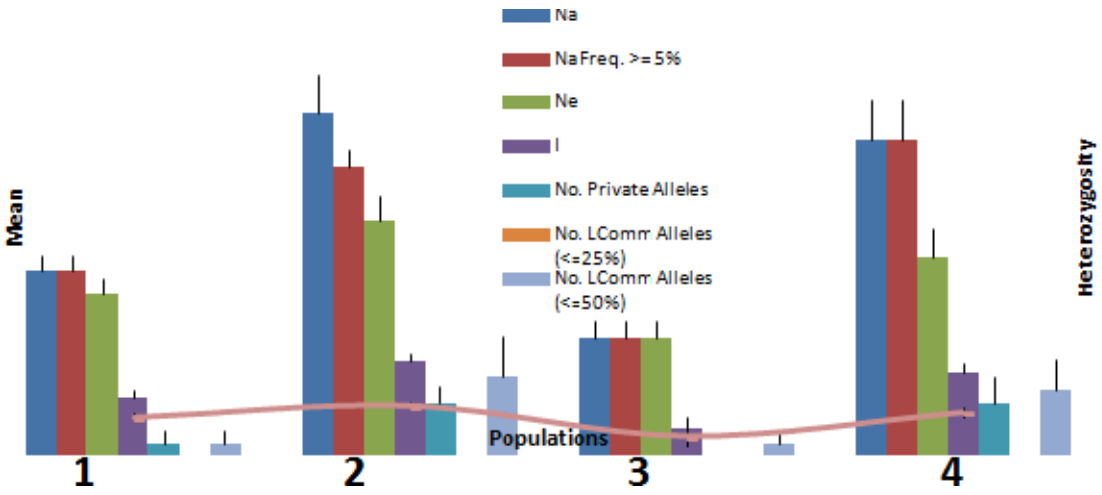

Figure 2. Allelic Patterns for Co dominant alleles among Kenya taro populations
Key: **Key: 1**=Central Kenya; **2**=Western Kenya; **3=**Rift valley Kenya; **4=**Nyanza Kenya.

higher similarity and lesser variation amongst its cultivars. However, a few showed greater variations like MT78, BS42 and ST12. Nyanza Kenya taro cultivars like LT21 and LT22 are genetically similar while the rest showed greater variations (Figure 1).

Allelic patterns across taro populations

The overall mean percentage of polymorphic loci across the taro population was 87.5% with standard error SE (±6.39). This was a true reflection of allelic diversity among taro varieties. Rift valley reflected fifty per cent (50%) polymorphic rate. The rest of the taro accessions showed 100% polymorphism among its loci**.** The result showed that the expected and unbiased expected heterozygosity of polymorphic loci for Kenyan taro populations were greater in Western, Nyanza and Central Kenya (uHe: 0.67; 0.60 and 0.59) respectively (Figure 2).

Nei's (1978) genetic identity (*I*) and Distance (*D*) among taro populations

Nei´s measures of genetic distance generated from the binary matrix varied from 0.185 to 0.794 while the

Table 8. Pair wise population matrix of Nei's unbiased measures of genetic identity and genetic distance of Kenya. taro populations.

Population	Central Kenya	Western Kenya	Rift valley Kenya	Nyanza Kenya
Genetic Distance Analysis				
1	0.000			
2	0.212			
3	0.228	0.502		
4	0.278	0.185	0.794	0.000
Unbiased Genetic Identity				
1	1.000			
2	0.809	1.000		
3	0.796	0.605	1.000	
4	0.757	0.831	0.452	1.000
Total	998	908		90

unbiased genetic identity ranged from 0.452 to 1.00 respectively for the 4 populations (Table 8). These reflect actual genetic distances and identity relationship between the populations used in the study. A lower value of Nei´s genetic distance between two or more groups represents a closer relationship between the populations (Nei, 1978). A higher genetic identity value between two or more groups indicates a proportion of similar genes or alleles in the germplasm.

The lowest genetic distance was amongst germplasm was recorded between Central and other populations while the highest genetic distance was recorded between Rift Valley and other populations. Thus Rift Valley germplasm was more divergent from the other taro populations. A higher Nei´s value (0.794) of genetic distance was observed between Rift valley and Nyanza Kenya taro genotypes followed by Western (0.502). However, the genetic distance between Central and Rift Valley germplasm was lower at 0.228. Overall, Rift valley Kenya taro showed a higher Nei´s value of genetic distance in comparison to the other populations (Table 8).

The allele similarity relationships between the germplasm were depicted by the genetic identity index which is a measure of the proportion of genes that are identical in two populations. The highest comparative proportion of identical alleles was recorded between Central and other regions as indicated in Table 8. Thus the allele similarity between Central and Western taro germplasm was 80.9%, 79.6% with Rift Valley and 75.7% with Nyanza. Allele similarity between Western germplasm and Rift Valley was 60.5% and 83.1% with Nyanza. The lowest proportion of identical alleles was recorded between Rift Valley and Nyanza germplasm.

UPGMA clustering dendrogram among taro accessions

A clustering dendogram analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on using average linkage between accessions revealed two genetic major groups with four clusters (Figure 3). One accession, KK12 was genetically dissimilar from the other taro germplasm and formed a distinctive group. In fact, genotype KK12 had the highest genetic distance. The taro genotypes revealed the same genetic relationships where western genotype KK12 had the highest genetic distance from the rest followed by Nyanza taro genotypes SI81, then western ST12 genotype. The taro genotypes SI81, ST12 and KK12 from Western Kenya and CG33 from Central are genetically distant from each other as also reflected by the PCoA on the farthest end of the co-ordinates. Genetic similarity from the clustering dendogram is also seen in LT23 (Nyanza around Lake Victoria), IS14 (Western Kenya) with CG32 from central Kenya. On the other hand, taro genotypes such as BS42 and MT78 from Western Kenya and CG31 taro genotype from central Kenya both were showing genetic closeness as shown in sub-cluster in dendrogram.

Analysis of molecular variance

Genetic partitioning of the total species diversity using analysis of molecular variance attributed 6% of the variation to diversity among the taro populations, 24% amongst individual taro accessions while majority of seventy per cent (70%) of the genetic diversity resided within taro genotype accession. The genetic diversity partition components that were among and within individuals were statistically significant. The results presented on table about $H0_3$ on the analysis of molecular variance (*Colocasia esculenta)* based on SSR markers shows existence of comparative significant genetic diversity differences between the Kenya and PIC taro germplasm accessions. In effect, this null hypothesis: $H0_3$ There exists no significant comparative genetic variability of Pacific Islands and Kenyan taro germplasm accessions using SSR molecular marker was rejected.

Discussion

The six polymorphic microsatellites molecular (SSR)

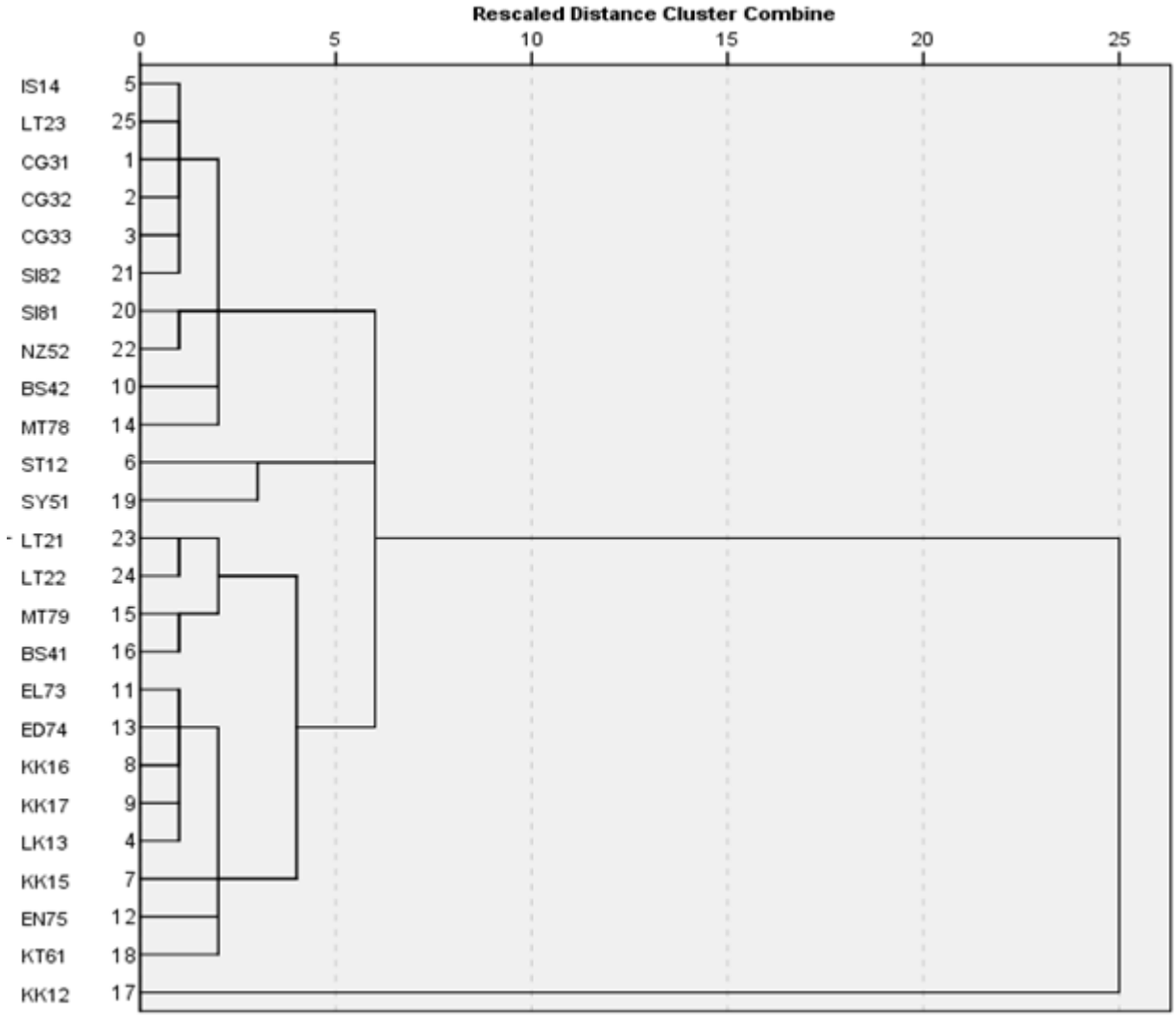

Figure 3. UPGMA Clustering dendrogram based using average linkage between groups indicating the genetic relationship among Kenya tarogen accessions using microsatellite markers.

markers representing each of the selected chromosomes indicated reasonably high levels of genetic diversity and richness among the assessed populations of taro genotypes thereby underscoring the significance of variability. Studies have indicated that germplasm variability at molecular level is important in their description and that molecular marker is a tag of a particular aspect of phenotype and genotype whose inheritance can be traced from one generation to another (Benchimol et al., 2000; Del Vicente and Fulton, 2004). This is significant in taro whose chromosome number is reported to include 2n = 22, 26, 28, 38 and 42 as the species is prone to unpredictable behavior during cell division (Coates et al., 1988; Omwueme, 1999).

Average genetic diversity values were high (He = 0.653 and He = 0.699) for Kenya revealing a clear indication of genetic richness of taro populations that can be used for conservation and breeding strategies. Western and Nyanza from Kenyan taro genotypes. The findings from this study are comparable to study of other crops like sweet potato which had genetic diversity (He) ranging from 0.21 to 0.75 (Karuri et al., 2009) in Kenya while cassava in the great lakes region showed a value above 0.5 that was considered sufficient for a conservation program (Pariyo et al., 2009; Tumwegamire et al., 2011). From the results, low genetic diversity values on taro accessions were revealed by Central and Rift valley parts of Kenya Macharia et al. (2014) also reported that higher genetic diversity in taro (He: 0.2783) and tannia (0.2478) from Lake Victoria basin populations as it was observed with Nyanza Kenya (He: 0.5432) taro accessions.

Genetic identity explained the genetic distances amongst populations observed in the study. Genetic identity is a measure of the proportion of genes that are

identical in two populations thus high values indicated greater similarity and lower values depict less allele similarity. Thus, the high proportion of similar alleles observed between Central and other germplasm indicated a high genetic similarity which was subsequently corroborated by the lower values of genetic distance. There were significantly lower similarities between the genes of Rift Valley and Nyanza germplasm as well as Western and Rift Valley. This indicated that the taro germplasm in these regions were quite dissimilar in comparison to other regions. Genetic distance is a measure of the genetic divergence between species or between populations within a species (Nei, 1987). Populations with many similar alleles have small genetic distances. This indicates that they are closely related and have a recent common ancestor. Therefore, genetic distance is useful for reconstructing the history of populations and understanding the origin of biodiversity.

The results have confirmed that the microsatellite markers chosen were relatively informative as depicted by the average values of polymorphic information content (PIC) with mean of 0.4120. SSRs were used to delineate common bean germplasm of Kenya (Nyakio, 2015). Elibariki et al. (2013) also reported that the closer the value to 1.0 the better the PIC value and the more the informative is the SRR marker. The most informative SSR markers with the highest PIC values were Xuqtem 88 (72.38%) and Xuqtem 97 (70.91%). The least informative marker with lowest polymorphic information value was Xuqtem73. This could attest to the fact that SSR molecular markers could detect high levels of genetic diversity in various crops including taro because of its effectiveness and efficient to use.

Conclusion

The results of this study propose that there are significant similarities and dissimilarities within and among taro accessions and populations in Kenya. In addition, molecular development initiatives, in particular SSR offers a dependable and real means of assessing genetic diversity within and between taro populations of Kenya. Variation of DNA patterns among accessions within taro germplasm in Kenya in respect to the AMOVA revealed a high within population variation than among populations. As such, germplasm from Rift Valley should be included in taro improvement programs to ensure broadening of the genetic base of taro germplasm in the region. There is need for the conservation of taro genetic resources as evidenced by the considerable thinning of genetic diversity amongst most populations underscoring the importance for genetic upgrade programs.

SSR markers offer a comparatively valuable tool for categorizing germplasm and are a good complementation to field trials for classifying groups of genetically similar cultivars. Germplasm classification and appraisal complimented by molecular studies generate the evidence base for more proficient exploitation of these valuable resources by conservationists and research scientists. Field trials for identification of perfect heterotic patterns can be planned more efficiently based on findings from SSR analyses. The present study aimed to genetically characterize the taro accessions of Kenya using molecular makers. The findings offer an opportunity for taro improvement programs to understand the genetics of the taro populations in Kenya and help generate new superior cultivars in the future.

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