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Genetic Structure and Geographical Relationship of Selected *Colocasia esculenta* [L. Schott] Germplasm Using SSRs

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Authors' contributions

This work was carried out in collaboration between both authors. Author VAPP designed the study and wrote the protocol. Author EPA performed the experiments and statistical analysis. Author VAPP wrote the first draft of the manuscript. Author EPA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: SSR markers were used to infer population genetic structure variability in taro cultivars with the objective of characterizing the allelic diversity of each geographical population.
Place and Duration of Study: Masinde Muliro University of Science and Technology and Beca Hub, ILRI, Nairobi.
Methodology: Six highly polymorphic SSR markers widely distributed in taro population genome were used in genotype 50 cultivars collected from Kenya and a taro genebank (SPC Tarogen).
Results: The average polymorphic loci was 87.88%. The highest Shannon information index was observed in the germplasm from Nyanza (1.04), Western (1.2) and Hawaii (1.11) and Malaysia (1.36). Only Malaysia and Thailand germplasm had allele unique to a single locus. The analysis of

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molecular variance (AMOVA) revealed that 70% of the variations found within individual taro accessions, 6% of variations among the taro populations and only 24% amongst individual taro genotypes and they were statistically significant ($p<0.001$). Principal component analysis clustered the taro germplam into different groups. In total 50.06% and 51.82% of the variation was explained by the first three principal components of the taro germplasm. Some of the Kenyan taro cultivars clustered together with the Tarogen germplasm.

Conclusion: The determination of genetic diversity is core function towards understanding taro genetic resources for varietal identification to rationalize its collection and safeguarding the existing genetic diversity for taro germplasm conservation, management and for potential utilization for food security.

Keywords: Cluster analysis; shannon diversity index; taro; principal component analysis; heterozygosity.

1. INTRODUCTION

Taro (*Colocasia esculenta)* is an ancient important root tuber crop grown throughout many parts of the world for its fleshy corms and nutritious leaves. Taro is a vegetatively propagated root crop species belonging to the monocotyledonous family, *Araceae*. It contributes to sustained food security in some domestic markets as well as a source of export earnings [1]. Taro hence has the potential to ameliorate household food hunger for small scale farmers. Despite the importance of taro in food crop systems, its crop improvement and development has been rather low. This impacts negatively on food production and security. Since about 10% of the world populations, mainly living in the developing tropical countries, use root and tuber crops as staple foods, there is an urgent need to understand the genetic diversity and preserve the remaining indigenous germplasm of native food crops for crop development and posterity [2].

The extent of genetic diversity and genotypes agronomic performance of various Kenyan taro varieties remains largely unknown indicating a largely untapped potential for research on this underutilized crop in the region. This is a clear indicator of lack of phenotypic and genotypic information towards identification of heritable and desirable traits important for improved taro productivity. Since there have been no prior formal germplasm introductions into the cropping systems in the region, the comparative performance of Kenyan germplasm and geographically isolated populations is not understood. The absence of this authentic information on comparative population biology of regional germplasm therefore hinders sustainable selection of cultivars.

For the effective conservation of taro genetic resources it is imperative to describe genetic variability in order to assist germplasm bank curators to preserve genetic diversity and for breeders to use it effectively [3]. Speed, reproducibility and the ability to detect genetic variation within and between accessions determine the utility of molecular techniques for germplasm bank management [4]. The increasing availability of highly polymorphic genetic markers and the decreasing cost of genotyping provide great tools for discovering the true biological association between individuals [5]. Simple sequence repeats (SSRs) markers provide an advanced level of information compared with other groups of molecular markers [6]. [7] has developed a set of SSR markers for use in taro diversity assessment. SSRs permits the selection of the most revealing and well-distributed SSR loci in the taro genome to be used in molecular analysis. Together these characteristics make the microsatellites loci one of the best genetic markers for mapping purposes [8]. In the study described in this paper, SSR markers were used to infer population genetic structure variability in taro cultivars with the objective of characterizing the allelic diversity of each geographical population.

2. MATERIALS AND METHODS

2.1 Plant Material

Fifty accessions of taro (*C. esculenta*) belonging to several populations were used to investigate the level of polymorphism detected by selected SSR markers (Table 1). Twenty five accessions were sampled from four different regions of Kenya. The other twenty five varieties were germplasm held by the Secretariat of Pacific Community's Tarogen (Taro gene bank) located in Vanuatu.

Table 1. Taro germplasm used in the study

S/No	CePaCT accession number	Study no.	Genotype variety/accession	Origin/Population	Latitude	Longitude
1	KCT/GHT/31	CG31	Kigoi	Central-Kenya	00.416666°	03 6.66666°
2	KCT/KGI/32	CG32	Kigirigasha	Central-Kenya	00.416666°	036.66666°
3	KCT/NGC/33	CG33	Ngirigacha	Central-Kenya	00.416666°	036.66666°
4	KWK/LKW/13	LK13	Lukuyw	Western Kenya	0.28135°	034 .75140°
5	KWK/ISW/14	IS14	Ishwa	Western Kenya	0.28135°	034. 75140°
6	KWK/SHT/12	ST12	Shitao	Western Kenya	00.28273°	0 34.75186°
7	KWK/KAK/15	KK15	Kakamega T15	Western Kenya	0.28135°	034.75140°
8	KWK/KAK/16	KK16	Kakamega T16	Western Kenya	0.28135°	034.75140°
9	KWK/KAK/17	KK17	Kakamega T17	Western Kenya	0.28135°	034.75140°
10	KWK/BSA/42	BS42	Amak Tar72	Western Kenya	00.33333°	034.48333°
11	KMM/ELU/73	EL73	Eluhya	Western Kenya	00.33333°	0 34.48333°
12	KMM/ENG/75	EN75	Mumias T75	Western Kenya	00.33333°	034.48333°
13	KMM/END/74	ED74	Enduma	Western Kenya	00.33333°	0 34.48333°
14	KMM/MMU/78	MT78	Mumias T78	Western Kenya	00.33333°	034.48333°
15	KMM/MMU/79	MT79	Mumias T79	Western Kenya	00.33333°	034.48333°
16	KRT/KTL/61	KT61	Kiminini	Rift Valley Kenya	00.89356°	034.92582°
17	KNY/SYA/51	SY51	Siaya	Nyanza Kenya	00.0623°	034 .28781°
18	KNY/KIS/81	SI81	Kisii T81	Nyanza Kenya	00.67831°	034.77197°
19	KNY/KIS/82	SI82	Kisii T 82	Nyanza Kenya	00.67831°	034.77197°
20	KNY/NYA/52	NZ52	Kisumu	Nyanza Kenya	00.09170°	03 4.76196°
21	KNY/LVT/21	LT21	Lake VictoriaT21	Nyanza Kenya	00.75578°	034.43835°
22	KNY/LVT/22	LT22	Lake Victoria T22	Nyanza Kenya	00.75578°	034.43835°
23	KWK/BSA/41	BS41	Amagoro Busia	Western Kenya	00.460769°	034.11146°
24	KWK/KAK/12	KK/12	Kakamega T12	Western Kenya	0.28135°	034.75140°
25	KWK/LVT/23	LT23	Lake Victoria	Nyanza Kenya	00.75578°	034.43835°
26	BL/WH/08	BH08	PEXPH15-6	Hawaii	19.89618°	0155.58 278°
27	BL/HW/26	BH26	BC99-11	Hawaii	19.89618°	0155.5827 8°
28	BL/HW/37	BH37	Pa'akala	Hawaii	19.89618°	0155.582 78°
29	BL/SM/43	BL43	Sama043	Samoa	13.75902°	172.10462°

S/No	CePaCT accession number	Study no.	Genotype variety/accession	Origin/Population	Latitude	Longitude
30	BL/SM/80	BL80	Alafua	Samoa	13.75902°	172.10462°
31	BL/SM/92	BL92	Silipisa	Samoa	13.75902°	172.10462 °
32	BL/SM/111	BL11	Pauli	Samoa	13.75902°	172.10462°
33	BL/SM/116	BL18	Manu	Samoa	13.75902°	172.10462°
34	BL/SM/120	BL20	Manono	Samoa	13.75902°	172.10462°
35	BL/SM/128	BL28	Nu'utele2	Samoa	13.75902°	172.104 62°
36	BL/SM/132	BL32	Fanuatupu	Samoa	13.75902°	172.104 62°
37	BL/SM/143	BL03	Vaimuga	Samoa	13.75902°	172.10462 °
38	BL/SM/149	BL49	Lepa	Samoa	13.75902°	172.10462°
39	BL/SM/151	BL51	Letoga	Samoa	13.75902°	172.10462°
40	BL/SM/152	BL52	Saleapaga	Samoa	13.75902°	172.104 62°
41	BL/SM/158	BL53	Lalomanu	Samoa	13.75902°	172.1046 2°
42	CA/JP/03	CJ23	Mayako	Japan	36.20482°	138.25292°
43	CE/IND/01	CN01	Kudo	Indonesia	00.78927°	113.9213 2°
44	CE/IND/06	CN06	IND155	Indonesia	00.78927°	113.92 132°
45	CE/MAL/14	CM14	Klauang	Malaysia	04.21048°	101.97 576°
46	CE/MAL/12	CM12	Klang	Malaysia	04.21048°	101.9757 6°
47	CE/THA/07	CT07	Srisamrong	Thailand	15.87003°	100 .99254°
48	CE/THA/09	CT09	Tadeang	Thailand	15.87003°	100.99 254°
49	CE/THA/24	CT24	Boklua	Thailand	15.87003°	100.99 254°
50	BL/PNG/10	BP10	C3-12	Papua New Guinea	6.31499°	1 43.95555°

2.2 DNA Isolation

A measure of 500 mg of fresh leaf material was washed in distilled water and rinsed with 80% ethanol. The surface sterilized leaves were ground in liquid nitrogen and extracted with 1.5 ml of CTAB extraction buffer as described by [9]. DNA was precipitated with isopropanol and washed with 76% ethanol washing solution and dissolved in TE buffer. DNA was quantified using spectrophotometer and diluted to 10 ng/µl.

2.3 PCR

Six highly polymorphic SSR markers widely distributed in taro population genome were used in genotyping [10]. PCR was carried out in a GeneAmp®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 X buffer (10 mM Tris-HCL pH 8.0, 1 mM EDTA pH 8.0), 0.25 mM dNTPs, 2.5 mM $MgCl_2$; 0.1 µl of each of forward and reverse primers and 0.25 µl Taq polymerase. PCR conditions were: Initial denaturation at 95℃ for 5 minutes followed by 35 cycles of denaturation at 94℃ for 30 seconds, annealing at 57℃ for 1 minute, extension at 72℃ for 2 minutes and final extension at 72℃ for 10 minutes. PCR amplicons were separated on polyacrylamide gel electrophoresis system.

2.4 Statistical Analysis

Polymorphic DNA bands were scored as present (1), absent (0) for each accession and data was compiled in a binary data matrix or as a missing observation for further analysis with NTSYS-pc version 2.1. To generate data for each population, the data of accessions from any one similar region were pooled together to represent the region. Each SSR amplification band was measured as an allele of the SSR locus. The number of alleles per locus was estimated using Powermarker software. The number of private alleles were considered as alleles found only in genotypes from one country and was determined by examination of the allele distribution. The quantities of the number of private alleles to the total number of alleles observed in genotypes were calculated. Monolocus diversity was assessed using Nei's unbiased gene diversity [11] while multilocus diversity was estimated using the Shannon diversity index. The estimates for probability of genetic identity and distance between all pairs of taro genotypes were obtained using the formula of [11]. Allelic frequencies of SSR markers were used to estimate the percentage of polymorphic loci with respect to Hardy-Weinberg equilibrium [12]. Principal Component Analysis (PCA) was performed within the different taro populations used in the study to reveal the level of clustering per population according to variance/covariance method. A table of Eigen vectors and values was generated containing fifty tarogen collections 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 using F-statistic [13] according to equations of [14]. Based on individual product profiles, a Euclidean distance matrix was generated and analyzed with the ARLEQUIN ver. 3.01 software package [15]. The dendogram for all individual cultivars was constructed based on average linkage between accessions using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) [16] as implemented in the Numerical Taxonomy and Multivariate Analysis System (NTSYS) program [17].

3. RESULTS AND DISCUSSION

Allelic diversity was evident in all the eleven populations of varieties with the level of polymorphism generated by the SSR markers varying (Table 2, Fig. 1). As expected, groups with the fewest members had the fewest alleles. The overall mean percentage of polymorphic loci was 87.88 % with standard error of ±6.39 which was a true reflection of the allelic diversity among the populations. Papua New Guinea and Rift valley reflected fifty per cent (50%) polymorphic rate among its loci while Indonesia showed 66.67%. The rest of the taro accessions showed 100% polymorphism among its loci. The expected and unbiased expected heterozygosity of polymorphic loci for SPC Tarogen germplasm were greater for Hawaii, Thailand and Malaysia (0.77; 0.75 and 0.73) while Kenyan taro populations were greater in Western, Nyanza and Central Kenya (0.67; 0.60 and 0.59) respectively (Table 3). Traces of private alleles unique to taro populations were only seen between Malaysia and Thailand of about 0.5 and 0.33 across taro germplasm accessions. Greatest diversity (Shanon diversity index) was observed in the germplasm from Nyanza (1.04), Western (1.2) and Hawaii (1.11).

Table 2. Taro cultivars and populations used in the study

Origin	No.	Population/Geographical region sampled	Cultivars	Percentage of polymorphic loci
Kenya	1	Central Kenya	CG31; CG32; CG33	100.00
	2	Western Kenya	LK13, IS14; ST12; KK15; KK16; KK17; BS42; EL73; EN75, ED74; MT78; MT79; BS41; KK12;	100.00
	3	Rift valley Kenya	KT61	50.00
	4	Nyanza Kenya	SY51; SI81; SI82; NZ52; LT21; LT22; LT23	100.00
SPC Tarogen	5	Hawaii	BH08; BH26; BH37.	100.00
	6	Malaysia	CM12; CM14	100.00
	7	Indonesia	CN01; CN06	66.67
	8	Samoa	BL03; BL11; BL80; BL18; BL20; BL28; BL32; BL43; BL49; BL51; BL52; BL58; BL92	100.00
	9	Thailand	CT07; CT09; CT24	100.00
	10	Japan	CJ23	100.00
	11	Papua New Guinea	BP10	50.00
Mean				**87.88±6.39**

Table 3. Summary of allelic patterns for locally co-dominant alleles across taro populations

Population	Na	Na Freq. >= 5%	Ne	I	No. 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
Hawaii	3.3	3.33	3.0	1.11	0.00	0.17	1.33	0.64	0.64
Malaysia	5.0	4.17	3.39	1.36	0.5	0.17	1.67	0.70	0.77
Indonesia	1.67	1.67	1.67	0.46	0.00	0.17	0.50	0.33	0.73
Samoa	2.5	2.5	2.31	0.82	0.00	0.00	0.50	0.52	0.69
Thailand	2.5	2.5	2.44	0.87	0.33	0.00	0.50	0.56	0.75
Japan	2.5	2.5	2.05	0.76	0.00	0.00	0.17	0.47	0.57
Papua New Guinea	1.5	1.5	1.5	0.35	0.00	0.00	0.17	0.25	0.5

Key: Na (Freq >= 5%) = No of different alleles with a Frequency >= 5%; Ne=No of effective alleles; I= Shannon's Information Index = -1 Sum (pi * Ln (pi)); 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*

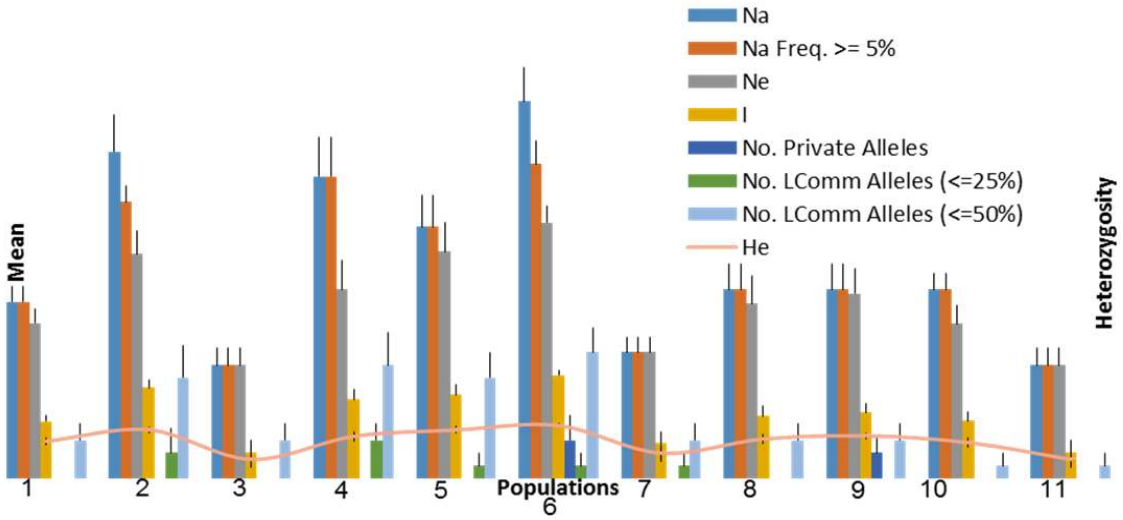

Fig. 1. Allelic Patterns for co-dominant alleles among Kenya and Pacific Islands tarogen populations

Key: 1=Central Kenya; 2=Western Kenya; 3=Rift valley Kenya; 4=Nyanza Kenya 5: Hawaii; 6=Malaysia; 7=Indonesia; 8=Samoa; 9=Thailand; 10=Japan 11=Papua New Guinea

Principal component analysis based on allele frequencies among the populations across mapped SSR markers clustered the taro germplasm (Fig. 2). PCA was performed for the different taro populations to reveal the level of clustering per population. The PCA reflected a successful clustering analysis, with the accessions separating out as per each taro population. Majority of taro accessions from western Kenya showed higher similarity and lesser variation amongst its cultivars while 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. Some of the taro accessions from Thailand (CT07, CT09), three from Samoa (BL92, BL32 and BL52), one from Indonesia (CN01), one Japan (CJ23) and Hawaii accession (BH26) exhibited less variations while the rest displayed more variations (Fig. 2). Variation was explained using of Eigen values (Table 4). The first three components of the axis had a cumulative percent 50.06% and 51.82 of the variation in the observed genetic relationships and reflecting species separation across the accessions for Kenya and SPC Tarogen germplasm respectively. 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 indicated while SPC Tarogen 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 PCA, the first and second principal coordinates were 22.04% and 15.2% respectively, accounting for over 37.86% for Kenya while SPC Tarogen were 21.13% and 17.87 accounting for over 39% of the genetic variation exhibited by the taro populations.

The populations were shown to differ in genetic variability with regard to genetic distance (Table 5). Nei´s unbiased measures of genetic distance varied from 0.185 to 0.794. The highest and lowest genetic distances for Kenyan populations were 0.794 (Rift Valley and Nyanza) and 0.185 (Western and Nyanza) respectively. For the SPC Tarogen germplasm, their lowest and highest genetic distances ranged from 0.111 (Papua New Guinea and Hawaii) to 0.794 (Japan and Thailand). This reflected the actual genetic distances between the taro populations. A lower value of Nei´s genetic distance between two or more groups represents a closer relationship between the populations. Thus the Western and Nyanza populations were closely related compared to Nyanza and Rift Valley. A higher Nei´s value of genetic distance was observed between Indonesia and Thailand, Indonesia and Samoa, Thailand and Papua New Guinea, Japan and Indonesia taro populations with values of 0.837, 0.783, 0.634 and 0.601 respectively**.** The smallest Nei´s value of genetic distance was clearly shown between Hawaii and Malaysian taro varieties, between Malaysian with Samoa and Thailand, between Thailand and Japan.

Overall, Rift valley Kenya taro showed a higher Nei´s value of genetic distance with Indonesia, Samoa, Japan and Papua New Guinea while Indonesia replicated higher value with Samoa and Thailand. Nei´s unbiased measures of genetic identity varied from 0.452 to 1.00 for Kenyan while SPC Tarogen ranged from 433 to 1.00 reflecting the actual similarity relationship between the taro populations. Western and Nyanza Kenya reflected a higher Nei´s value (0.831) of genetic identity within taro genotypes.

Table 4. Percentage of genetic variation expressed from Eigen values using SSR markers among Kenyan and Tarogen taro populations

Origin	Axis	Eigen value*	Percent	Cumulative percent
SPC Tarogen	1	5.662	21.13	21.13
	2	4.789	17.87	39.00
	3	3.436	12.82	51.82
	4	2.600	17.87	69.69
	5	2.001	7.47	77.16
	6	1.634	6.10	83.26
Kenya	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*

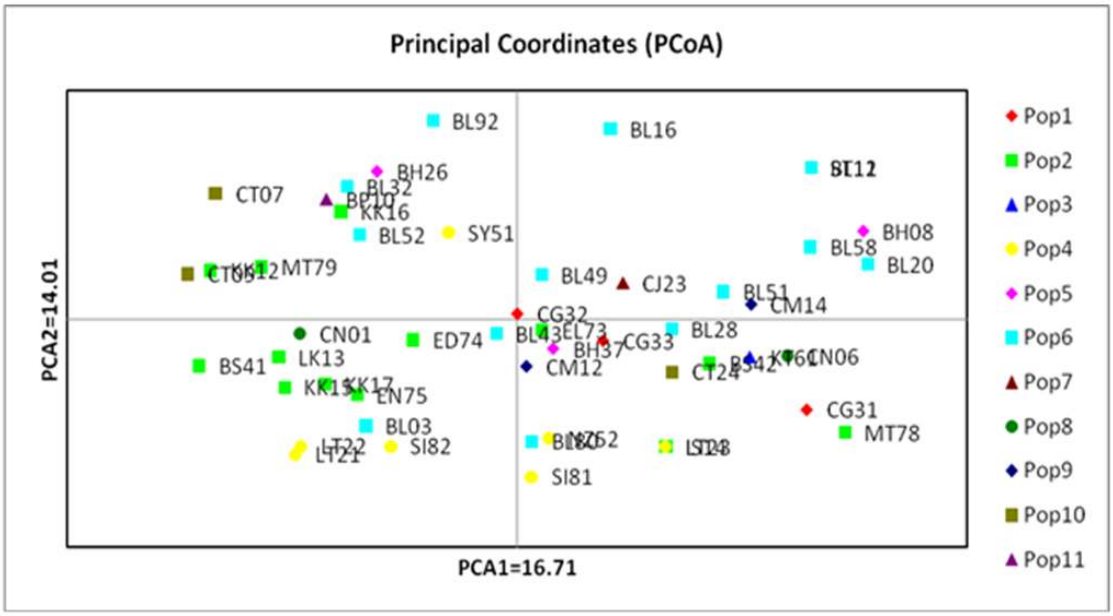

Fig. 2. Principal coordinate analysis of individuals from eleven taro populations based on Jaccards' similarity coefficients. PC1 and PC2 are the first and second principal coordinate, respectively

Key: Pop-Population; KEY: 1=Central Kenya; 2=Western Kenya; 3=Rift valley Kenya; 4=Nyanza Kenya 5: Hawaii; 6=Malaysia; 7=Indonesia; 8=Samoa; 9=Thailand; 10=Japan 11=Papua New Guinea

Table 5. Pair wise population matrix of Nei's unbiased measures of genetic distance for nine populations of geographically isolated taro cultivars using six primer combinations

Popn. ID	1	2	3	4	5	6	7	8	9	10	11
1	0.000										
2	0.212										
3	0.228	0.502									
4	0.278	0.185	0.794								
5	0.160	0.125	0.494	0.538							
6	0.234	0.150	0.390	0.478	0.000						
7	0.238	0.414	0.490	0.024	0.227	0.227					
8	0.295	0.075	0.735	0.257	0.123	0.001	0.783				
9	0.169	0.162	0.123	0.540	0.023	0.000	0.837	0.000			
10	0.542	0.082	0.622	0.413	0.241	0.054	0.601	0.047	0.097		
11	0.308	0.212	1.099	0.409	0.111	0.093	0.085	0.447	0.634	0.015	0.000

Key: Popn ID = Population identity; 1=Central Kenya; 2=Western Kenya; 3=Rift valley Kenya; 4=Nyanza Kenya; 5 =Hawaii; 6=Malaysia; 7=Indonesia; 8=Samoa; 9=Thailand; 10=Japan; 11=Papua New Guinea

Table 6. Pair wise population matrix of Nei's unbiased measures of genetic identity of nine Kenya and SPC Tarogen taro population using six primer combinations

Popn. ID	1	2	3	4	5	6	7	8	9	10	11
1	1.000										
2	0.809	1.000									
3	0.796	0.605	1.000								
4	0.757	0.831	0.452	1.000							
5	0.852	0.882	0.610	0.584	1.000						
6	0.791	0.861	0.677	0.620	1.118	1.000					
7	0.788	0.661	0.612	0.977	0.797	0.797	1.000				
8	0.745	0.928	0.480	0.774	0.884	0.999	0.457	1.000			
9	0.845	0.851	0.884	0.583	0.978	1.043	0.433	1.244	1.000		
10	0.581	0.921	0.537	0.662	0.786	0.948	0.548	0.954	0.907	1.000	
11	0.735	0.809	0.333	0.665	0.895	0.911	0.919	0.640	0.530	0.985	1.000

Key: Popn. ID = Population; 1=Central Kenya; 2=Western Kenya; 3=Rift valley Kenya; 4=Nyanza Kenya; 5 =Hawaii; 6=Malaysia; 7=Indonesia; 8=Samoa; 9=Thailand; 10=Japan; 11=Papua New Guinea

The dendogram generated revealed the clustering of the taro accessions and how closely related or diverse are the cultivars in relation to their genotype accessions. Clustering dendogram analysis using the UPGMA based on using average linkage between accessions revealed two genetic major groups with four clusters (Fig. 3). Findings revealed that the Malaysian CM14 genotype is genetically distant from all other the taro varieties followed by Thailand CT09 genotype. This is depicted from PCA where CM14 and CM12 varieties are on different axes. Samoa taro genotype BL49, Thailand's CT24 and Malaysia's CM12 are closely related as shown in both the cluster dendogram and PCA. This is similar to Nei's unbiased genetic identity that ranged from 0.797 to 1.00. Papua New Guinea's BL10 and Samoa's BL32 genotypes are genetically similar as indicated by PCA. Hawaii's Genetic distance ranged from 0.00 to 0.24 revealing a closer genetic relatedness with other genotypes such as Indonesian CN06 and CN01. Kenyan genotypes displayed interesting relationships with the Tarogen genotypes. For example Western's KK12 was closely related to Thailand's CT09 and yet distantly related to the other genotypes thereby forming a distinct cluster. NZ62 was also closely related to a number of Tarogen germplasm.

Most Kenyan genotypes were closely related with Western's KK12 having the highest genetic distance from the rest followed by Nyanza's SI81 and then Western's ST12 genotype. Genotypes SI81, ST12 and KK12 from Western Kenya and CG33 from Central are genetically distant from each other as shown by PCA on the farthest end of the co-ordinates. Genetic similarity is also shown between LT23 (Nyanza), IS14 (Western Kenya) and CG32 (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 dendogram. Comparatively, Malaysian (CM 14) and Thailand CT09 showed the highest genetic distance from the other Tarogen germplasm. Majority of taro genotypes fall within sub-clusters where genetic distance is very close to each other. The cluster analysis also revealed that Japan (CJ23) and Papua New Guinea (BP10), Thailand (CT07) and Samoa (BL52, BL51), Malaysia (CM12) and Hawaii (BH08) taro genotypes have closer genetic relatedness while Indonesia taro population has a distant genetic relationship with other varieties. Indonesia's (CN01), Nyanza's (NZ52, SI81 and SY51), Western's (IS14, MT78) and Japan (CJ23) depicted distinctive similarity and closer genetic relatedness. A two dimension PCA using the first two axes analysis and UPGMA dendogram tree results correlated to a greater extent.

The hierarchical analysis of molecular variance was performed with the eleven populations used in this study. Partitioning of the entire species diversity using analysis of molecular variance accredited 6% of the disparity to diversity among the populations, 24% amongst individual taro accessions while majority (70%) of the genetic diversity resided within taro populations (Table 7). Though both the diversity separation component, that is, between and within populations was statistically significant at $p \leq 0.05$, the figures suggest that most of the genetic diversity of taro exists within the populations.

4. DISCUSSION

The assessment of genetic diversity is essential for the conservation of germplasm. The use of DNA data pools from the different geographical regions has been very effective at evaluating the allelic diversity of the taro germplasm. Pooling germplasm is a very important strategy for speeding up the genotyping of germplasm, which due to their higher genetic variability requires the analysis of more individual plants for each accession to produce a more realistic evaluation of the variability within the germplasm of a particular cultivar. The six SSRs analyzed in the present study were located on chromosomal regions, detecting alleles in the 50 cultivars. Allelic diversity is vital for delineating the potential of germplasm for further conservation studies and crop improvement. Previous reports have used SSRs in determining the number of alleles and alleles per locus in various food crops [18,19,10]. In general, as the number of cultivars in any given population increased so did the diversity and the number of alleles/locus. The allele number generated for this study was 64 that is 30 for Kenyan and 34 for SPC Tarogen germplam. [20] In which 31 alleles were identified across 98 taro accessions. However, the allele number in this study was relatively lower than those of previous reports including [9] whose average allele number was 41 alleles using AFLP markers. The average polymorphism observed in this study (87.5%) was comparable to previous studies [20] which reported 85%. This indicates the reliability of SSR markers and especially those described by [7] in the study of taro evolution and population biology.

Table 7. Molecular variance of the fifty taro populations grouped in eleven populations based on SSR markers

Source of variation	df	SS	MS	Estimated Variance	%	p-value
Among Population	1	8.740	8.740	0.122	6%	0.001
Among Individuals	48	127.040	2.647	0.538	24%	0.001
Within Individuals	50	78.500	1.570	1.570	70%	0.001
Total	99	214.280		2.230	100%	

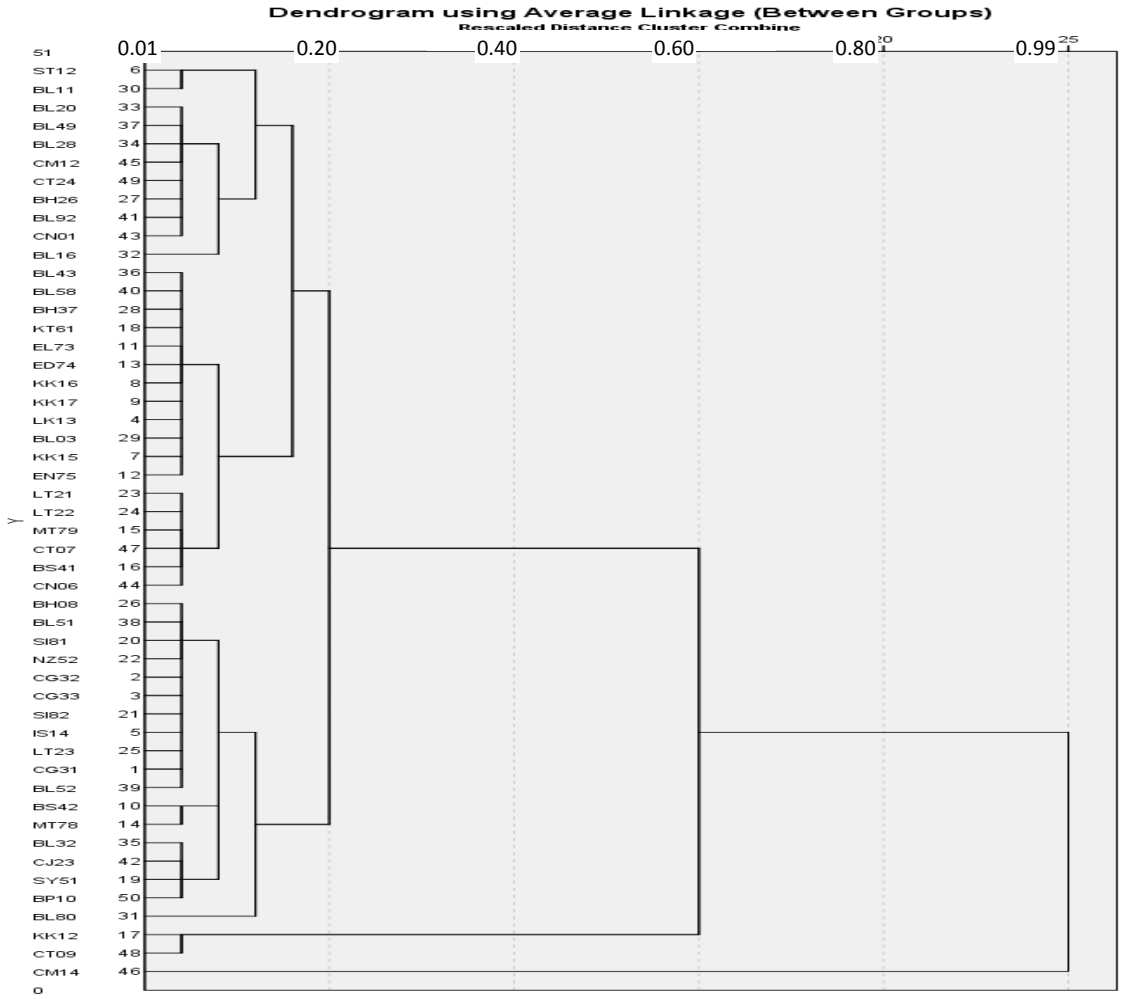

Fig. 3. UPGMA clustering dendogram based on Nei's Genetic distance of the 50 taro cultivars

Results from the first three PCA analysis plot corroborated to a greater extent the findings of UPGMA dendogram clustering. The first three components of the axis accounted for over 51.82% for Kenya and 50.06% for PC Tarogen germplasm variation. A number of studies have been reported on the assessment of genetic diversity of other crops like beans and cowpeas. [21] evaluated genetic diversity of dry bean *P. vulgaris* of Kenya using SSR markers, observed that the first and second Principal coordinates were 49.37% and 15.2% respectively, and accounting for over 64.59% of the variation exhibited by the bean populations. Studies of [22] evaluated genetic divergence among 29 genotypes of dry beans using SSR primer pairs and observed 45% (PCA) of the total variation. This concurs with [23] who reported that the larger the PCA value reflects how analysis was successful. The PCA provided

further information on the genetic distance and similarity between the Kenya and Pacific Island tarogen collection genotypes.

The UPGMA cluster analysis revealed that closely related cultivars sampled from same geographical region can be undoubtedly differentiated and that the genetic distance can also be established. The majority of taro accessions fall within sub-clusters where genetic distance is very close to each other. With these findings, dendograms generated could clearly show the clustering of the taro accessions and how closely are related or diverse in relation to their taro populations. This has also confirmed the presence of taro cultivars with genetic similarity or relatedness or duplicates showing very strong association and thus eliminated a chance that the rest of the taro genotypes could contain any duplicates. The considerable variation within the germplasm point to the extent to which variation exist in nature. The genetic differentiation evident in the germplasm therefore indicates genetic diversification in progress. Findings from [24] generated four genetic clusters with similarity coefficient of 33% that was used to resolve the phylogenetic relationships among the different deepwater rice genotypes. The results are consistent with results observed by [25] with cassava landraces whereby the principle coordinate analysis provided similar information on the genetic distance between the Tanzanian and non-Tanzanian cassava landraces from geographically distinct regions. Their PCA plot demonstrated trends similar to the clustering revealed in a dendrogram. Findings from analysis of molecular variance analysis revealed low genetic variations among taro populations but high genetic variation within taro genotypes. This concurs with observations also reported by [26] and [27] which was attributed by the limited number of taro accessions introduced to their populations. These results were consistent with previous studies on DNA characterization that showed greater differences between agro-morphological and molecular marker characterization (SSR) primers on taro landraces. [28] reported that agro-morphological characterization was useful in reflecting differences' between taro cultivated landraces while SSR primer characterization was very useful for characterizing taro landraces.

One of the consequences of SSR analysis for cultivar conservation is that the genotyping of cultivars can detect variances that are otherwise difficult to identify using traditional methods of phenotype characterization. Another appropriate facet of genotyping using SSR molecular markers is the ability of this methodology not only to scrutinize many accessions simultaneously but also to study individual plants of a specific accession. The information of within-accession variability is vital for conservation purposes, because it is probable to determine the most genetically variable accessions which would demand an additional effort of sampling a higher quantity of seeds in order to preserve this genetic variability and prevent genetic drift during routine periodic germplasm multiplication.

5. CONCLUSION

The evaluated SSR molecular markers have revealed comparative genetic diversity that exists between Kenya and SPC Tarogen germplam. SSR is as such an efficient co-dominant marker which expresses the homozygous and heterozygous state of the DNA. The informative molecular marker generate higher reproducible results compared to other markers. This could serve as a genetic benchmark towards identification of genetically distant taro genotypes as well as in sorting of duplication for morphologically close taro accessions. In addition, the taro cultivars with wide genetic distance can be cross fertilized to widen the genetic base to improve heterosis that could help in taro productivity and improvement of varieties by breeders.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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