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Evaluation of Polymorphism Generated By Single Sequence Repeat Markers in Selected Taro Populations

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ABSTRACT

Genetic structure of Kenya and Secretariat of Pacific Community's Tarogen taro germplasm was investigated. Fifty cultivars were evaluated using six SSR primer combinations. Significant polymorphic variations within the Kenyan and Tarogen taro sub-populations were revealed. A total of 64 alleles were detected with sizes from 99 to 294 bp. Kenyan germplasm generated 30 alleles and averaged 5.0 alleles/ locus while the Tarogen revealed 34 alleles with an average of 5.70 alleles/ locus. Primers generated a higher polymorphism information content (PIC) in Tarogen (0.6508) compared to Kenyan (0.6108) genotypes and genetic diversity index mean in Tarogen (0.6989) than Kenyan (0.6530) genotypes. Genetic diversity ranged between 0.25 to 0.6218 and 0.25 to 0.06204 for Kenyan and Tarogen genotypes. Allele frequency ranged from 42.52 to 75% across the populations. Generally, observed mean heterozygosity was lower than the expected mean genetic diversity indexes of 0.6530 and 0.6989 for Kenyan and Tarogen. Kenya recorded the highest mean genetic diversity (0.4735) and richness (3.04), allele frequency (0.62) and PIC (0.412) while Tarogen recorded 2.0, 0.4527, 0.5988 and 0.3917, respectively. Observed genetic diversity was significantly greater than the expected diversity. All the evaluated primers displayed distinct polymorphisms among the taro accessions studied indicating the robust nature of microsatellites in revealing polymorphism variability.

Key words: Primers, Genetic richness, Genetic diversity, Allele frequency, Tarogen germplasm and simple sequence repeat.

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INTRODUCTION

Taro (*Colocasia esculenta L. (Schott),* commonly known as arrowroots, and locally known as "Nduma" is a well-balanced food highly nutritious and compares favorably with other foods rich in carbohydrates, proteins, vitamins and minerals. Its corms, cormels, leaves, stalks and inflorescence are utilized for human consumption (Jirarat et al., 2006; Vishnu et al., 2012). Despite reported genetic variability existing in local in taro germplam (Macharia et al., 2014) only three varieties are recognized in Kenya. Distinguishing variables are colour of the flesh, water requirement during growing period and length of time to cook. Production is mainly along riverine but farmers also cite 'rain fed' varieties that require considerably low amounts of water. Genetic diversity assessment between individuals within a species or between different species or populations is very important in every crop improvement program for selection of genetically diverse parental lines to obtain superior recombinants. This information is highly vital in formulating breeding programs aimed at improving the quality of production systems of taro. The study of relationships between genotypes is important for evaluating the likelihood of joint selection of two or more traits and hence for estimating the effect of selection for

secondary traits on phenotypes for traits under consideration. Comparative assessment analysis of the genotypic and genetic diversity would quantify the level of the distribution among germplasm collections. Studies with simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) markers have confirmed the existence of these two distinct gene pools in taro (Noyer et al., 2003; Kreike et al., 2004).

This variation can be targeted by polyacrylamide gel (PCR), by placing the primers either side of the block. This leads to highly reproducible, co-dominant, easily analyzed and polymorphic markers (Powell et al., 1996; Hedrick, 2001). Together these characteristics make the microsatellites loci one of the best genetic markers for mapping purposes (Oliveira et al., 2010; Verma and Cho, 2004; Toth et al. 2000; Scotti et al., 2000). Limited studies on taro genetic diversity in powerful nations like China and Kenya is not an exceptional, yet there are several microsatellites loci for taro that have been already developed and published by many authors (Mace and Godwin, 2002; Mace et al., 2006). These microsatellite loci have been widely used for characterizing genetic diversity in several crops species like rice (Sadia et al., 2012; Brondani et al., 2005; Siwach et al., 2004), sorghum (Smith et al., 2000; Dean et al.,1999), maize (Senior et al.,1998), cotton (Liu et al., 2000) and wheat (Prasad et al., 2000). Molecular markers have been widely used in the assessment of genetic diversity studies among various crops to detect high levels of allelic diversity because they are efficient, cost-effective to use. They are some of the most powerful tools for genetic diversity and genetic relationships assessment among species. Researchers have also showed that significant correlations exist between yield and several vegetative traits. This reinforces the suitability of agronomic characters in selecting genotypes (Garcia et al., 2006; Broughton et al., (2003); Dwivedi and Sen, 1999).

These results attest to the fact that, the usefulness of microsatellite marker based molecular genotyping. De Vicente and Fulton (2004) showed that the existence of variations within a population means that some individuals within that population can adapt to certain environmental conditions and can be selected for production in adapted conditions. Benchimol et al. (2000) showed that germplasm variability at molecular level is important in their description and molecular marker is a tag of a particular aspect of phenotype and genotype whose inheritance can be traced from one generation to another. This could serve as a genetic benchmark based on the assessment of genetic diversity amongst taro genotypes. Previous work on cassava (Roa et al., 2000), maize (Buckler et al. 2006) and taro (Mace and Godwin, 2002) indicate the usefulness of SSR molecular markers in crops with low levels of intraspecific diversity. SSRs molecular markers developed by Mace and Godwin (2002) are useful in delineating polymorphism in taro. They are highly preferred genetic markers because of rapid processing and abundant throughout the genome.

Since the markers are highly variable within various populations, they can be used to study the genetic variations in taro populations. Their small size range allows multiplex development and discrete alleles allow digital record of data hence formed the fundamental basis for this research work. In view of the above, this research is aimed at analyzing the relationships existing between polymorphic characters of the selected local and introduced taro varieties using genetic markers.

MATERIALS AND METHODS

Plant Material

Fifty accessions of *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 germplasm) located in Vanuatu.

DNA Isolation

Five hundred milligrams 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 Cetyl trimethylammonium bromide (CTAB) extraction buffer as described by Sharma et al. (2008). DNA was precipitated with isopropanol and washed with 76% ethanol washing solution and dissolved in Tris-EDTA (TE) buffer. DNA was quantified using spectrophotometer (XNanoDrop® ND1000, Thermo Scientific) and diluted to 10 ng/µl.

Polyacrylamide Gel (PCR)

Six highly polymorphic SSR markers widely distributed in taro population genome were used in genotyping (Mace et al., 2010). 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 mMTris-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 to 59°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. PCR amplicons were separated on polyacrylamide gel electrophoresis system.

Statistical Analysis

The genetic diversity, number of alleles, highest

Table 1. Taro germplasm used in the study.

S/no	CePaCT accession number	Genotype variety/accession	Origin	Latitude	Longitude
1	KCT/GHT/31	Kigoi	Central-Kenya	00.416666°	036.66666°
2	KCT/KGI/32	Kigirigasha	Central-Kenya	00.416666°	036.66666°
3	KCT/NGC/33	Ngirigacha	Central-Kenya	00.416666°	036.66666°
4	KWK/LKW/13	Lukuyw	Western Kenya	0.28135°	034.75140°
5	KWK/ISW/14	Ishwa	Western Kenya	0.28135°	034.75140°
6	KWK/SHT/12	Shitao	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	Eluhya	Western Kenya	00.33333°	034.48333°
12	KMM/ENG/75	Mumias T75	Western Kenya	00.33333°	034.48333°
13	KMM/END/74	Enduma	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	Kiminini	Rift Valley Kenya	00.89356°	034.92582°
17	KNY/SYA/51	Siaya	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	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	AmagoroBusia	Western Kenya	00.460769°	034.11146°
24	KWK/KAK/12	Kakamega T12	Western Kenya	0.28135°	034.75140°
25	KWK/LVT/23	Lake Victoria	Nyanza Kenya	00.75578°	034.43835°
26	BL/WH/08	PEXPH15-6	Hawaii	19.89618°	0155.58278°
27	BL/HW/26	BC99-11	Hawaii	19.89618°	0155.58278°
28	BL/HW/37	Pa'akala	Hawaii	19.89618°	0155.58278°
29	BL/SM/43	Sama043	Samoa	13.75902°	172.10462°
30	BL/SM/80	Alafua	Samoa	13.75902°	172.10462°
31	BL/SM/92	Silipisa	Samoa	13.75902°	172.10462°
32	BL/SM/111	Pauli	Samoa	13.75902°	172.10462°
33	BL/SM/116	Manu	Samoa	13.75902°	172.10462°
34	BL/SM/120	Manono	Samoa	13.75902°	172.10462°
35	BL/SM/128	Nu'utele2	Samoa	13.75902°	172.10462°
36	BL/SM/132	Fanuatupu	Samoa	13.75902°	172.10462°
37	BL/SM/143	Vaimuga	Samoa	13.75902°	172.10462°
38	BL/SM/149	Lepa	Samoa	13.75902°	172.10462°
39	BL/SM/151	Letoga	Samoa	13.75902°	172.10462°
40	BL/SM/152	Saleapaga	Samoa	13.75902°	172.10462°
41	BL/SM/158	Lalomanu	Samoa	13.75902°	172.10462°
42	CA/JP/03	Mayako	Japan	36.20482°	138.25292°
43	CE/IND/01	Kudo	Indonesia	00.78927°	113.92132°
44	CE/IND/06	IND155	Indonesia	00.78927°	113.92132°
45	CE/MAL/14	Klauang	Malaysia	04.21048°	101.97576°
46	CE/MAL/12	Klang	Malaysia	04.21048°	101.97576°
47	CE/THA/07	Srisamrong	Thailand	15.87003°	100.99254°
48	CE/THA/09	Tadeang	Thailand	15.87003°	100.99254°
49	CE/THA/24	Boklua	Thailand	15.87003°	100.99254°
50	BL/PNG/10	C3-12	Papua New Guinea	6.31499°	143.95555°

frequency allele and polymorphism Content (PITCs) were estimated. The number of alleles per locus and PIC were estimated using the power marker version 3.25 (Liu and Muse, 2005).The estimates of genetic richness and genetic diversity index test of significance X^2 (Chi-Square test) were calculated at α = 0.01 level of significance based on the power marker version 3.25 (Liu and Muse, 2005). The critical-value was obtained from Chi –square table on each microsatellite loci based on alleles size.

Limitations

The study is limited by the fact that the SSR primers used were drawn from one study (Mace, 2002). Sampling of

Table 2. Genetic diversity, number of alleles, highest frequency allele and PITCs values found among twenty five Kenyan taro collections for Six SSR Markers.

Primer names	Loc.	N	Allele No.	Amp.size	Alle. size	Freq.	Het.	PIC val.	He
Xuqtem 55	Kenyan	4	4	99-109	109	0.4773	1.0000	0.6097	0.6643
	Tarogen	7	6	99-139	105	0.4565	0.9565	0.6656	0.7060
Xuqtem 73	Kenyan	4	3	153-167	161	0.7619	0.1905	0.3360	0.3810
	Tarogen	5	5	153-200	161	0.4565	0.1739	0.5901	0.6541
Xuqtem 84	Kenyan	6	5	167-190	167	0.5600	0.3200	0.5919	0.6296
	Tarogen	6	5	167-190	184	0.4000	0.3600	0.7124	0.7480
Xuqtem 88	Kenyan	10	6	65-87	83	0.3750	0.3333	0.7238	0.7587
	Tarogen	7	5	71-91	85	0.5000	0.2500	0.5489	0.6172
Xuqtem 91	Kenyan	7	6	206-268	212	0.4048	0.6667	0.6945	0.7336
	Tarogen	7	5	206-268	268	0.4167	0.7500	0.6519	0.7023
Xuqtem 97	Kenyan	9	6	206-268	212	0.3333	0.7500	0.7091	0.7509
	Tarogen	11	8	206-294	268	0.3913	0.7391	0.7361	0.7656
Total	Kenyan		30	Mean		0.4854	0.5434	0.6108	0.6530
	Tarogen		34			0.4368	0.5383	0.6508	0.6989

Loc. – location of germplasm origin; He – gene diversity; Het – heterozygosity; N- chromosome number; freq – major allele frequency; amp. Size - amplicon fragment size range; alle. size – major allele size; PIC val. - polymorphic information content.

Kenyan taro germplasm was limited to mainly Western Kenya. Therefore, care should be exercised in extrapolating these results to other geographical regions.

RESULTS

Analysis of SSR Data

The amplification products revealed a distribution of amplified fragments unique for each primer combination. Generally, our analysis of SSR markers detected significant polymorphic variations within the Kenyan and Tarogen taro populations. A total of 64 alleles were detected and scored as polymorphic bands in the 50 accessions investigated. The number of alleles per marker ranged between 3 (Xuqtem 73) and 8 (Xuqtem 97). The six primer combinations produced bands ranging in size from 99 to 294 bp. However, the major allele bands ranged in size from 83 to 268 bp per primer pair. The population specific bands were discernable from the fragment patterns produced. These bands were treated as genetic loci. Each band generated by the primer combinations was discrete and reproducible. The polymorphic bands produced were proficient in evaluating the genetic diversity among and within the populations. The polymorphism generated in the Kenyan and Tarogen germplasm was variant (Table 2). The Kenyan taro germplasm generated a total of 30 alleles with an average of 5.0 alleles per locus while the Tarogen core collection revealed a total of 36 alleles with an average of 6.0 alleles per locus. The number of alleles per marker ranged from 4 to 6 in the Kenyan accessions and 5 to 8 in the Tarogen varieties.

The amplified fragments sizes were comparatively different with the Tarogen germplasm revealing higher base pairs sized fragments than the Kenyan accessions. The smallest size of amplified fragments was generated by Xuqtem 88 with sizes ranging from 65 to 87 bp for Kenyan and 71 to 91 bp for Tarogen germplasm. The largest amplified fragment sizes (206 to 294 bp) were generated by Xuqtem 91 and 97.The major allele sizes varied across the two sets of germplasm and ranged between 83 to 268 bp. The major alleles with the smallest size was generated by Xuqtem88 which produced major fragment sizes of about 83 and 85 bp in the Kenyan and Tarogen germplasm, respectively. The largest sized major alleles were produced by Xuqtem 91 and 97 with 268 bp (Table 2). Generally, the allele frequency of most the common allele within-group at each locus ranged from 33.33 to 76.19%. The Tarogen germplasm generated a frequency range of 39.13 to 50% with an average of 43.68%. The Kenyan germplasm produced allele frequency ranges of between 33.33 and 76.19% and averaging 48.54%. These frequency indexes denoted the frequency shared by a common major allele at any given locus. The Tarogen germplasm revealed the highest number of polymorphic alleles (8) which the Kenyan germplasm generated 6. The PIC generated revealed the presence of dissimilar levels of polymorphism (Table 2). Tarogen germplasm revealed a higher level of PIC (0.6508) compared to the Kenyan genotypes (0.6108). Generally, the highest and least polymorphic information was generated by Xuqtem 97 (PIC values 0.7509 and 0.7656) and Xuqtem 73 (PIC values 0.3360 and 0.5901) for both sets of germplasm. The Tarogen PIC values ranged from 0.7361 to 0.5489.

The PIC values for Kenyan taro genotypes ranged from 0.7238 to 0.3360. For Tarogen germplasm, the highest PIC values were 0.7364 and 0.7124 and were generated by Xuqtem 97 and 84 primers. On the overall polymorphism analysis, the rate of polymorphism among

Table 3. Cluster analysis of the total number of alleles mapped from microsatellite SSR markers in Kenyan germplasm collections.

Locus†	Chromosome	Number of Alleles (N) in Kenyan accessions				Mean
		Western	Central	Rift Valley	Nyanza	
Xuqtem 55	1H	3	3	2	4	3
Xuqtem 73	2H	3	2	1	2	2
Xuqtem 84	3H	5	2	1	4	3
Xuqtem 88	4H	6	2	2	2	3
Xuqtem 91	5H	5	2	2	4	3.25
Xuqtem 97	6H	4	3	1	6	3.5
	Total (N=73)	28	14	9	22	18.25
	Mean	4.67	2.33	1.5	3.67	

Locus†	Chromosome	Number of Alleles (N) in Tarogen core collection							Mean
		Samoa	Thailand	PNG	Malaysia	Japan	Indonesia	Hawaii	
Xuqtem55	1H	4	3	2	3	2	3	4	3
Xuqtem73	2H	5	2	1	2	1	2	2	2.14
Xuqtem84	3H	5	3	1	3	2	2	3	2.71
Xuqtem88	4H	5	2	1	2	1	2	2	2.14
Xuqtem91	5H	4	2	2	2	1	2	5	2.57
Xuqtem97	6H	7	3	2	2	2	4	3	3.29
	Total (N=111)	30	15	9	14	9	15	19	15.86
	Mean	5.0	2.5	1.5	2.33	1.5	2.5	3.17	

the Kenyan taro accessions, three polymorphic markers (Xuqtem 91, 97 and 88) produced six alleles each, Xuqtem 55 four alleles, Xuqtem 77 three alleles while Xuqtem 84 produced 5 alleles at each locus. The number of alleles per locus ranged from three to six. For Tarogen germplasm markers Xuqtem 77, 84, 88, and Xuqtem 91 each produced five alleles while Xuqtem 55 produced six alleles each while the highest polymorphic marker produced 8 alleles each. Thus the level of polymorphism among Tarogen taro genotypes was moderately higher than Kenyan genotypes. Genetic diversity was evident amongst the tested fifty taro cultivars (Table 2). Comparatively, the Tarogen germplasm revealed a comparatively higher genetic diversity index mean (0.6989) than Kenyan genotypes (0.6530). The highest genetic diversity index (0.7656) was generated by Xuqtem 97 in the Tarogen genotypes. The least genetic diversity index (0.3820) was produced by marker Xuqtem 93 in the Kenyan taro genotypes. The genetic diversity index generated in the Kenyan genotypes by the markers ranged from 0.3810 to 0.7587 while for Tarogen was 0.6172 to 0.7656. Values of observed heterozygosity for the Tarogen genotypes were lower with a mean index of 0.5383 compared to the Kenyan germplasm which had 0.5434. Both sets of observed mean heterozygosity was lower than the expected mean genetic diversity indexes of 0.6530 and 0.6989 for Kenyan and Tarogen germplasms, respectively.

Population Genetic Analysis

The SSR markers amplification products revealed a distribution of amplified fragments unique for each primer combination within and among the taro genotypes in the Kenyan and Tarogen germplasm (Table 3). Thus significant polymorphic variations within the Kenyan and Tarogen taro sub-populations were revealed. For the Kenyan germplasm, the SSR markers generated the highest number of alleles in taro genotypes from Western Kenya (28) while the least was produced by Rift Valley germplasm (9). The mean allele numbers generated per marker ranged between 1.5 (Rift Valley) and 4.67 (Western). For the Tarogen germplasm, Samoa generated the highest number of alleles (30) while germplasm originating from Papua New Guinea and Japan recorded the lowest number of alleles (9). The mean allele numbers generated per marker ranged between 1.5 (Papua New Guinea and Japan) and 5.0 (Samoa). The genetic diversity index varied across populations and ranged between 0.25 to 0.6218 and 0.25 to 0.06204 for Kenyan and Tarogen genotypes (Table 4). For the Kenyan germplasm, genetic diversity was significantly higher in taro genotypes of Western Kenya (0.6218) and Nyanza (0.5432) while the least diversity index was recorded in the Central Kenya (0.479) and Rift valley Kenya (0.25) populations. Similarly, the highest genetic diversity index amongst the Tarogen germplasm was recorded in Hawaii (0.6204), Samoa (0.611) and Thailand (0.5208) while the lowest was with germplams from Indonesia (0.25), Malaysia (0.25), Papua New Guinea (0.25) and Japan (0.25). Variations were observed in the genetic richness values of the populations (Table 4).

The genetic richness values ranged between 1.5 (Rift Valley) to 4.67 (Western Kenya) and 1.5 (Japan and Papua New Guinea) to 5.0 (Samoa) for Kenyan and

Table 4. Analysis of the genetic richness and genetic diversity mapped from Microsatellite SSR Markers in Kenyan taro collections.

Parameter	Kenyan germplasm populations				Mean
	Western	Central	Rift Valley	Nyanza	
Genetic richness	4.67	2.33	1.5	3.67	3.04
Genetic diversity (*He*)	0.6218	0.479	0.25	0.5432	0.4735
Allele frequency	0.5121	0.639	0.7500	0.5789	0.62
PIC Value	0.5731	0.398	0.1875	0.4895	0.412

	Tarogen core collection							Mean
	Samoa	Thailand	Papua New Guinea	Malaysia	Japan	Indonesia	Hawaii	
Genetic richness	5.0	2.5	1.5	2.33	1.5	2.5	3.17	2.0
Genetic diversity (He)	0.611	0.4722	0.25	0.5208	0.25	0.5208	0.6204	0.4527
Allele frequency	0.4252	0.6387	0.7500	0.5417	0.7500	0.5417	0.5440	0.5988
PIC Value	0.6579	0.4051	0.1875	0.4232	0.1875	0.4362	0.4444	0.3917

Table 5. Analysis of genetic diversity test of Chi-Square test of significance among SSR Markers for PITCs collections.

Marker		X^2 value (Observed)	df	Critical value (expected)	p-value
Xuqtem 55	Tarogen	19.3492	15	30.578	0.0400
	Kenyan	19.4246	6	16.812	*0.0000
Xuqtem73	Tarogen	78.9322	10	23.209	*0.0000
	Kenyan	23.9531	3	11.345	*0.0100
Xuqtem 84	Tarogen	67.9141	10	23.209	*0.0000
	Kenyan	47.0663	10	23.209	*0.0000
Xuqtem 88	Tarogen	46.1690	10	23.209	*0.0000
	Kenyan	45.7963	15	30.578	*0.0000
Xuqtem 91	Tarogen	35.4012	10	23.209	*0.0000
	Kenyan	26.1936	15	30.578	*0.0020
Xuqtem 97	Tarogen	29.4932	28	44.314	0.0680
	Kenyan	26.9733	15	30.578	*0.0110
	Tarogen	46.2098	15	30.578	*0.0180
	Kenyan	31.5679	11	24.725	*0.0038

Tarogen genotypes. The genetic richness value was significantly higher in those from Western Kenya (4.67) and Nyanza (3.67) and lowest in Central Kenya (2.33) and Rift valley (1.5). For Tarogen the genetic richness value was comparatively higher in Samoa (5.0); Hawaii (3.17), Thailand (2.5) and lowest in Indonesia (2.5); Malaysia (2.33); Papua New Guinea (1.5) and Japan (1.5). Differences in the allele frequencies across the populations were observed. The allele frequency values ranged from 42.52 to 75% across the populations. The allele frequencies in the Kenya germplasm were between 51.221 and 75 and 42.52 and 75% in Tarogen germplasm. The allele frequency was significantly higher in PNG and Rift Valley and lowest in Samoa. The PIC values varied between 0.5731 (western Kenya) to 0.1875 (Rift valley Kenya) among the Kenyan taro genotypes accessions while in Tarogen germplasm ranged from 0.6579 (Samoa) to 0.1875 (Papua New Guinea). The most polymorphic information was generated in Samoa and Western Kenya taro populations. The genetic diversity test of significance $X^2$was determined using the critical-value obtained from X^2 tables for each microsatellite loci based on alleles size (Table 5). The application of Chi-square test on analysis of genetic diversity index *(H)* of test of significance (X^2) revealed a significant difference between the microsatellite loci of Kenyan and Pacific Island taro accessions with reference to use of molecular marker based (SSR). The Chi-square values at $p<0.05$ and $p<0.001$ were greater than Chi-square critical values.

DISCUSSION

Molecular assessments of any new germplasm are important to enhance our understanding on the capabilities of current germplasms, so that we can estimate agronomic performances and select parental for crop improvement programs. The Tarogen taro germplasm tested had a significant level of genetic heterogeneity compared to the local germplasm. Introduction of this genetic variability in the cropping systems is thus vital for the sustainability of small scale farmers, because despite the low yield capacity, the cultivars present high yield stability. The evaluation of the genetic variability of accessions of isolated populations

can provide the basic information essential to help genbanks multiply and properly conserve these genetic resources. This will also help breeding programs to design crosses to integrate this variability into the genetic background of elite taro lines, which in turn will produce new taro cultivars. SSR was effective in distinguishing polymorphism and genetic variation in the genomes of taro genotypes. None of the studied populations were monormorphic as all the SSR loci were polymorphic. SSR revealed a large number of polymorphic DNA fragments. An average of 5 to 6 alleles per locus was detected for across the taro germplasm.

The molecular data is largely correlated with variation at the agronomic performance in the crop plants and hence offer good guidance on the distribution of valuable variation as well as on the presence of co-adapted gene complexes (Hawtin et al., 1997). Despite the fact that the SSR markers used in our study are considered not functionally related to any trait and therefore neutral, the genomic fingerprinting profile of each plant is not expected to vary from one environment to another, indicating the ability of plant populations to adapt (Virk et al., 1996). The direct association between the fingerprint of an accession and the phenotypic response to a target environment is caused by linkage disequilibrium (Ford-Lloyd et al., 1997). Neutral markers can therefore be used to delineate the evolutionary past of varietal groups and populations and to account for pre-selection of the germplasm to be used in breeding programs (Glaszmann et al., 1996). Several authors have reported similar average number of alleles of about 6.0 in the genetic diversity studies of many crops such as cassava and sweet potato (Elibariki et al*.,* 2013; Lokko et al*.,* 2006; Elameen et al., 2008). Other similar studies on use of microsatellites in cassava (Chavarriaga-Aguirre et al., 1998) noted twenty percent (20%) increase in both the number of alleles observed and the levels of polymorphism for (GA) microsatellite locus, when the number of accessions screened increased from 38 to over 500. In a similar study using microsatellites to assess genetic diversity in coconut (Rivera et al*.,* 1999), it was found out that the number of alleles per locus increased from 5.2 to 8 when the number of accessions screened was increased from 20 to 40 which is comparable to fifty taro accessions. Mace and Godwin (2002) also used similar SSR markers to assess genetic diversity based on previous assays of PCR product amplification and polymorphism in taro accessions.

It has been found that SSR primers have previously shown to reveal high level of polymorphism in Polynesian taro cultivars (Mace and Godwin, 2002). They are co-dominant markers that are more powerful than dominant markers, enabling allele frequencies to be estimated and require a smaller sample sizes to achieve equivalent analytical power. The numbers of repeats are highly variable between individuals or genotypes hence forming the basis for diversity indices and polymorphism.

The results have shown that the observed levels of genetic richness and diversity were related to the number of alleles in both regions. The molecular markers representing each of the chromosomes indicated high levels of genetic diversity and richness among taro populations. This indicates that the germplasm is a good source of genetic variability. Average genetic diversity values were high hence revealing a clear indication of genetic variability abundance within the taro populations which could be harnessed for conservation and breeding programs. Western and Nyanza from Kenyan taro genotypes, Samoa and Hawaii from Tarogen taro accessions were leading in terms of genetic richness and diversity. The high genetic richness observed in Western Kenya is understandable as the crop is highly popular and widely cultivated. The high genetic richness has been reported in previous reports (Tumwegamire et al*.,* 2011; Pariyo et al*.,* 2009). The low genetic diversity values on taro accessions were revealed by Central and Rift valley parts of Kenya similar to the findings for Indonesia, Malaysia, Papua New Guinea and Japan from Tarogen collections indicated that there was lower genetic variation amongst these populations' correlating with the low taro production and utilization in Central and Rift Valley regions of Kenya.

The results have confirmed that the microsatellite markers chosen were highly informative as depicted by the average values of polymorphic information content. Elibariki et al*.* (2013) also reported that the closer the value to 1.0 the better the PIC value and the more the informative the SRR marker. The most informative SSR markers with the highest PIC values were Xuqtem 88 (72.38%) and Xuqtem 97 (70.91%) for the Kenyan populations and Xuqtem 84 (73.64%) and Xuqtem 97 (71.24%) for the Tarogen populations. The least informative marker with lowest polymorphic information value was Xuqtem 73. Bered et al*.* (2005) reported similar results by use of molecular markers have become important tools in studies of genetic diversity among and within sweet corn populations due to the high resolution and reliability in the identification of cultivars. Previous studies indicate that PCR-based markers are abundant, co-dominant, highly reproducible and interspersed throughout the genome (Panaud et al., 1996; Temnykh et al*.,* 2000; McCouch et al*.*, 1997; Paul et al., 2012). There are factors that might account for the relatively moderate level of polymorphism revealed amongst Rift valley and Central parts of Kenya as well as Japan, Papua New Guinea taro genotypes from Tarogen collection in comparison with other groups with the highest polymorphism rates. Consequently, Mace and Godwin (2002) also found out that the SSR markers isolated Pacific Island region are expected to be highly successful in revealing heterozygosity in taro genotypes despite relatively low levels of polymorphism revealed and the low number of alleles per locus. They further concluded that taro cultivars in the Pacific Islands Community exhibit

remarkable morphological variation while their genetic base appears to be very narrow. The results in this study on taro genotypes showed an average of 5 and 6 alleles per locus of microsatellite markers for Kenyan and Tarogen collections, respectively.

The results by Sadia et al*.* (2012) also reported that genetic diversity studies would be very useful for the selection of the parents for developing submergence tolerant and flash flood tolerant rice variety through molecular breeding program. Varshney et al. (2013) also reported on the significance progress that has been met in recent years in the application of molecular markers such as SSR to genome sequencing, plant genetic resource characterization and cultivation, conservation of the flanking sequence of each microsatellite locus allow the design of primers for PCR amplification. From these analyses, determination of genetic diversity and genetic richness is very fundamental towards understanding taro genetic resources for varietal identification and rationalizing its collection. This would be safeguarding the existing genetic diversity for taro germplasm conservation, management and for potential utilization for food security. Therefore, comparative assessment of genetic diversity among taro accessions is very important toward improving the quality of taro productions in Kenya and whole Africa. PIC was a key indicator of allelic diversity and frequency. From the results, the levels of polymorphism among Tarogen collections were higher compared to Kenyan taro genotypes. These results have revealed that all the primers showed distinct polymorphisms existing amongst the taro accessions studied indicating the robust nature of microsatellites in revealing polymorphism among taro accessions.

These results PIC of allelic diversity frequency are consistent with the results observed among deep water rice genotypes varieties, which ranged from 0.477 to 0.782, with an average of 0.634 (Sadia et al*.,* 2012; Ni et al., 2002; Okoshi et al*.,* 2004) which becomes fundamental and ideal for studies on genetic diversity and intensive genetic mapping (Cho et al*.,* 2000). This study has pointed out the most informative and exhaustive primers that could be useful in marker-assisted selection, gene mapping and tagging for useful traits. This information could greatly assist in the selection decisions in taro breeding. Conclusively, SSR molecular markers have proven to be fundamental and instrumental as the markers of choice for molecular characterization due to high levels of polymorphism, co-dominant character, their abundance and uniform distribution throughout the plant genome. Stereotype character of transferability and reproducibility with high simplicity detection through PCR has made its applicability for vegetative root tuber crops.

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