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Analysis Of Genetic Diversity And Richness Of Western, Nyanza And Central Kenya Taro Genotypes {Colocasia Esculenta L. (Schott)} Using Simple Sequence Repeat Markers (SSR Molecular Based Markers)

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Abstract: ***Six Simple Sequence Repeat (SSR) primer pairs were used to characterize Western, Nyanza and Central Kenya taro genotypes of Kenya. A modified CTAB protocol method was used for genomic DNA extraction. Genemapper scoring software v4.0 and powermarker v3.25 were used to analyze genetic diversity using simple sequence repeat (SSR) markers. A total number of 64 alleles were revealed by cluster analysis mapped from microsatellite SSR Markers. Western Kenya had the highest genetic diversity and richness of (He: 0.6218; 4.6667) followed by Nyanza Kenya taro genotypes (He: 0.5432; 3.67) and was moderately high in Central Kenya taro genotypes (He: 0.479, 2.33). Polymorphism information content (PIC) from six molecular markers varied among the taro accessions from 0.5731 to 0.398. Similarly, the number of alleles per locus followed the same trend of genetic diversity and genetic richness. Taro genotypes displayed a wider genetic diversity among taro accessions. Western and Nyanza Kenya taro genotypes stood significantly with the highest genetic diversity. These findings of genetic diversity and genetic richness are very useful to taro breeders for identification and rationalization of taro germplasm collections. This would help them to incorporate information on genetic diversity towards improving taro productivity for food security and breeding purposes.***

Keywords: ***Taro (Colocasia esculenta), genetic diversity, alleles, genetic richness. Polymorphism information content (PIC)***

ABBREVIATION

AFLP- Amplified Fragment Length Polymorphism
CTAB- Cetyl trimethylammonium bromide (CTAB)
DNA- Deoxyribonucleic Acid
DArTs- Diversity Arrays Technology
FAO- Food and Agricultural Organization
NaCl- Sodium Chloride
PIC- Polymorphism Information Content
PCR- Polymerase Chain Reaction
SSR- Simple Sequence Repeat Markers

I. INTRODUCTION

Taro (*Colocasia esculenta L. Schott),* is a highly nutritious crop rich in carbohydrates, proteins, vitamins and minerals (Vishnu *et al.*, 2012). It is one of the most important food crops in Africa for food security and income but its production has been very low. The inability of the African continent to adequately feed its growing population is pegged on several factors (FAO, 2010). One of these factors is the genetic erosion of resources of indigenous African underutilized crops including taro (Padulosi *et al.*, 2013). Such a crop has a great potential to contribute to food security by reducing poverty levels, hunger and malnutrition (Harvestplus,

2011; Massawe *et al.*, 2013). It has a rich source of calcium, phosphorus, iron, thiamine, riboflavin and niacin which are important constituents of human diet (Paul and Bari, 2011). It's an ancient important food crop grown throughout many Pacific Island countries, parts of Africa, Southeast Asia, Madagascar and the Caribbean for its fleshy corms and nutritious leaves (Ivancic and Lebot, 2000). However, in spite of its importance as a staple food in many countries, taro crop has received very little research attention to enhance its production and utilization potential yet increasing poverty levels and food insecurity are major concerns (Goenaga and Heperly, 1990; Krishna *et al.*, 2013; Watanabe, 2002).

Taro cultivation in East Africa and South Africa is mainly practiced by small scale farmers that grow a few popular taro cultivars mainly propagated vegetatively leading to the fixation of a few plants of a particular genetic base and potentially, loss of some valuable genetic resources (Hu *et al.*, 2008; Lebot and Aradhya, 1991; Tafadzwanashe and Albert, 2013). Very little is known of the genetic diversity that exists among taro genotypes (Hu *et al.*, 2008). In East Africa region, there is limited documented information on taro cultivation and small scale rural farmers experience constraints such as low productivity, lack of improved varieties, lack of extension services , pest and diseases, scarcity of labour, lack of post-harvest knowledge, and declining soil fertility (Cheema, 2006; Twalana *et al.*, 2009).

In Kenya, studies geared towards improving taro genotypes is lacking and little has been documented on its research profile and conservation (Tumuhimbise *et al.*, 2009). There is limited information regarding taro its genetic diversity, genetic richness and high yielding varieties. As a result of this, taro breeders are faced with difficult choices of selecting the right parents due to lack of accurate genetic constitution assessment (Quero *et al.*, 2004). In Kenya, taro production does not translate into improved yields for sustained food security. There is lack of information on taro its genetic diversity and genetic richness which is vital for its sustainable cultivation productivity. The lack of its genetic diversity information could lead to loss of its genetic resilience in terms of climate change and biodiversity loss. Studies with microsatellites (SSR) markers 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).

Microsatellites markers are highly preferred genetic markers to be used in this research study because of its rapid processing and abundant throughout the genome (Mace and Godwin, 2002; Oliveira *et al.*, 2010; Babu *et al.*, 2004). They are highly variable within various populations hence could be used to study the genetic diversity 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 (Mace and Godwin, 2002; Korzu, 2003; Hedrick, 2001). Microsatellites (SSR) markers have been continued to be relied upon because of its genomic sequences and they have been proven to be the most widely used DNA marker type in characterizing germplasm collections of crops, because of their easy use, relatively low cost, and high degree of polymorphism provided by the large number of alleles per locus (Noli *et al.*, 2008; Van *et al.*, 2010). In the view of the above, The SSR technique was ideal for this study where genomic heterogeneity is high and amplification of PCR products could be used to ascertain an accurate measure of genomic diversity and richness of taro genotypes.

II. MATERIALS AND METHODS

Twenty four taro germplasm accessions of Kenya were collected from Western, Central and Nyanza Kenya (Table 1). The study was conducted in a farm field station at Masinde Muliro University of Science and Technology (MMUST) Main campus, located 00^{O} 17.30' North and 34^{o}45' East (GPS receiver) at Kakamega county. Young leaf tissue (500 mg) of fresh leaf material was collected and 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 to10 ng/µl. Six highly polymorphic SSR markers widely distributed in taro population genome were used in genotyping (Mace et al., 2010) as shown on Table 2. 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 MgCl2; 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. One microlitre of each PCR sample was mixed with a mixture of HiDi formamide and a size standard Liz (GeneScan™-500LIZ, Applied Biosciences, UK) in a 96-well plate. The taro samples were denatured at 95 °C for 3 minutes and immediately chilled on ice ready for capillary electrophoresis wrapped in a clean adhesive film and placed in ice bags for fragment separation by capillary electrophoresis. ABI 3500 DNA genetic analyser was used for fragment separation and allele calls made using Genemapper software V.4.0. Analysis of genetic diversity and richness, allele frequency allele and polymorphism Content (PIC) was analyzed using the power marker version 3.25 (Liu and Muse, 2005).

SSR. No.	Kenya Accession Number	Germplasm	Region
CG31	KCT/GHT/31	Kigoi	Central Kenya
CG32	KCT/KGI/32	Kigirigasha	Central Kenya
CG33	KCT/NGC/33	Ngirigacha	Central Kenya
LK13	KWK/LKW/13	Lukuywa	Western Kenya
IS14	KWK/ISW/14	Ishwa	Western Kenya
ST12	KWK/SHT/12	Shitao	Western Kenya
KK15	KWK/KAK/15	Kakamega T15	Western Kenya
KK16	KWK/KAK/16	Kakamega T16	Western Kenya
KK17	KWK/KAK/17	Kakamega T17	Western Kenya

BS42	KWK/BSA/42	Amagoro Teso	Western Kenya
EL73	KMM/ELU/73	Eluhya	Western Kenya
EN75	KMM/ENG/75	MumiasEN75	Western Kenya
ED74	KMM/END/74	Enduma	Western Kenya
MT78	KMM/MMU/78	Mumias T78	Western Kenya
MT79	KMM/MMU/79	Mumias T79	Western Kenya
SY51	KNY/SYA/51	Siaya	Nyanza Kenya
SI81	KNY/KIS/81	Kisii T81	Nyanza Kenya
SI82	KNY/KIS/82	Kisii T 82	Nyanza Kenya
NZ52	KNY/NYA/52	Kisumu	Nyanza Kenya
LT21	KNY/LVT/21	Lake VictoriaT21	Nyanza Kenya
LT22	KNY/LVT/22	Lake Victoria T22	Nyanza Kenya
BS41	KWK/BSA/41	Amagoro Busia	Western Kenya
KK12	KWK/KAK/12	Kakamega T12	Western Kenya
LT23	KWK/LVT/23	Lake Victoria	Nyanza Kenya

Table 1: Taro genotypes identification (Colocasia esculenta L. (Schott) used for SSR molecular markers genetic diversity analysis

SSR Primers	Allele Size Repeat Motif	Primer Sequence Forward Primer: (5´→3´) ; Reverse primer: (3´→5´)
Xuqtem55_F	(CAC)5	FWD: CTTTTGTGACATTTGTGGAGC
Xuqtem55_R		RVS: CAATAATGGTGGTGGAAGTGG
Xuqtem73_F	(CT)15	FWD: ATGCCAATGGAGGATGGCAG
Xuqtem73_R		RVS: CGTCTAGCTTAGGACAACATGC
Xuqtem84_F	(CT)18	FWD: AGGACAAAATAGCATCAGCAC
Xuqtem84_R		RVS: CCCATTGGAGAGATAGAGAGC
Xuqtem88_F	(CAT)9	FWD: CACACATACCCACATACACG
Xuqtem88_R		RVS: CCAGGCTCTAATGATGATGATG
Xuqtem91_F	(TG)6(GA)4	FWD: GTCCAGTGTAGAGAAAAACCG
Xuqtem91_R		RVS: CACAACCAAACATACGGAAAC
Xuqtem97_F	(CA)8	FWD: GTAATCTATTCAACCCCCCTTC
Xuqtem97_R		RVS: TCAACCTTCTCCATCAGTCC

Source: Mace and Godwin, (2002).

Table 2: The six profiles microsatellites loci (SSR markers) used in genetic diversity for tarogen collections (Colocasia esculenta L. (Schott)

III. RESULTS AND DISCUSSIONS

A. GENETIC DIVERSITY AND RICHNESS AMONG GENOTYPES

Western Kenya taro genotypes showed the highest genetic diversity and richness (*He:* 0.6218, 4.6667), followed by Nyanza Kenya taro genotypes (*He:* 0.5432, 3.67) and moderately high in Central Kenya taro genotypes (*He:* 0.479, 2.33) as shown on Table 3. A total of 64 alleles were mapped from three taro genotypes with Western Kenya leading with 28 alleles with a mean of 4.67 alleles per locus and Central Kenya genotypes had a total of 14 alleles mapped from its taro population with a mean average of 2.33 alleles per locus (Table 4). These findings have revealed a clear indication of genetic richness of taro populations that can be used for conservation and breeding strategies. 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 (Tumwegamire *et al.* 2011; Pariyo *et al.* 2009). 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 genotypes.

Parameter	Western	Central	Nyanza
Genetic richness	4.67	2.33	3.67
Genetic diversity (*He*)	0.6218	0.479	0.5432
Allele frequency	0.5121	0.639	0.5789
PIC Value	0.5731	0.398	0.4895

Table 3: Analysis of the genetic richness and genetic diversity mapped from
Microsatellite SSR Markers in Kenyan taro collections

Locus†	**Chromosome**	**Western**	**Central**	**Nyanza**
Xuqtem55	1H	3	3	4
Xuqtem73	2H	3	2	2
Xuqtem84	3H	5	2	4
Xuqtem88	4H	6	2	2
Xuqtem91	5H	5	2	4
Xuqtem97	6H	4	3	6
	Total (N=64)	28	14	22
	Mean	**4.67**	**2.33**	**3.67**

Table 4: Cluster analysis of the total number of Alleles mapped from Microsatellite SSR Markers in Kenyan germplasm collections

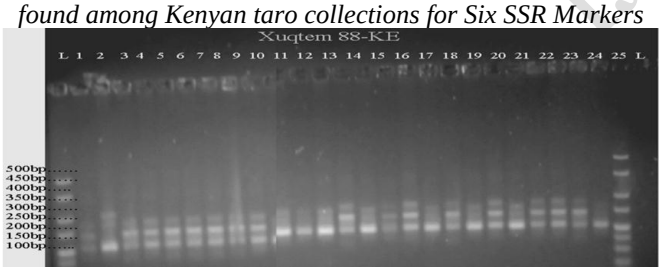
B. POLYMORPHISM INFORMATION CONTENT (PIC)

Majority of simple sequence markers revealed heterozygozity at loci by producing clear and sharp bands that were visible on two per cent agarose gel electrophoresis with expected base pairs of DNA fragments radiolabelled with fluorescent dyes (Fig. 1). These included the following microsatellite loci: Xuqtem55, Xuqtem73, Xuqtem84, Xuqtem8,; Xuqtem91 and Xuqtem 97 were among the best SSR primers. Polymorphic information content (PIC) from six molecular markers varied among the taro accessions. Overall, polymorphism information content (PIC) reflected allelic diversity among Kenyan taro genotypes was 0.6108 with a total genetic diversity of (*He*) 0.653 across mapped SSR markers (Table 5). Western Kenya taro genotypes had the highest PIC value of 0.5731 followed by Nyanza Kenya (PIC=0.4895) and the least was from Central Kenya taro genotypes (PIC=0.398). Similarly, the number of alleles per locus followed the same trend of genetic diversity and genetic richness, polymorphism information content (PITCs) value among the groups with significant differences. These results have revealed that all the primers showed distinct polymorphisms existing among the taro accessions studied indicating the robust nature of microsatellites in revealing polymorphism variability among taro accessions. These results of polymorphism information content 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). SSR markers revealed an average of 4.67, 2.33 and 3.67 alleles per locus in this study for Western, Central and Nyanza Kenya at the loci of six microsatellite markers across the taro germplasm accessions. The results have shown that the genetic richness and diversity followed the same pattern as the number of alleles in both regions. The six polymorphic microsatellites molecular (SSR) markers have indicated high levels of genetic diversity and richness among clusters of taro genotypes in Kenya. Several authors have reported a similar average number of alleles of 6.0 in the genetic diversity study of many crops such as cassava (Elibariki *et al.* 2013). 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. These results have revealed that all the primers showed distinct polymorphisms existing among the taro accessions studied indicating the robust nature of microsatellites in revealing polymorphism variability among taro accessions.

Primer names	Chromosome No.	Allele No.	Amplicon size range	Major allele	Major allele frequency	Heterozygosity	PIC Value	Gene Diversity(*He*)
Xuqtem 55	4	4	99-109	109	0.4773	1.0000	0.6097	0.6643
Xuqtem 73	4	3	153-167	161	0.7619	0.1905	0.3360	0.3810
Xuqtem 84	6	5	167-190	167	0.5600	0.3200	0.5919	0.6296
Xuqtem 88	10	6	65-87	83	0.3750	0.3333	0.7238	0.7587
Xuqtem 91	7	6	206-268	212	0.4048	0.6667	0.6945	0.7336
Xuqtem 97	9	6	206-268	212	0.3333	0.7500	0.7091	0.7509
		30			**0.4854**	**0.5434**	**0.6108**	**0.6530**

Table 5: Genetic diversity, Number of alleles, highest frequency allele and polymorphism Content (PITCs) values found among Kenyan taro collections for Six SSR Markers



Key: Central Kenya taro genotypes: 1=Kigoi 2=Kigirigasha 3=Ngirigacha, Western Kenya taro genotypes:4=Lukuywa 5=Ishwa 6=Shitao 7=KakamegT15 8=KakamegaT16; 9=KakamegaT17 10=Amagoro Teso 11=Eluhya 12=MumiasEN75 13=Enduma 14=Mumias T78 15=Mumias T79 23=Amagoro Busia 24=Kakamega T12, Nyanza Kenya taro genotypes: 17=Siaya 18=Kisii T81 19=Kisii T82; 20=Kisumu; 21=Lake Victoria T21 22=Lake VictoriaT22 25=Lake Victoria

Figure 1: Gel electrophoresis showing PCR amplified fragment using microsatellite primer designed for the SSR marker Xuqtem88 for Kenya taro germplasm collections

IV. CONCLUSIONS

Molecular markers have proven to be useful in assessing the genetic diversity taro crop. SSR Markers were able to detect high levels of allelic diversity among taro genotypes because they are efficient, cost-effective to use (Mace and Godwin, 2002). They are the most powerful tools for genetic diversity and genetic relationships assessment among species. Taro genotypes displayed a wider genetic diversity and richness among Western, Nyanza and Central Kenya taro accessions using molecular marked based SSR markers. Western and Nyanza Kenya taro genotypes stood significantly with the highest genetic diversity. The findings of genetic diversity and richness values of taro genotypes could be used as a springboard to improve heterosis that could help in taro productivity and improvement of genotypes by breeders.

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