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Genetic structure and diversity of East African taro [*Colocasia esculenta* (L.) Schott]

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Taro [*Colocasia esculenta* (L) Schott] is mainly produced in Africa by small holder farmers and plays an important role in the livelihood of millions of poor people in less developed countries. The genetic diversity of East African taro has not been determined. This study utilizes six microsatellite primers to analyze five populations of taro from three different regions of East Africa. Plant material consisted of 98 taro cultivars obtained from East Africa (Kenya, Tanzania and Uganda). Principal component analysis of microsatellite data indicated variation but did not show any distinct structure. Population diversity estimate was relatively low with the highest being 0.27, for accessions sourced from Lake Victoria basin. Analysis of molecular variance (AMOVA) revealed most variation among individuals within population at 79%. Nei's genetic distance showed that relatedness is not based on geographical proximity. Based on these findings, this study proposes establishment of a regional collection that will be conserved and ensure a broad genetic base for available varieties and enable development of improved varieties through breeding programmes.

Key words: Genetic diversity, simple sequence repeats (SSRs), taro.

INTRODUCTION

Taro, *Colocasia esculenta* (L.) Schott is a member of the plant family Araceae. It is one of the most important food crops worldwide. The family comprises at least 100 genera and more than 1500 species (Cho et al., 2007). Taro is a traditional root crop of the tropics grown for its edible corms and leaves, and is believed to be one of the earliest cultivated root crops in the world (Plucknett, 1976). Worldwide production is on the increase, with Food and Agriculture Organization (FAO) records indicating that taro production has doubled over the past decade (FAOSTAT, 2000), and it is now the fifth most-consumed root vegetable worldwide. Cultivated types are mostly diploid ($2n = 2x = 28$), although some triploids are also found ($2n = 3x = 42$) (Singh et al., 2007). Taro plays an important role in the livelihood of millions of relatively poor people in less developed countries. Taro leaves and corms are used for human food in most producing countries. Its peels and wastes are fed to domestic

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Table 1. SSR primers used in the analysis of taro (*colocasia esculenta*) collected from East Africa regions.

Primer	Repeat type	Sequence(5'-3')
Xqutem110	(TGA)6(TGGA)4	AGCCACGACACTCAACTATC GCCCAGTATATCTTGCATCTCC
Xqutem73	(CT)15	ATGCCAATGGAGGATGGCAG CGTCTAGCTTAGGACAACATGC
Xqutem55	(CAC)5	CTTTTGTGACATTTGTGGAGC CAATAATGGTGGTGGAAGTGG
Xqutem88	(CAT)9	CACACATACCCACATACACG CCAGGCTCTAATGATGATGATG
Xqutem97	(CA)8	GTAATCTATTCAACCCCCCTTC TCAACCTTCTCCATCAGTCC
Xqutem91	(TG)6(GA)4	GTCCAGTGTAGAGAAAAACCAG CACAACCAAACATACGGAAAC

livestock. Efforts have been made to produce silage from the large quantities of taro tops which are left after the corms are harvested (Kuruvilla and Singh, 1981). Taro corms are highly priced in urban markets hence generates extra income to the rural farmers while its trade provides employment for many people. The crop also maintains ground cover in the fields (Tumuhimbise et al., 2009). However, there is very limited local research on taro in East Africa and its actual contribution to food security and economy is underestimated. Also, its profile on the national research and conservation agenda is low. In Uganda for example, the average taro yields is less than 1 t ha^{-1}. The majority of smallholder produces annually (Tumuhimbise et al., 2009) as compared to the African and world average of 5.9 and 6.6 t ha^{-1}, respectively (FAO, 2008). It is possible to increase the status of taro in east Africa to the level of potato and sweet potato. This can be done by diversifying the taro germplam grown in East Africa including the dryland (unflooded) or upland taro varieties.

Morphological characters in tubers are highly variable among the genotypes. This high variability is a result of natural mutations for traits like root and skin color, leaf and vine characteristics (Karuri et al., 2009). However morphological variability may be as a result of environmental changes and therefore it is important to carry out a genetic diversity study using molecular markers. This study aims to quantify the level of genetic diversity and distribution of taro germplasm within the East African region SSR molecular markers. This information is crucial in formulating breeding programs aimed in improving the quality of taro germplasm in East Africa.

MATERIALS AND METHODS

Plant materials and DNA extraction

Plant materials consisted of taro cultivars obtained from Lake Victoria basin that covers three countries: Kenya, Tanzania and Uganda. Additional samples from Central and Eastern Kenya were incorporated in the study. The different cultivars were collected from farmers. Collections involved both principal botanical varieties of taro - *Colocasia esculenta* var. *esculenta*, commonly known as dasheen, and *Colocasia esculenta* var. *antiquorum*, commonly known as eddoe. The planting material was collected as apices, 1-2 cm of the corm with the basal 15-20 cm of the petioles attached; use of apices as planting material is particularly advantageous because it does not entail the utilization of much material that is otherwise edible. Moreover, apices establish very quickly and result in vigorous plants. The apices attached to part of the corms were planted in pots in the green house and appropriately labeled. The plants were watered every day to ensure growth, after a month, young shoots had already formed. DNA was extracted from young leaves according to the CTAB protocol modified and optimised by Sharma et al. (2008).

PCR amplification

The present study used six SSR primers (Table 1) that revealed high level of polymorphism in Polynesian taro cultivars (Mace and Godwin, 2002). The PCR mixture (10 µl) contained 1 µl of template DNA, 0.05 µl forward primer and 0.05 µl reverse primer, 0.05 µl of each dNTP (A, T, C, and G), 1 µl of reaction buffer that contained magnesium chloride and 0.25 µl *Taq* DNA polymerase. The PCR regime consisted of initial denaturation (94°C for 5 min), 35 cycles each consisting of 30 s denaturation (94°C), 1 min annealing temperature ranging from 55 to 59°C and 2 min elongation (72°C). Finally, an extension period of 10 min was included. A 10% native PAGE gel was used to resolve the SSR products as described by Andrus and Kuimelis (2001).

Data analysis

The number of individual bands generated by each primer was checked visually and scored as presence and absence matrix. The resulting presence/absence data matrix was analyzed using POPGENE v. 1.31 (Yeh and Yang., 1999), assuming Hardy-Weinberg equilibrium, to estimate three genetic diversity parameters: the percentage of polymorphic loci (PPL), Shannon's information index of diversity (*I*), Nei's gene diversity (*H*) and Nei's genetic distance (Nei and Li,1979). Analysis of molecular variance (AMOVA) was used to partition the total SSR variation into within-

Table 2. Mean diversity estimates (H) for 5 populations of *C. esculenta* generated from 6 SSR markers; N: sample size, H: Gene diversity and I Shannon Index.

Ecotype	N	*H*	I	Polymorphic loci (%)
Lake Victoria basin (Taro)	33	0.2783	0.4871	90.32
Lake Victoria basin (Tannia)	22	0.2478	0.4672	80.65
Central Kenya (Taro-Girigasha)	10	0.1774	0.3590	64.52
Central Kenya (Taro-Kigoi)	10	0.1261	0.2476	48.39
Eastern Kenya (Taro)	23	0.1863	0.3406	58.06

Table 3. Analysis of molecular variance (AMOVA) for SSR among *C. esculenta* sampled from Kenya, Uganda and Tanzania. Degrees of freedom (d.f) and significance (P) of the variance components, Sum of square (Ss), mean of square (Ms), and estimated variance (Est.Var) are shown.

Source of variation	df	Ss	Ms	Est.var	percentage	P-value
All population						
Among regions	2	61.083	30.541	0.357	6	0.01
Among population	4	39.088	19.544	0.824	14	0.01
Within population	93	422.676	4.545	4.545	79	0.01
Total	99	522.847		5.726	99	

population and between-population (Excoffierer et al., 2005). Principal coordinate analysis was done using GenAlEx 6.1 this is a multivariate technique that allows one to find and plot the major patterns within a multivariate data set (Peakall and Smouse, 2001).

RESULTS

In this study, six primers developed by Mace and Godwin (2002) were used and showed different levels of polymorphism. The six primers used differed in the ability to identify unique multiband phenotypes among the 98 accessions. In total, 31 alleles were amplified from six SSR primers across 98 accessions of which 85% were polymorphic.

No region specific markers were amplified. The gene diversity values (H) ranged from 0.2783 for Lake Victoria basin (taro) to 0.1863 for Eastern Kenya (taro) as shown in Table 2. The highest proportion of percentage polymorphic loci was found in taro from Lake Victoria basin while the lowest was found in Central Kenyan taro (Kigoi variety). Girigasha variety from Central Kenya had 64% of polymorphic loci while taro from Eastern Kenya had 58.06% polymorphic loci (Table 2). A general trend between the highest % polymorphic loci and highest gene diversity was observed. The standard error for all the population was generally high (± 0.1859) in taro from private alleles across the entire collection. Accessions of taro from Lake Victoria basin were the most distinguishable based on the SSR data set.

Genetic structure

In this study, there was no significant differentiation among the populations of *C. esculenta*, using SSR markers (AMOVA, P>0.01) (Table 3). The overall genetic variation (79%) was ascribed to differences between samples within varieties. The variation among regions was 6 and 14% among populations. A plot of the first two principal components of analysis of SSR variation represented an overall variation of 32% in the first axis and 19% in the second axis (Figure 1). There was no distinguishable pattern of clustering of accessions from a certain region. Taro-Kigoi samples from central Kenya revealed little variation in the first axis. Taro-Girigasha from Central Kenya was also in the first axis. Tannia and Taro from Lake Victoria basin showed high variation across the two axes, this was also true for the Eastern Kenya taro. Genetic distance based on Nei and Li (1979) is shown in Table 4.

DISCUSSION

An understanding of the extent and distribution of genetic variation within and among taro populations is essential for determining appropriate genetic management strategies for the species. The amount of molecular marker information has considerable impact on the results of studies of germplasm genetic relationships in crop (Cheng-lai et al., 2010). In this study, a total of 31 alleles were amplified with an average of 6.1 alleles per locus, this agrees with results obtained by Mace et al. (2006) who used similar primers to rationalize taro germplasm in the Pacific; in their study, a total of 38 alleles were amplified in 515 accessions of taro. No allele was found to be specific for any population.

Average genetic diversity (H) for all the taro populations assessed was 0.20318 with the highest being taro and Tannia from Lake Victoria basin populations (H=0.2783

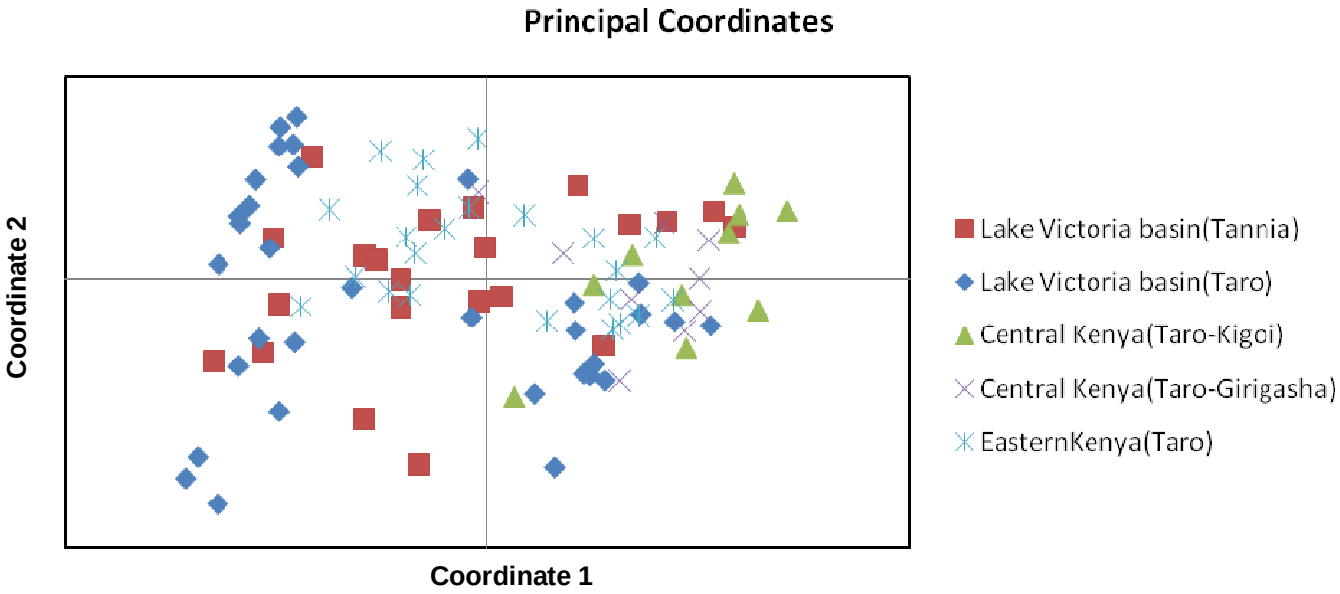

Figure 1. Distribution of 98 accessions on the first and second coordinates of PCA performed with six markers. The accessions are represented according to their geographical region.

Table 4. Matrix of unbiased genetic distance according to Nei (1978) among 5 populations of *C. esculenta* based on 6 SSR markers; where, 1- Population from Lake Victoria basin (Taro), 2- Population from Lake Victoria basin (Taro-tannia), 3- Population from Central Kenya (Taro-girigasha), 4- Population from Central Kenya (Taro-Kigoi) and 5- Population from Eastern Kenya (Taro).

Pop 1D	1	2	3	4	5
1					
2	0.0766				
3	0.2733	0.1931			
4	0.1597	0.128	0.1265		
5	0.0876	0.0371	0.1753	0.0894	

and H=0.2478, respectively). This is low as compared to genetic diversity of sweet potato in Kenya whose average was 0.75 with the lowest being 0.21 (Karuri et al., 2009). In the genetic diversity of cassava in the great lakes region, value of H was above 0.5 which was considered sufficient for a conservation program (Pariyo et al., 2009; Tumwegamire et al., 2011). The highest genetic diversity was in Lake Victoria basin, this could be attributed to the fact that the Lake Victoria basin covers three countries namely: Kenya, Uganda and Tanzania. The three countries are separated by a lake, this feature is a probable reason as the individual countries germplasm is not exchanged among farmers in the three countries.

Taro from central Kenya harbored low genetic diversity; girigasha (H=0.1774) and Kigoi (H=0.1261). These two populations consist of two different varieties. None of the accessions from Kigoi overlap with cultivars from girigasha, this is clearly shown by the PCA analysis. The low genetic diversity in the central region is attributed to clonal propagation indicating the use of planting material from a common source. It is worth noting that clonal plants have lower genetic diversity than non-clonal plants (Harper, 1977). This phenomenon was reported by Fajardo et al. (2002) and Gichuki et al. (2003), who also studied genetic diversity in the clonally propagated sweet potato. Despite these findings, Pujol et al. (2005) while studying genetic diversity of cassava emphasizes the incorporation of volunteer seedlings, of predominantly vegetatively propagated crops, by traditional farmers as an important mechanism for increasing genetic variability and a potential avenue for avoiding genetic erosion. Diversity in clonally propagated crops is attributed to accumulation of random mutations resulting via stem cuttings and adventitious buds arising from storage (Karuri

et al., 2009). Low levels of genetic diversity are detrimental to populations as they lead to inbreeding depression. However, they can be of interest in evolutionary studies as they may indicate ongoing evolution and speciation (Shepherd, 1999). The results signify the importance of assessing populations for variability for conservation purposes.

Results from AMOVA analysis showed low genetic variation among populations but high genetic variation within population. This agrees well with report of Kreike et al. (2004) and Mace et al. (2006). This may be attributed to the limited number of taro accessions introduced to populations. The accessions collected from the three countries were grouped into populations across the regions, namely: Lake Victoria basin (which covers Kenya, Uganda and Tanzania) Central Kenya and Eastern Kenya. In general, no population was found to be unique and some varieties had duplicates in the collection.

From the principle component analysis, a few accessions from the Lake Victoria basin were distinct and formed one cluster displaying divergence from other regions. Varieties from Eastern region have a higher similarity with the tannia varieties from the Lake Victoria basin as compared to varieties from central region implying that planting material must have been sourced from the same area. The genetic distances between taro from Lake Victoria basin and taro-girigasha from central Kenya was the highest (0.2733) as shown in Table 4. The smallest genetic distance was between tannia from Lake Victoria basin and taro from Eastern Kenya (0.0371) as shown in Table 4.

Overall results indicated that accessions did not cluster as dasheens or eddoes and neither did they cluster according to geographical region and cultivars known by the popular names did not always cluster together. This indicates diversity within the variety or misidentification. This study has contributed critical knowledge about the distribution of genetic variation within and among the taro germplasm collections of the East African regions. There is a narrow genetic base among and within taro cultivars in the East African taro and only the accessions from Lake Victoria basin analyzed have sufficient genetic diversity that can be used to enhance breeding and conservation.

This agrees with a study conducted by Elameen et al. (2008) on sweet potato varieties who found that only few of the populations studied had sufficient diversity. Lack of genetic diversity implies that the crop may be susceptible to pest and diseases as there is no enough material to evaluate superior varieties and thus leading to enormous loss of crop. This was the case of Samoan Archipelago in 1993, seriously damaging the crop. The severity of this epidemic was mainly due to extensive plantings of the same susceptible taro cultivar. A USDA program, Agricultural Development in the American Pacific (ADAP), financed from 1994 to 1996 the collection, evaluation and distribution of leaf blight resistant taro to American Samoa. Taro production quickly recovered, but almost 10 years later, the number of different varieties remained (TaroGen, 2000).

Conclusion

This study will further help to support the use of molecular markers for the successful development of a core collection of taro germplasm for the East African region. This study provided insights into the genetic composition of the taro crop in East African regions with Lake Victoria basin hosting the greatest diversity and Central Kenya having the lowest diversity.

Partitioning of genetic variation of the populations indicated that the species is characterized by high within population genetic diversity. The study has also demonstrated the usefulness of SSR markers in genetic diversity analysis of clonally propagated crop. SSR results showed that the sampled populations of *C. esculenta* are not significantly different. Despite concerns of genetic erosion due to clonal propagation, the accessions obtained from the same variety harbored some genetic differences. Partitioning of genetic variation of the populations indicated that the species is characterized by high within population genetic diversity.

Conflict of Interests

The author(s) declare no conflict of interests.

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REFERENCES

Andrus A, Kuimelis R (2001). Polyacrylamide gel electrophoresis (PAGE) of nucleic acids. Curr Protoc Nucleic Acid Chem.10: 4.

Cheng-lai W, Sheng-fu L, Bing-xue D, Qian-qian Z, Chun-qing Z (2010). Determination of the number of SSR alleles necessary for the analysis of genetic relationships between maize inbred lines. Agric. Sci. in China. 9(12):1713-1725.

Cho JJ, Yamakawa RA, Hol J (2007). Hawaiian kalo, past and future. College of Tropical Agriculture and Human Resources (CTAHR).

Elameen A, Siri F, Arild L, Odd A, Rognli LS, Susan M, et al. (2008). Analysis of genetic diversity in a sweet potato (*Ipomoea batatas* L.) germplasm collection from Tanzania as revealed by AFLP. Genet. Resour. Crop Evol. 50 (55):397-408.

Excofferier L, Smouse P, Quattro M. (2005). Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondria DNA restriction data. Genetics 131:479-491.

Fajardo DD, La Bonte DR, Jarret RL. (2002). Identifying and selecting genetic diversity in Papua New Guinea sweet Ipomea batatas (L.)Lam.Germplasm collected as botanical seed. Genet. Resour. Crop Evol. 49:463-470.

FAOSTAT (2000). FAO statistical database: agricultural production of primary crops.

FAO (2008). Climate change adaptation and mitigation: challenges and opportunities for food security. Information document prepared for the High-level Conference on World Food Security: the Challenges of Climate Change and Bioenergy, Rome, 3-5 June 2008 (available at ftp://ftp.fao.org/docrep/fao/ meeting/013/k2545e.pdf).

Gichuki ST, Berenyi M, Zhang D P, Hermann M, Schmidt J, Glossl J, et al. (2003). Genetic diversity in sweet potato (Ipomoea batatas (L.) in relationship to geographic sources as assessed with RAPD markers. Genet. Resour. Crop Evol. 50:429-437.

Harper JL (1977). Population biology of plants. Academic Press, London.

Karuri HW, Ateka EM, Amata R, Nyende AB, Muigai AWT, Mwasame E, et al. (2009). Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. Int. J. Agric. Biol. 12:33-38

Kreike CM, Van Eck HJ, Lebot V (2004). Genetic diversity of taro, *Colocasia esculenta* (L.) Schott, in Southeast Asia and the Pacific. Theor. Appl. Genet. 109:761-768.

Kuruvilla KM, Singh A (1981). Karyotypic and electrophoretic studies on taro and its origins. Euphytic. 30:405-412.

Mace ES, Godwin ID. (2002). Development and characterization of polymorphic microsatellite markers in taro, *Colocasia esculenta* (L.) Schott. Genome 45:823-832.

Mace ES, Mathur PN, Izquierdo L, Hunter D, Taylor MB, Singh D, et al. (2006). Rationalization of taro germplasm collections in the pacific island region using simple sequence (SS) markers. Plant Genet. Resour. 4:210-220.

Nei M, Li WH. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. National Academic Science USA. 76(5269-5273).

Pariyo A, Baguma YK, Kawuki RS, Omongo CA, Alicai T, Edema R, et al. (2009). Genetic diversity of cassava landraces in the great lakes region of Africa assessed using SSR markers. Centro Internacional de Agricultura Tropical (CIAT), Cali, CO. 1.

Peakall R, Smouse P. (2001). GENALEX 5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. The Australian National University, Canberra, Australia.

Plucknett DL (1976). Edible aroids: *Alocasia, Colocasia, Cyrtosperma, Xanthosoma*. In: Simmonds NW (ed.). Evol. Crop Plants, London: Longman. 10-12.

Pujol B, Gigot G, Laurent G, Pinheiro-Kluppel M, Elias M, McKey HM, McKey D (2005). Germination ecology of cassava (*Manihot esculenta* Crantz, Euphorbiaceae) in traditional agroecosystems: Seed and seedling biology of a vegetatively propagated domesticated plant. Econ Bot. 56:366-379.

Sharma K, Mishra AK, Misra, RS (2008). Identification and characterization of differentially expressed genes in the resistance reaction in taro infected with *P. colocasiae*. Molecular Biology Reporter, 93.

Singh D, Mace ES, Godwin ID, Mathur PN, Okpul T, Taylor, M, et al. (2007). Assessment and rationalization of genetic diversity of Papua New Guinea taro (*Colocasia esculenta*) using SSR DNA fingerprinting. Genet. Resour. Crop Evol. 55(6):811-822.

TaroGen (2000). Taro Genetic Resources: Conservation and Utilisation Project.Annual Report 1999/2000. Secretariat of the Pacific Community, Noumea, New Caledonia. Tumuhimbise R, Talwana HL, Osiru, DSO., Serem AK, Ndabikunze, B. K., Nandi , J. O. M., et al. (2009). Growth and development of wetland-grown taro under different plant populations and seed bed types in Uganda. Afr. Crop Sci. J. 17:49-60.

Tumwegamire, S., Rubaihayo, P. R., LaBonte, D. R., Diaz, F., Kapinga, R., Mwanga, R. O. M. (2011). Genetic diversity in white- and orange-fleshed sweetpotato farmer varieties from East Africa evaluated by simple sequence repeat markers. Crop Science, 51(3), 1132-1142.

Yeh FC, Yang R. (1999). Microsoft window based freeware for population genetic analysis (POPGENE version 1.31). University of Alberta (Canada).