



Molecular Characterisation of Traditional Cashew (*Anacardium occidentale* L.) Populations from Nachingwea and Newala Districts in Tanzania

Hussein R. Chiumbi¹ and Hashim M. Mangosongo²

¹Dar es Salaam University College of Education (DUCE), Tanzania.

²Department of Botany, University of Dar es Salaam, Tanzania.

Corresponding author's postal address: P.O. Box 2329, Dar es Salaam, Tanzania.

Email: hchiumbi@gmail.com

Received 18 April 2023, Revised 10 Dec 2023, Accepted 9 Jan 2024, Published Jan 2024

<https://dx.doi.org/10.4314/tjs.v49i5.4>

Abstract

This study assessed the genetic diversity of 120 selected cashew plants from Nachingwea and Newala traditional cashew populations, comprising of 60 cashew plants from each population. Eight Simple Sequence Repeats (SSR) makers were used to ascertain the extent of genetic variation within and between the two traditional cashew populations. Genomic DNA extraction and Polymerase Chain Reaction (PCR) were performed using standard protocols. The results showed that all SSR markers were polymorphic. Average Polymorphic Information Content (PIC), heterozygosity, number of alleles per locus, allelic frequency and Shannon's Index were 0.540, 0.721, 3.391, 0.666 and 0.649, respectively. Analysis of molecular variance results showed that genetic variation within populations was 88% while genetic variation between populations was 12%, implying gene flow between the populations. The results further showed that the two cashew populations are genetically diverse and that Nachingwea traditional cashew population was genetically more diverse than Newala population. High genetic variation observed in the cashew populations shows that they are suitable for use in cashew breeding programmes. Thus, conservation of traditional cashew populations is recommended.

Key Words: SSR marker, Genetic diversity, *Anacardium occidentale*, Polymorphism

Introduction

The cashew (*Anacardium occidentale* L.) is a plant that belongs to a genus *Anacardium* and the family Anacardiaceae, which also includes the mango, pepper, pistachio, poison oak and poison ivy (Aliyu 2012). It is an evergreen, broad leafed, perennial plant that prefers poor soils that are well drained with acidic pH, ranging from 4.5 to 6.5. It grows well in areas with elevations below 2000 m above the sea level, temperature ranging from 15 °C to 35 °C and an average annual rainfall of about 400 mm (Kapinga et al. 2017). These plants reproduce mainly through cross-pollination by insect pollinators such as honey bees. Cashew plants are distributed in

various countries in the world, particularly in the tropical and subtropical countries such as Benin, Ivory Coast, Nigeria, Malawi, Mozambique, Kenya, India and Vietnam (Sika et al. 2015b). In Tanzania, commercial cultivation of traditional cashew started in 1953 in Lindi, Mtwara and Ruvuma regions by household farmers. Currently, some areas are growing both improved and traditional cashew. However, the traditional cashew population is cultivated in large scale in Lindi and Mtwara regions, particularly in Nachingwea and Newala districts respectively. Molecular characterisation of the traditional cashew populations from the two districts would help to provide

information on genetic diversity of the population which is needed for not only cashew breeding, but also for conservation of the species germplasm.

Genetic diversity refers to variation in genetic makeup among individuals of the same species, population or community (Charlotte 2022). The genetic diversity of plant species is caused by many factors, including mutation, gene flow, genetic drift and recombination of genes (Charlotte 2022). Environmental factors, particularly climate, altitude and soil physicochemical properties such as soil pH and nutrient content also cause genetic diversity (Balogoun et al. 2016). Genetic diversity can be assessed using genetic markers, such as molecular markers, biochemical markers and morphological markers (Sethi et al. 2016). Examples of molecular markers include Simple Sequence Repeats (SSR), Inter-Simple Sequence Repeats (ISRR) and RAPID. The SSR markers are useful for evaluation of the genetic diversity of cashew plant populations (Ahmad et al. 2012) and form a strong theoretical inference for developing strategies for conservation and breeding (Dar et al. 2019). The genetic variation in a species or a population is commonly assessed based on the genetic diversity indices such as percentage polymorphic loci, polymorphic information content (PIC), heterozygosity, allele frequency, Shannon's information Index (I) and number of effective alleles (Sika et al. 2015b, Mangosongo et al. 2020).

The detailed understanding or knowledge on genetic diversity of traditional cashew population is important for improving crop yield (Pullaiah 2015), future breeding

programmes and conservation of cashew germplasm (Bhadra et al. 2019). Before the present study, no comprehensive study had been conducted to assess the genetic diversity of traditional cashew populations in the study area. Therefore, this study was intended to assess genetic diversity of traditional cashew populations from Nachingwea and Newala using SSR markers. These molecular markers were preferred in this study due to their loci specificity (Sika et al. 2015b), high reproducibility, codominance nature (Kouakou et al. 2020) and high polymorphic information content (PIC).

Material and Methods

The study area

This study was conducted in two districts, namely; Nachingwea district and Newala district located in Lindi and Mtwara regions respectively. The two study sites are about 114 kilometres apart. Nachingwea district is actually located between latitude 10° and 11° south of Equator and longitude 38° and 39° east of Greenwich Meridian (Nachingwea District Council [NDC] 2015). Newala district is located between latitude 10° and 11° south of Equator, and longitude 39° and 40° east of Greenwich Meridian (Newala Town Council [NTC] 2019). The two study areas differ slightly in terms of environmental factors especially elevation, climate (temperature and rainfall) and soil physicochemical factors. Based on altitude, Nachingwea district is located in the low land while Newala district is located in higher landscape of the Makonde plateau. The location of the study sites is shown in Figure 1.



Figure 1: A map of Tanzania showing location of the study sites (Modified from tawasenet.or.tz)

Sampling method

A total of 120 cashew individual plants were randomly selected from the two traditional cashew populations, comprising of 60 individuals from each population. In each study site, samples were collected from four different points, which were located at a distance not exceeding 10 km apart. A maximum distance of 10 km was maintained between the sampling points in order to allow the gene flow between individuals from two or more points that might be caused by insect

pollinators, which are reported to travel a distance not exceeding 13.5 kilometres (Beekman and Ratnieks 2000). The first cashew tree at each sampling point was randomly selected and other 14 plants were systematically selected according to Chipojola et al. (2009). A diagonal distance of 10 m was maintained between the sampled trees according to Sika et al. (2015a). The diagonal crossing sampling technique is shown in Figure 2.

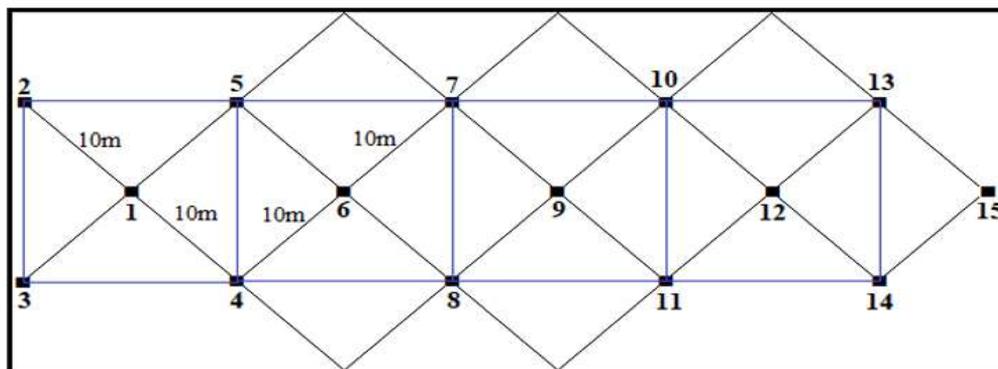


Figure 2: A diagonal-crossing sampling technique

Leaf sample collection

Three young leaves were randomly selected and collected from each of the 120 traditional cashew trees. The collected leaf samples were preserved in zip-locked plastic bags containing silica gel and properly labelled using a permanent marker pen. The leaf samples were transported to the molecular laboratory housed in the Department of Botany of the University of Dar es salaam for DNA extraction and PCR analysis.

DNA Extraction

DNA was extracted according to standard protocol using Quick-DNA™ Plant/Seed Miniprep Kit developed by Zymo Research Corporation (SA) without modification. The DNA pellets were air-dried for about 10 minutes by inversion of the microcentrifuge tube on the clean tissue paper for quick absorption of alcohol used for washing. The DNA was dissolved in 50 µl double distilled water and stored at -20 °C for future use.

PCR Analysis

Polymerase Chain Reaction (PCR) analysis was carried out using standard method, according to Sika et al. (2015b) with some minor modifications. The total reaction volume of PCR mixture was 25 µl, which contained 12.5 µl of master mix [AccuStart II PCR ToughMix (2×)], 2 µl template DNA, 1 µl forward primer, 1 µl reverse primer and 8.5 µl nuclease free water. The PCR reaction was performed in a programmable thermal cycler (Q cycler II - Quanta Biotech, UK). The content was initially denatured at 95 °C for 2 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing for 45 seconds at gradient temperatures of 56.1 °C, 58.2 °C, and 60.3 °C, followed by extension at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes. The forward sequence, reverse sequence and number of bases of the primers used in this study are shown in Table 1.

Table 1: SSR markers used for molecular characterisation of traditional cashew populations

SN	SSR Marker	Forward sequence	Reverse sequence
1	mAoR3	CAGAACCGTCACTCCACTCC	ATCCAGACGAAGAAGCGATG
2	mAoR6	CAAAACTAGCCGGAATCTAGC	CCCCATCAAACCCTTATGAC
3	mAoR7	AACCTTCACTCCTCTGAAGC	GTGAATCCAAAGCGTGTG
4	mAoR11	ATCCAACAGCCACAATCCTC	CTTACAGCCCCAAACTCTCG
5	mAoR17	GCAATGTGCAGACATGGTTC	GGTTTCGCATGGAAGAAGAG
6	mAoR42	ACTGTCACGTCAATGGCATC	GCGAAGGTCAAAGAGCAGTC
7	mAoR48	CAGCGAGTGGCTTACGAAAT	GACCATGGGCTTGATACGTC
8	mAoR52	GCTATGACCCTTGGGAACTC	GTGACACAACCAAAACCACA

Gel electrophoresis of the PCR products

The PCR products were run on 1.5% agarose gel stained with GR-Green dye for observation of the polymorphic bands formed by every sample. The GR-Green dye was used because it induces clear visibility of the bands when observed under UV-trans illuminator. The DNA samples were loaded in the electrophoresis machine using Purple loading dye (Thermo scientific, UK). The electrophoresis was run for 45 minutes at 120 V in electrophoresis machine (NanoPAC-300P, Cleaver Scientific LTD, UK) in order to allow enough time for band formation. A DNA ladder (O'geneRuler 100 bp Plus, Ready-to-use) served as a standard marker in order to determine the amplification sites and measure the size (length) of the bands in base pairs. The polymorphic bands were manually scored whereby presence was rated 1 and absence was rated 0. The bands that appeared in all individuals were treated as monomorphic bands while the bands that appeared in one or more individuals but not in all were treated as polymorphic bands (Bhadra et al. 2019).

Data analysis

Genetic diversity of the traditional cashew populations was determined based on the indices of genetic diversity generated using GenA1Ex software package, version 6.51 (Peakall and Smouse 2012). The genetic

diversity indices included Polymorphic Information Content (PIC), number of alleles per locus (Na), number of effective alleles per locus (Ae), observed heterozygosity (Ho), expected heterozygosity (He), allele frequency (Af) and Shannon's information index (H). Analysis of Molecular Variance (AMOVA) was performed to ascertain the extent of genetic variation between and within traditional cashew populations. Principal Component Analysis (PCA) was performed using PAST software package (version 4.03) to determine the population structure and distribution within the traditional cashew populations based on the genetic similarity or dissimilarity.

Results

SSR markers' amplification

The amplification results showed that all 8 SSR markers generated polymorphic bands with all 120 DNA samples. The bands produced by the eight primers varied in size, ranging from 150 bp to 600 bp. Primer P2 produced smallest band of 150 bp while the primer P7 produced largest band of 600 bp. Moreover, Primer P7 amplified twice, producing two bands one with 390 bp and another one with 600 bp. The SSR marker profiles for eight representative DNA samples using all eight primers are shown in Plate 1.

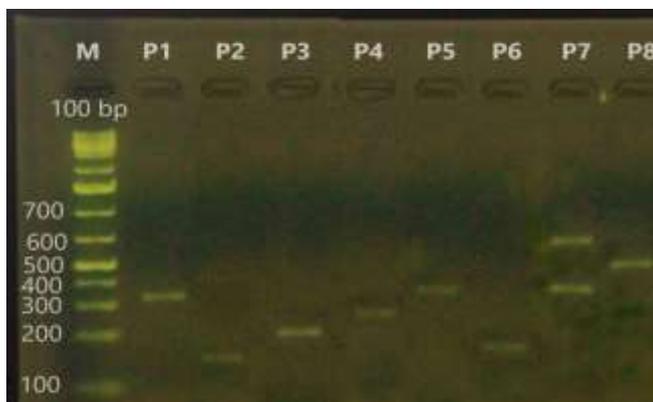


Plate 1: SSR marker profiles for DNA polymorphic bands produced by eight representative samples of traditional cashew using eight primers.

Legend: P1 = primer 1 (mAoR3) amplification in NA17, P2 = primer 2 (mAoR6) amplification in NA32, P3 = Primer 3 (mAoR7) amplification in NA51, P4 = primer 4 (mAoR11) amplification in NA59, P5 = primer 5 (mAoR17) amplification in NE05, P6 = primer 6 (mAoR42) amplification in NE25, P7 = primer 7 (mAoR48) amplification in NE36 and P8 = primer 8 (mAoR52) amplification in NE60, NA = individual from Nachingwea cashew population, and NE = individual from Newala cashew population.

Polymorphism and SSR markers informativeness

The present study revealed that all eight SSR markers were highly informative as demonstrated by high levels of polymorphism. The PIC value of the samples from the two traditional cashew populations ranged from 0.292 (mAoR6) to 0.743 (mAoR11). Other markers mAoR42, mAoR7, mAoR17, mAoR3, mAoR52 and mAoR48 detected average PIC values of 0.499, 0.450, 0.505, 0.569, 0.571 and 0.689, respectively.

cashew population has high genetic diversity as represented by high levels of genetic diversity indices. Shannon’s Information Index ranged from 0.570 to 0.731. Observed heterozygosity ranged from 0.284 to 0.491, while expected heterozygosity ranged from 0.543 to 0.876. The number of alleles per locus ranged from 2.500 to 4.625 and the effective number of alleles ranged from 2.186 to 8.086. The allele frequency per locus ranged from 0.488 to 0.902. The genetic diversity indices of the traditional cashew samples from Nachingwea generated by eight markers are shown in Table 2.

Genetic diversity of Nachingwea traditional cashew population based on various indices of diversity

The results on genetic diversity based on various indices showed that the Nachingwea

Table 2: The genetic diversity indices of Nachingwea traditional cashew population detected by the eight SSR markers

Marker	Genetic diversity indices						
	Ho	He	Na	Ae	Af	H	PIC
mAoR3	0.345	0.728	3.125	3.678	0.610	0.570	0.628
mAoR6	0.491	0.543	4.625	2.186	0.902	0.661	0.186
mAoR7	0.394	0.700	3.625	3.329	0.707	0.662	0.500
mAoR11	0.284	0.876	2.500	8.082	0.488	0.678	0.762
mAoR17	0.394	0.747	3.625	3.955	0.707	0.731	0.500
mAoR42	0.357	0.794	3.250	4.858	0.634	0.721	0.598
mAoR48	0.296	0.861	2.625	7.215	0.512	0.683	0.738
mAoR52	0.357	0.769	3.250	4.332	0.634	0.680	0.598
Average	0.365	0.752	3.328	4.704	0.649	0.673	0.564

Legend: Ho = Observed heterozygosity, He = Expected heterozygosity, Na = Number of alleles per locus, Ae = Effective number of alleles per locus, Af = Allele frequency, H = Shannon’s information index and PIC = Polymorphic Information Content.

Genetic diversity of Newala traditional cashew population based on various indices of diversity

The results on genetic diversity based on various indices revealed that the Newala cashew population had high genetic diversity

as represented by high levels of genetic diversity indices. Observed heterozygosity ranged from 0.263 to 0.388 while the expected heterozygosity ranged from 0.534 to 0.809. The allele frequency was between 0.525 and 0.775. The number of alleles per

locus ranged from 3.000 to 3.875, whereas the effective number of alleles per locus ranged from 2.148 to 5.246. Shannon's Information Index ranged from 0.496 to

0.706. The genetic diversity indices of all markers are shown in table 3.

Table 3: The genetic diversity indices of Newala traditional cashew population detected by the eight SSR markers

Marker	Genetic diversity indices						
	Ho	He	Na	Ae	Af	H	PIC
mAoR3	0.350	0.675	3.500	3.077	0.700	0.613	0.510
mAorR6	0.388	0.534	3.875	2.148	0.775	0.496	0.399
mAoR7	0.388	0.609	3.875	2.560	0.775	0.612	0.399
mAoR11	0.263	0.809	2.625	5.246	0.525	0.594	0.724
mAoR17	0.350	0.735	3.500	3.774	0.700	0.706	0.510
mAoR42	0.388	0.662	3.875	2.957	0.775	0.685	0.399
mAoR48	0.300	0.789	3.000	4.734	0.600	0.669	0.640
mAoR52	0.338	0.702	3.375	3.354	0.675	0.623	0.544
Average	0.345	0.689	3.453	3.481	0.691	0.625	0.516

Legend: Ho = Observed heterozygosity, He = Expected heterozygosity, Na = Number of available alleles per locus, Ae = Effective number of alleles per locus, Af = Allele frequency, H = Shannon's information index and PIC = Polymorphic Information Content.

Comparison of genetic diversity of the traditional cashew populations based on various genetic diversity indices

Comparative analysis of the two traditional cashew populations based on various measures (indices) of genetic diversity showed that Nachingwea traditional cashew

population had higher values than Newala population, implying that Nachingwea population is more diverse than Newala population. The genetic diversity indices of Nachingwea and Newala traditional cashew populations are shown in Figure 3.

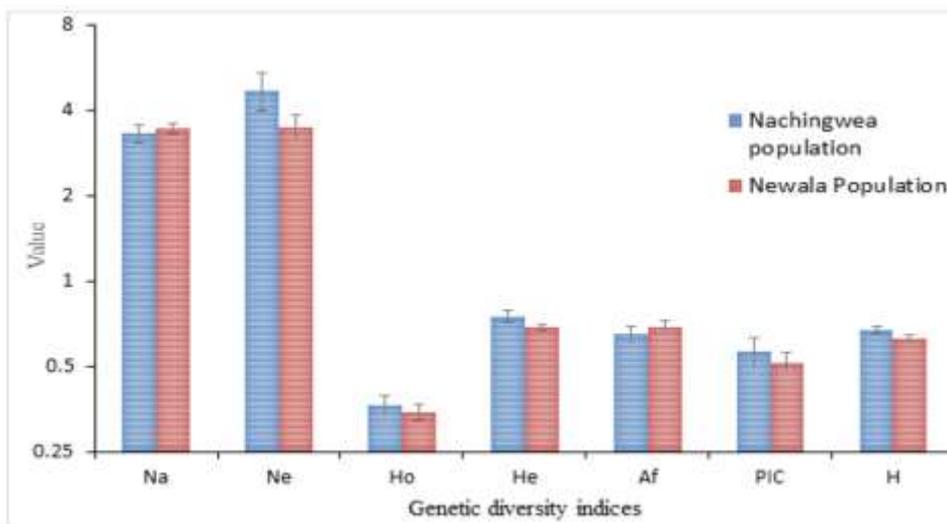


Figure 3: A bar chart showing comparison of the two traditional cashew populations based on different indices of genetic diversity

Analysis of Molecular Variance (AMOVA)

The results on Analysis of Molecular Variance (AMOVA) of the two traditional cashew populations (Nachingwea and Newala populations) based on the eight SSR

markers showed that the genetic variation within populations was 88% while genetic variation between populations was 12%, as shown in Figure 4.

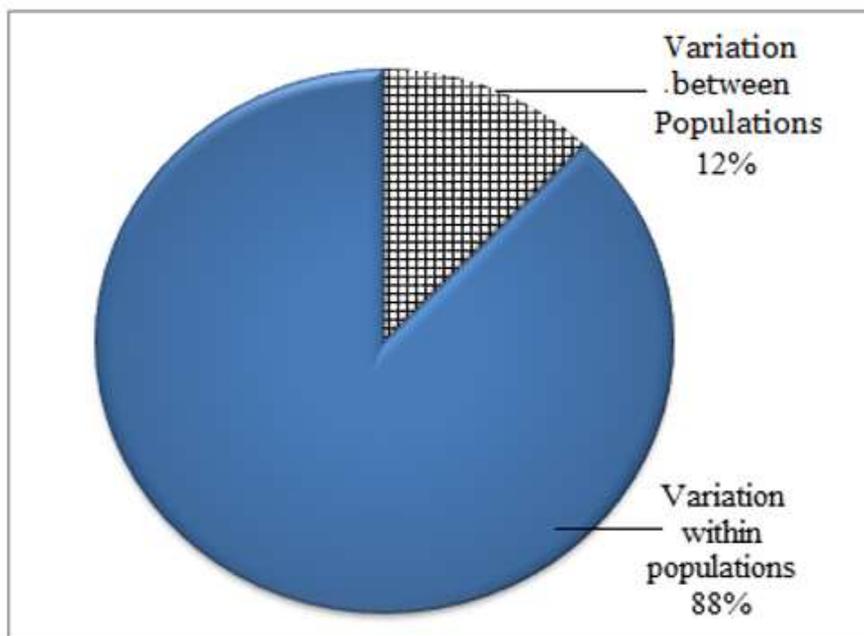


Figure 4: Percentages of molecular variance within and between the two traditional cashew populations

Principal Component Analysis (PCA) of the cashew populations

The principal component analysis results revealed that 100% of the variation in the traditional cashew population is explained by the eight Eigen vectors. The first five Eigen vectors accounted for 75.33% of the variation. The eight primers used in this study showed different levels of efficacy in

detecting genetic variation in the traditional cashew populations as depicted by the differences in lengths of the biplot lines. Clustering together of the individuals from Nachingwea and Newala traditional cashew populations was based on genetic similarity or dissimilarity, and not geographical origin, as shown in Figure 5.

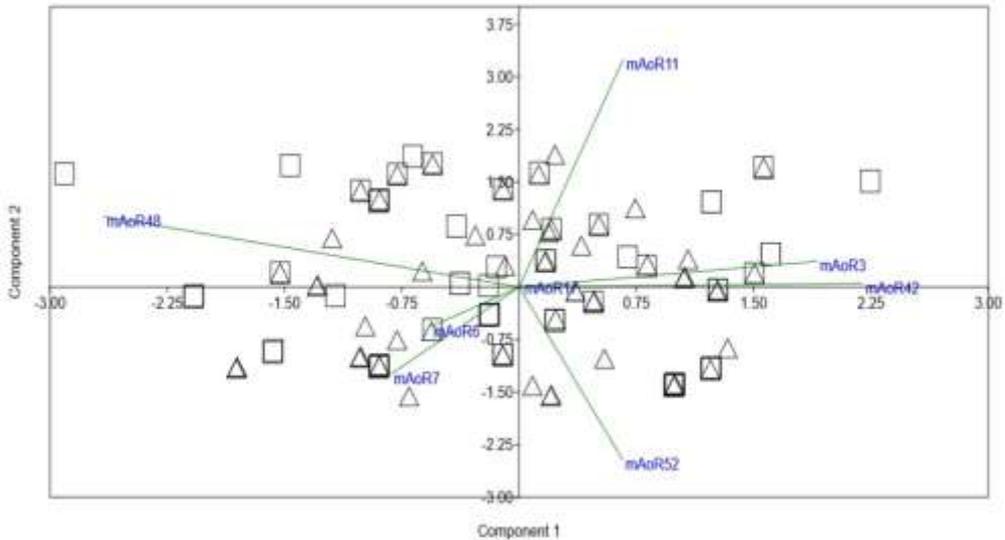


Figure 5: PCA plot showing the distribution of 120 sampled traditional cashew plants from Nachingwea and Newala cashew populations. Legend: □ = individual from Nachingwea cashew population, Δ = individual from Newala cashew population.

Discussion

In this study, molecular characterisation of the traditional cashew population was done using eight SSR markers. All the markers used in the present study generated polymorphic bands with all 120 cashew samples and the PIC of the markers used in the present study was 0.540. According to Dashab et al. (2011), a PIC value that is greater or equal to 0.5 denotes high genetic polymorphism of a marker. Thus, all the markers were very informative to detect genetic variations within and among traditional cashew populations (Kouakou et al. 2020). Aliyu (2012) showed that the markers which are more informative are efficient for molecular characterisation and management of cashew germplasm. Similar findings were reported by Moumouni et al. (2022) in the study on genetic variability of elite cashew trees in Burkina Faso, which reported a PIC value of 0.534.

In the present study, the genetic diversity was assessed based on various measures of genetic diversity heterozygosity, Shannon's information index, number of alleles per locus and allele frequency. Based on heterozygosity, the two traditional cashew populations were found to have high

heterozygosity (0.721), which might indicate high mutation rates occurring within the populations (Dasmohapatra et al. 2014) or probably because of cross pollination (Chipojola et al. 2009) and self-incompatibility in reproduction (Shen et al. 2017). On the other hand, Sika et al. (2015b) studied Benin cashew cultivars using SSR markers, and reported low heterozygosity (0.40), which was predicted to be contributed by the presence of a lower number of alleles in the population possibly due to small population size and inbreeding.

Based on the mean Shannon information index (H), this study revealed higher genetic variation within the two cashew populations probably caused by strong cross-pollination by insect pollinators (Vanitha and Raviprasad 2019). A study by Moumouni et al. (2022) who studied genetic diversity among elite cashew trees in western Burkina Faso reported a lower value of Shannon's Information Index which was 0.217 ± 0.074 . Sika et al. (2015b) studied the genetic diversity among Benin cashew cultivars and found Shannon's information Index of 0.04.

On the basis of number of alleles, the findings of the present study showed that the mean number of alleles per locus was $3.391 \pm$

0.198 and differed from the mean number of alleles of 3.56 ± 1.45 reported by Kouakou et al. (2020) who studied genetic diversity among cashew populations from Cote d'Ivoire. The low number of alleles observed in this study implies a close genetic relationship between the two traditional cashew populations under study (Savadi et al. 2020). According to the results on the allele frequency, the two traditional cashew populations had allele frequency of 0.666, which suggests high genetic variation within the cashew populations, possibly contributed by higher polymorphism of the SSR markers. Savadi et al. (2020) reported a relatively low heterozygosity of 0.630 in the study of genetic diversity of hybrid species of *Anacardium* in India using microsatellites.

In addition, AMOVA results showed that genetic variation within populations was 88% and variation between populations 12%. The lower genetic variation between Nachingwea and Newala populations implies that the two populations share some genetic characteristics. Usually, sharing of allele types between populations reduces variations (dos Santos et al 2019). On the other hand, higher genetic variation within the populations implies a strong cross-pollination taking place within the populations (Shen et al. 2017), possibly due to pollination by insects. The cross-pollination causes interchange of alleles between individuals within the population (dos Santos et al. 2019) and leads to new genetic recombination (Vanitha and Raviprasad 2019). The high genetic variation within cashew populations offers adaptive advantages to the environment (Adu-Gyamfi et al.2020). These findings of the present study differed from those reported by Kouakou et al. (2020) in the study of genetic diversity of four high-yielding cashew populations from Cote d'Ivoire in which the genetic variation within populations was 98.6% and the genetic variation among populations was 1.4%.

Based on the Principal Component Analysis (PCA) results, it was revealed that the individuals from the two traditional cashew populations clustered together irrespective of their geographical origin, indicating that they

are genetically similar. The similarity between the individuals from the two cashew populations might be caused by gene flow between the two cashew populations. According to Chipojola et al. (2009), other factors that contribute to genetic similarity in cashew populations include agronomic selection and ecogeography.

Moreover, when the two traditional cashew populations were compared based on various indices or measures of genetic diversity such as Polymorphic Information Content (PIC), heterozygosity (observed and expected heterozygosity), number of effective alleles and Shannon's information index showed that Nachingwea cashew population had higher levels of the genetic diversity than Newala cashew population. This observation implies that the Nachingwea cashew population was more diverse than the Newala cashew population. The observed difference in genetic diversity between the two cashew populations might be caused by environmental factors, such as climate, altitude, soil physicochemical factors (Balogoun et al. 2016) and spatial isolation (Kuss et al. 2008).

Conclusion

From the findings of this study, it can be concluded that the SSR markers used in this study were very informative and thus are suitable for studying the genetic diversity of cashew populations. The two traditional cashew populations studied have considerable level of genetic diversity and the extent of genetic variation was higher within the populations than between the populations. Although the two traditional cashew populations were genetically diverse, Nachingwea traditional cashew population was slightly more diverse than the Newala traditional cashew population. The two traditional cashew populations are potentially useful or important for cashew breeding programmes. Moreover, appropriate conservation measures for the traditional cashew germplasms from the two populations are recommended.

References

- Adu-Gyamfi PKK, Akperterey A, Barnnor M, Ofori A and Padi F 2020 Genotypic characterization of cashew (*Anacardium occidentale* L.) clones using agromorphological traits. *Plant-Environ. Interac.* 3:196-206.
- Ahmad S, Singh M, Lamb-Palmer ND, Lefsrud M and Singh J 2012 Assessment of genetic diversity in 35 *Pisum sativum* accessions using microsatellite markers. *Can. J. Plant Sci.* 92: 1075-1081.
- Aliyu OM 2012 Genetic Diversity of Nigerian Cashew Germplasm. In *Genetic diversity in Plants*. 6: 163-184. Research Gate.
- Balogoun I, Ahoton EL, Saidou A, Bello OD, Ezin V, Amadji GL, Ahohuendo BC, Babatounde S, Chougourou DC and Ahanchede A. 2016 Effect of climatic factors on cashew (*Anacardium occidentale* L.) productivity in Benin (West Africa). *J. Earth Sci. Clim. Change* 7:329
- Beekman M and Ratnieks FLW 2000 Long-range foraging by the honey bee, *Apis mellifera* L. In functional ecology. 14: 490-496. British Ecological Society.
- Bhadra T, Obaidullah AZM, Sultana S, Ahmed M and Islam MM 2019 Genetic analysis in cashew (*Anacardium occidentale* L.) germplasm using RAPD marker. *J. Bangladesh Agril. Univ* 17(4): 461-465.
- Charlotte C. 2022 Genetic diversity. http://www.coastalwiki.org/wiki/Genetic_diversity. Visited on 4th March 2022.
- Chipojola FM, Mwase WF, Kwapata MB, Bokosi JM, Njoloma JP and Maliro MF 2009 Morphological characterization of cashew (*Anacardium occidentale*) in four populations in Malawi. *Afri. J. Biotechnol.* 8(20): 5173-5181.
- Dar AA, Mahajan R and Sharma S 2019 Molecular markers for characterization and conservation of plant genetic resources. *Indian J. Agr Sci.* 89(11): 1755-1763.
- Dashab GR, Aslaminejad A, Nassiri M, Esmailzadeh AK and Saghi DA 2011 Analysis of Genetic diversity and structure of Baluchi sheep by microsatellite markers. *Trop. Subtrop. Agroecosystems* 14: 1047-1054.
- Dasmohapatra R, Rath S, Pradhan B, and Rout RS 2014 Molecular and agromorphological assessment of cashew (*Anacardium occidentale*) genotypes of India. *J. Appl. Hortic.* 16 (3): 215-221.
- dos Santos JO, Mayo SJ, Bittencourt CB and de Andrade IM 2019 Genetic diversity in wild populations of the restinga ecotype of the cashew (*Anacardium occidentale*) in coastal Piauí, Brazil.
- Kapinga FA, Kasuga LJJ and Kafiriti EM 2017 Growth and Production of Cashewnut. In soils, plant growth and crop production. *Encyclopedia of Life Support Systems (EOLSS)*.
- Kouakou CK, Adopo AN, Djaha AJ, N'da DP, N'da HA, Zoro Bi IR, Koffi KK, Djidji H, Minhobo MY, Dosso M and N'Guessan AE 2020 Genetic characterization of promising high-yielding cashew (*Anacardium occidentale* L.) cultivars from Cote d'Ivoire. *Biotechnol. Agron. Soc. Environ.* 24(1): 46-58.
- Kuss P, Pluess AR, Aegisdottir HH and Stocklin J 2008 Spatial isolation and genetic differentiation in naturally fragmented plant populations of the Swiss Alps. *J. Plant Ecol.* 1(3): 149-159.
- Mangosongo HM, Mneney EE, Harvey J and Kyallo M 2020 Assessment of genetic diversity of the wild rice (*Oryza longistaminata*) populations in Tanzania using microsatellite markers. *Tanz. J Sci.* 46(3): 828-839.
- Moumouni K, Vianney TW, Larbouga B, and Issa W 2022 Genetic diversity assessment among 18 elite cashew tree genotypes (*Anacardium occidentale*) selected in western Burkina Faso. *J. Plant Breed. Crop Sci.* 14(1): 1-11.
- NDC 2015 Medium term rolling strategic plan for the years 2016/2017 - 2020/2021. Nachingwea District Executive Director.
- NTC 2019 Medium term strategic plan 2015/2016-2020/2021. Council

- Management Team, Newala Town Council.
- Peakall R and Smouse PE 2012 GenAlEx 6.5: Genetic Analysis in Excel. Population genetic software for teaching and research - an update. *J. Bioinform.* 28(19): 2537-2539.
- Pullaiah T, Bahadur B, and Krishnamurthy KV 2015 (eds.) Plant biology and Biotechnology. *Plant Diver. Org. Func. Improv.* 1(177): 22-28.
- Savadi S, Sowmya K, Megha VS, MuralidharaS BM and Mohana GS 2020 Genetic diversity and identification of interspecific hybrids of *Anacardium* species using microsatellites. *Braz. J. Bot.* 44: 139-148.
- Sethi K, Lenka PC, Tripathy SK and Mohanty P 2016 Assessment of molecular genetic diversity and identification of elite cashew hybrids. *Inter. Jour. Hort.* 6(2): 1-10.
- Shen Z, Zhang K, Ma L, Duan J and Ao Y 2017 Analysis of genetic relationships and diversity among 11 populations of *Xanthoceras sorbifolia* using phenotypic and microsatellite marker data. *Electron. J. Biotechnol.* 26: 33-39.
- Sika CK, Adoukonou-Sagbadja H, Photon LE, Adebo I, Adigoun FA, Saidou A, Ahanchede A, Kotchoni SO, and Baba-Moussa L 2015a Morphological characterization and agronomical performances of cashew (*Anacardium occidentale* L.) accession from Benin. *J. Hortic.* 3(2): 27-40.
- Sika KC, Adoukonou-Sagbadja H, Ahoton L, Saidou A, Ahanchede A, Kefela T, Gachomo EW, Baba-Moussa L and Kotchoni SO 2015b Genetic characterization of cashew (*Anacardium occidentale* L.) cultivars from Benin. *J. Hortic.* 2(3): 153-159.
- Tanzania Water and Sanitation Network (TAWASANET) Southern zone map. Retrieved from <https://www.tawasanet.or.tz>
- Vanitha K and Raviprasad TN 2019 Diversity, species richness and foraging behaviour of pollinators in cashew. *J. Agric. Res.* 8(2):197-206.