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Genotypic Characterization of *Catha edulis* in Mt. Kenya Region, Kenya

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Abstract

Khat (*Catha edulis Vahl*) is a plant that is habitually ingested for its euphoric and stimulatory effects. This study aimed at evaluating genetic diversity of selected khat cultivars grown in Embu and Meru Counties Mt Kenya region. This will ensure that genetic resources are preserved and secured for future so that benefits from such plants continue to flow. DNA extraction was done using CTAB method and genetic diversity determined using 5 SSR markers. The number of alleles per locus ranged from 2 to 4 with an average of 2.4 across the 5 markers used. Gene diversity per locus ranged from 0.2296 (CE50) to 0.3344 (CE64) with an average of 0.2883 and Polymorphic Information Content (PIC) ranged from 0.2024 (CE50) to 0.2878 (CE37) with an average of 0.2475. A two dimensional scatter plot was generated and the two PCoA axis accounted for 43.33 and 28.19% of genetic variation. The AMOVA indicated intra-population variation of 93% while inter-population variation was 7%. The unweighted neighbour joining tree clustered khat cultivars into three major clusters and subsequent sub-clusters. This study revealed that there is a considerable level of genetic diversity among the Mt. Kenya khat cultivars. This was indicated by the alleles observed and clusters generated.

Keywords: Khat; Genetic diversity; Cultivar; SSRs

Introduction

Catha edulis (Vahl.) is an edible ever green plant [1,2]. It's classified in family Celastraceae, genus *Catha* and species *edulis* [3]. Various countries and communities have different names for the plant such as *Chat* and *Qat* in Yemen and Ethiopia respectively, *Qaad* and *Jaad* in Somalia, *Miraa* and *Muguka* in Kenya and *Jimma* in the Oromo language. In most western countries, it is known as khat [4].

Khat is said to have originated from Ethiopia and then spread to East Africa and Yemen [5]. However, there is a contentious belief the plant originated from Yemen before it spread to Ethiopia and neighboring states [6-8]. In Eastern Africa, the main khat markets are found in Kenya and Ethiopia near the growing zones [9]. Khat is not only consumed locally but also exported to generate income. In Kenya, it's mainly grown in Mt. Kenya region in Meru and Embu Counties. It is also grown in small quantities for local consumption in other areas such as Chyulu Hills, Nyeri and Taita hills [10]. Approximately 20 million people chew khat on a daily basis worldwide while hundreds of millions depend on it as a main source of livelihood [11,12].

Cathinone is the main component of khat that causes psycho-active effects. It induces its activity through the central and peripheral nervous system [13,14]. Khat cultivars are distinguished by the amount of cathionine (or the narcotic effect of the plant) that is present in the plant material and morphological differences. The varying morphological features and cathionine levels within khat may be also caused by genetic variations. Genotypic studies have not been conducted for this crop in Mt. Kenya region where it has become a major income earner. Farmers plant different cultivars obtained from various sources resulting to mixed populations.

It is not known the extent to which khat cultivars vary genetically within the study region. Therefore, it would be difficult to estimate the stability of such populations. The level of genetic variability can be a good indicator of productivity especially when different forms of biotic or abiotic stresses confront the plant [15]. Further, large gene pool, that is, if the genetic composition of individuals in the population varies significantly, such group has a greater chance of surviving and flourishing than a population with limited genetic variability. The information on intra-specific gene variability is, therefore, important to policy makers and khat crop farmers in formulation and implementation of sound strategies, such as breeding or propagation programmes that ensure preservation of genetic resources [16].

Genetic diversity information is essential for the germplasm management and developing conservation approaches [17,18] combination of factors such as the environment pressure, fitness selection, genetic drift and mutations [19]. Molecular characterization is an important tool used in genetic diversity studies to detect variations that exist in DNA sequences or specific genes or modifying factors [20,21].

Various molecular markers have been used in differentiating morphologically similar individuals among cultivars of the same plant species [22,23]. Molecular markers are independent or influenced by the environmental factors. They are a source of reliable information for genetic analysis and genotyping of different cultivars within a species [24]. Various molecular markers have been used in population genetics studies including simple sequence repeats (SSR) [25], restriction fragment length polymorphisms (RFLP) [26,24] random amplified polymorphic DNAs or (RAPDs) [27] amplified fragment length polymorphism (AFLP) [28] and inter simple sequence repeats (ISSR) [20,21,24].

In this study, we used SSRs markers to assess the genetic diversity of *C. edulis* in Embu and Meru counties. These markers were preferred

because they have been used effectively for various genetic diversity studies of many tropical species [20,21]. The main objective of this study was to evaluate the genetic relationship of 30 khat samples collected from different region in Embu and Meru Counties. The main objective of this study was to evaluate the genetic relationship of 30 khat samples collected from different region in Embu and Meru Counties.

Materials and Methods

Collection sites

The germplasm was collected from Meru and Embu Counties in 12 major khat producing wards (Figure 1). The wards in Meru County included Maua, Kangeta, Kianjai, Gaiti and Muthaara. Embu County wards included Kaaga South, Kaaga North, Kithimu, Mbeti South, Mbeti North, Mavuria and Muminji. Meru County is located at 0.047036 degrees North and 37.649808 degrees East on northeast slope of Mt. Kenya and its altitude is approximately 1500 metres. The area receives about 1366 mm per annum. Embu County lies between latitude 0°8" and 0°35" South and longitude 37°40" East.

Area of study

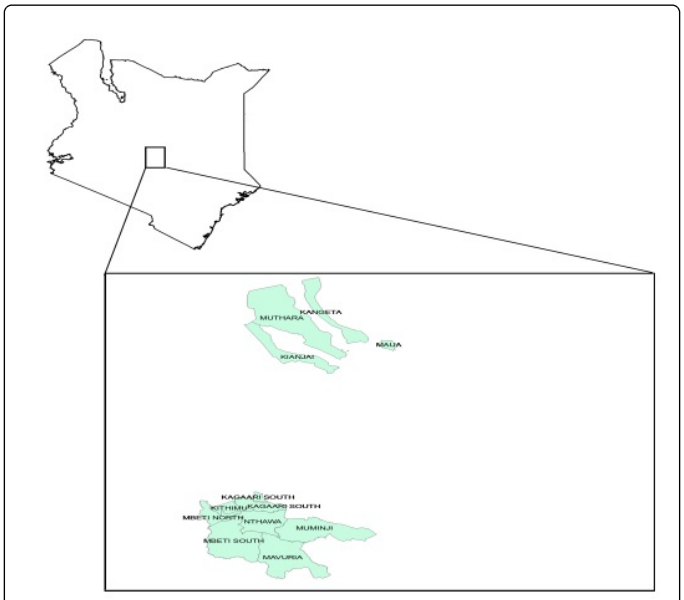

Figure 1: A map of Kenya showing the location of Embu and Meru Counties Meru County Wards included: Maua, Kangeta, Kianjai, Gaiti, Muthaara while Embu County Wards included; Kaaga South, Kaaga North, Kithimu, Mbeti south, Mbeti North, Mavuria, and Muminji

Specimen collection

Leaves used for molecular studies were picked with aseptic scalpel and placed in a cooler box then transported to National Museums of Kenya, Molecular Genetics Laboratory where molecular analysis was done. They were then kept at -20°C awaiting DNA extraction. All the information on these plants was recorded based on local names given by Meru and Embu communities and geographic distribution (Appendix1). The local names given by farmers included; *Kira kieru*-1, *Kira kieru*-2, *Kira kieru*-3, *Kira kiiru*-1, *Kira kiiru*-2, *Kira gitune*-1, *Kira gitune*-2, *Muchuri*, *Kithara*, *Mutimutiri*, *Mugiza*-1, *Mugiza*-2, *Mugumo*-1, *Mugumo*-2, *Mugumo* 3, *Mugumo*-4, *Mugumo*-5, *Muguka*-1, *Muguka*-2, *Muguka*-3, *Muguka*-4, *Muguka*-5, *Muguka wakarimi*, *Gitu,Mutamucii*, *Mukurukuru*, *Muruti*, *Muceke*, *Mitune*, and *Mumbu*.

Deoxyribonucleic acids (DNA) extraction and microsettlite analysis

A Modified Cetyltrimethy Ammonium Bromide (CTAB) protocol described by Doyle and Doyle [29] was used for khat genomic DNA extraction. The DNA was dissolved in 100 µl DNAse-free water and tapped to ensure that the pellet was completely dissolved. It was then incubated at 35°C for 2 hours and stored at -20°C. The quality of DNA was determined by 1% agarose gel electrophoresis. A total of 5 µl of each DNA sample was added to 2 µl of bromophenol blue dye and then loaded. The gel was run at a constant voltage of 100 volts for 30 minutes, then visualized under UV Trans-illuminator and images taken by a digital camera. Presence of distinct and bright bands in the gel was an indication of good quality DNA. Five SSR markers were used and showed clear and distinguishable bands between various khat individual samples. Reproducibility of the primers was tested by replicating the PCR amplification. Details of the marker used in this study are listed in the Table 1.

Polymerase chain reaction (PCR) amplification

Genomic DNA from each sample was used as a template for amplification. Amplification was carried out in 25 µl reaction volume prepared by adding 16µl of PCR sterilized water in PCR premix tube, 4 µl DNA templates and 0.5 µl of primer both forward and reverse was used.as per AccuPrep ® PCR amplification Kit. PCR reactions were carried out in Thermal cycler (Gene Amp PCR system 2400) perkin elmer. The PCR cycles were programmed as denaturation 95°C for 1 min, 94°C for 30 sec, annealing temperature of 50°C-60°C (depending on the primer used) for 30 sec, elongation of 72°C for 1 min for 35 cycles and an additional temperature of 72°C for 5 min for final extension. The reaction was maintained at 4°C and PCR products stored at -2°C after completion.

The PCR amplicons were resolved by horizontal gel electrophoresis on 2.0% agarose gels stained with ethidium bromide. This was then allowed to run for 1 hour. A volume of 2 µl of loading dye was added to 7 µl of each PCR product, thoroughly mixed then loaded onto the wells of the gel. One well of the gel was loaded with 5 µl of 100 bp DNA ladder (Invitrogen®). The gel was run for a period of 1.5 hours at a constant voltage of 100 V. The PCR amplicons were viewed under UV light and gel photos taken. The sizes of the bands were determined using l00 bp DNA ladder. This was repeated for all markers sets used. Clearly resolved unambiguous bands were scored as 1 and 0 for presence and absence respectively.

Data analysis

Genetic data was analysed using DARwin version 6.0.12, powermarker version 3.25 software [30] and GenALEx version 6.5 [31] statistical software. DARwin version 6.0.12 software, calculated genetic distance using Jacards dissimilarity coefficient followed by a dendrogram reconstruction using unweighted Neighbor Joining as implemented with 1000 permutations bootstraps. Powermarker version 3.25 software was used to analyze khat samples on the basis of

four statistical parameters; major allele frequency, allele number, gene diversity and polymorphism information content (PIC). Using GenALEx 6.5 software. Analysis of Molecular Variance (AMOVA) was carried out to reveal the partition and variation within and among the populations. Principle coordinate analysis (PCoA) was also conducted to visualize the relationship between the sample populations among 30 khat individual samples.

Marker	Forward primer	Reverse primer	Repeated motif	Estimated bp
CE37	ACTCGAAAAACATGGTGCAG	TGAGCCTCAATCTGGAGACA	(ATCT)11	208-276
CE39	AGCAGCAGCAACAACAAGAA	AGCAAGGGAGGCCTTATTA	(AAC)6	171-185
CE34	GCCAACCTCTTGTTCTGGAG	TAGGTTTGGCCATTCGATTC	(CT)12	179-218
CE50	CGGATGCCAAAACACTATCA	ATCCAAGAGGTTTTGGTTGC	(CT)12	208-218
CE64	CCTTCTATCACCCTCCCACA	CCCTCTGTATTGCACGGTTT	(CT)11	251-263

Table 1: SSR Markers used in the study.

Results

Assessment SSR profile

In the five microsatellite markers used on 30 khat samples, a total of 22 alleles were detected. The number of alleles per locus ranged from 2 to 4, with an average of 2.4 alleles and the size of the bands ranged from 200 bp to 500 bp. The highest number of alleles was seen in marker CE 37, and the least was seen in all the other markers (Table 2). The average gene diversity among all the selected samples was 0.2883. The Polymorphism Information Content (PIC) value of each marker varied for all tested SSR loci. The values ranged from 0.2024 (CE 50) to 0.2878 (CE 37) with an average of 0.2475 per locus (Table 2). Some bands presented by microsatellite markers were shared (Figures 2 and 3).

Marker	Major allele frequency	Allele no	Gene diversity	PIC
CE37	0.8166	4	0.3044	0.2878
CE39	0.7963	2	0.3044	0.2509
CE34	0.8111	2	0.2688	0.2194
CE50	0.8666	2	0.2296	0.2024
CE64	0.7833	2	0.3344	0.2769
Mean	0.8148	2.4	0.2883	0.2475

Table 2: Major Allele Frequency, Allele Number, Gene Diversity and PIC Values of the SSR Markers.

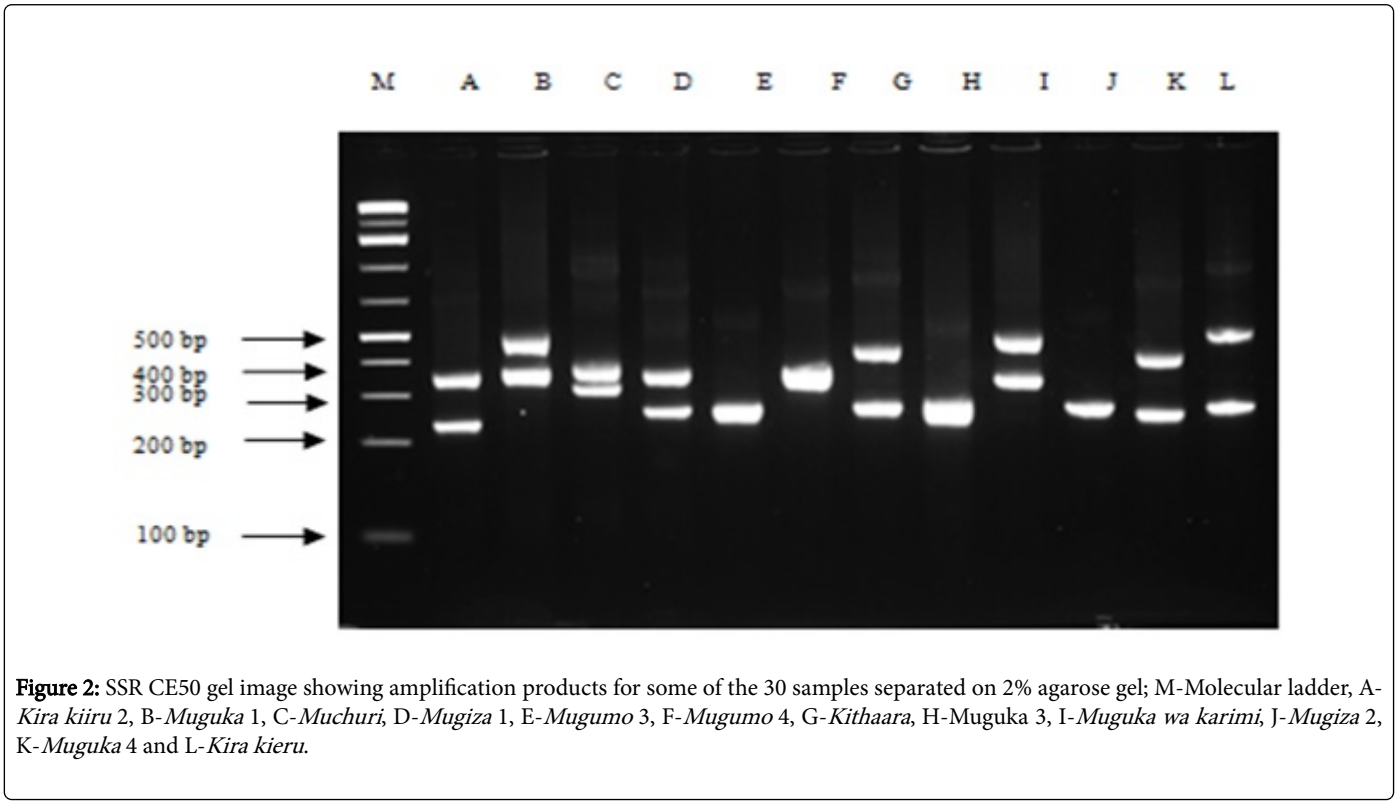

Figure 2: SSR CE50 gel image showing amplification products for some of the 30 samples separated on 2% agarose gel; M-Molecular ladder, A-*Kira kiiru* 2, B-*Muguka* 1, C-*Muchuri*, D-*Mugiza* 1, E-*Mugumo* 3, F-*Mugumo* 4, G-*Kithaara*, H-Muguka 3, I-*Muguka wa karimi*, J-*Mugiza* 2, K-*Muguka* 4 and L-*Kira kieru*.

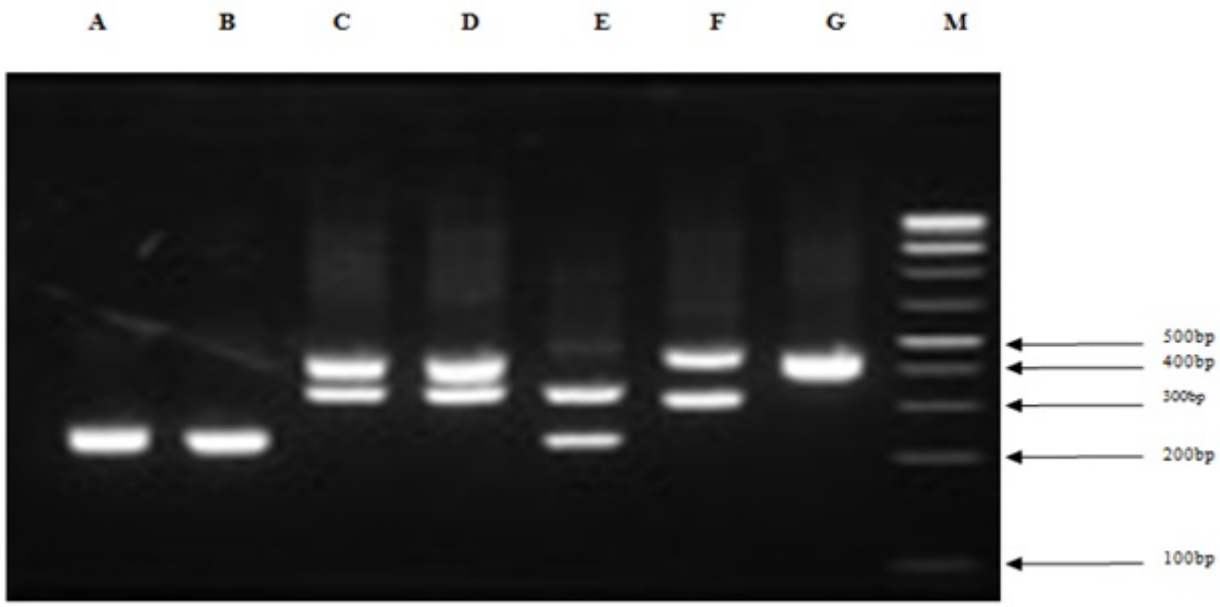

Figure 3: SSR CE64 gel image showing amplification products for some of the 30 samples separated on 2% agarose gel. A: Kira kieru 1; B: Kira kiiru 1; C: Kira gitune 1; D: *Kira kieru* 2; E: *Kira gitune* 2; F: *Kirakiiru* 3; G: *Mugiza* 2; M: Molecular ladder (100)

Genetic dissimilarity

A dissimilarity matrix based on Jaccard's dissimilarity index was calculated targeting shared microsatellite alleles and was used to determine the relatedness among the 30 *C.edulis* samples. The pairwise genetic dissimilarity values ranged from 1.000 to 0.000 (Table 3). The highest level of dissimilarity (100%) was in cultivars *Muguka*-2, *Kira kieru*-1, *Muchuri, Muguka wa karimi, Mugumo*-1, *Kira kiiru*-1, *Muguka*-2, *Mugumo*-1, *Mutimutiri, Mugiza*-2 *Mugiza*-2 *and Muguka*-1. On the other hand, the dissimilarity matrix of 0.000% was seen in cultivar *Kira gitune*-1, *Mugiza*-1, *Mugumo*-3, *Kithara, Kiira kieru*-3, *Mugumo*-3, *Mugiza*-1, *Mukurukuru* and *Kirakiru*-1.

	kirakieru1	kirakiiru1	kiragitune1	mutimutiri	kiragitune2	kirakieru3	mugiza2	kirakiiru2	mugumo2	mugumo1	muguka1	muguka2	muchuri	mugiza1	mugumo3	mugumo4	kiithara	muguka3	mugukawakarimi	gitu	muguka4	mutamucii	kirakieru2	mukurukuru	muruti	muguka5	muceke	mugumo5	gitune	mombo
kira kiiru 1	0.3333																													
kira gitune 1	0.3333	0																												
muti mutiri	0.7778	0.75	0.75																											
kira gitune 2	0.5	0.3333	0.3333	0.625																										
kira kieru 3	0.3333	0	0	0.75																										
mugiza 2	0.5714	0.7143	0.7143	0.75	0.7143																									
kira kiiru 2	0.7692	0.8462	0.8462	0.3846	0.8462	0.4615																								
mugumo 2	0.5	0.6667	0.6667	0.8333	0.6667	0.1429	0.5385																							
mugumo 1	1	1	1	0.875	1	0.8571	0.9231	1																						
muguka 1	0.7	0.5	0	0.2	0.8	0.6923	0.2308	0.7692	0.9																					
muguka 2	1	1	1	1	1	1	1	1	1	1																				
muchuri	1	1	1	1	1	1	1	1	1	1	1																			
mugiza 1	0.333	0	0	0.75	0	0.7143	0.8462	0.6667	1	0.8	1	1																		
mugumo 3	0.3333	0	0	0.75	0	0.7143	0.8462	0.6667	1	0.8	1	1	0																	
mugumo 4	0.6667	0	0	0.5556	0.6	0.5	0.6154	0.625	0.8	0.6364	1	1	0.6	0.6																
kiithara	0.5	0.3333	0.3333	0.625	0.3333	0.5714	0.7692	0.7143	0.6667	0.7	1	1	0.3333	0.3333	0.4															
muguka 3	0.7692	0.8462	0.8462	0.6	0.8462	0.4615	0.2667	0.5385	0.9231	0.4667	1	1	0.8462	0.8462	0.7143	0.7692														
muguka wa karimi	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1													
gitu	0.6	0.5	0.5	0.6667	0.5	0.7778	0.7857	0.75	1	0.7273	1	1	0.5	0.5	0.5	0.6	0.8667	1												
muguka 4	0.7143	0.6667	0.6667	0.4444	0.6667	0.7	0.5385	0.8	0.8333	0.4	1	1	0.6667	0.6667	0.625	0.5	0.5385	1	0.75											
muta mucii	1	1	1	0.9091	1	0.9	0.8667	1	0.75	0.8333	1	1	1	1	0.875	0.833	0.7857	1	1	0.75										
kira kieru 2	0.6667	1	0.6	0.7	0.6	0.8	0.8	0.7778	1	0.75	1	1	0.6	0.6	0.5714	0.6667	0.8	1	0.2	0.7778	0.875									
mukurukuru	0.3333	0	0	0.75	0	0.7143	0.8462	0.6667	1	0.8	1	1	0	0	0.6	0.3333	0.8462	1	0.5	0.6667	1	0.6								
muruti	1	1	1	1	1	1	0.9231	1	1	0.9	1	1	1	1	1	1	0.9231	1	1	0.8333	0.75	1	1							
muguka 5	1	1	1	1	1	1	0.9286	1	1	0.9091	1	1	1	1	1	1	0.9286	1	1	0.8571	0.5	1	1	0.5						
muceke	1	1	1	1	1	0.9	0.9375	0.8889	1	1	1	1	1	1	1	1	0.9375	1	1	1	1	1	1	1	1					
mugumo 5	0.8182	0.9091	0.9091	0.9412	0.9091	0.5833	0.7222	0.5455	1	0.8889	1	1	0.9091	0.9091	0.8462	0.9167	0.5625	1	0.9231	0.9333	0.9231	0.8462	0.9091	1	1	0.7273				
gitune	1	1	1	1	1	0.9091	0.875	0.9	1	0.9286	1	1	1	1	1	1	0.8	1	1	0.9	0.5	0.8889	1	0.8	0.6	0.7143	0.75			
mumbu	1	1	1	1	1	0.9	0.9375	0.8889	1	1	1	1	1	1	1	1	0.8667	1	0.8571	1	1	0.875	1	1	1	0.8571	0.7273	0.875		

Table 3: Jaccards Coefficients Dissimilarity Matrix among Pairs of 30 *Catha edulis* Genotypes.

Phylogenetic clustering of the 30 Khat genotypes

Unweighted neighbour joining dendrogram based on Jaccard's dissimilarity matrix was used to determine genetic relatedness among and within the different populations based on the 5 polymorphic SSR markers. The cultivars were clustered into three major clusters namely:clusters A, B and C (Figure 4). Cluster A grouped two cultivar together; Gitu from Embu county and Kira kieru-2 from Meru County with a bootstrap value of 76%.

Cluster B comprised of 9 cultivars, which clustered into 2 sub clusters at a bootstrap value of 24%. Two cultivars from Meru County clustered together (Kiithara and Kira gitune-2) in one ofthe sub-clusters. The other sub-cluster grouped *Mugiza-1, Mukurukuru and Mugumo-3* from Embu County along with *Kira gitune-1, Kira kieru-3, Kira kiiru-1 and Kira kieru-1* from Meru County. Cluster C consisted of 19 khat cultivars and segregated into several sub-clusters at a bootstrap value of 16%. These included *Mugumo-4, Mugiza-2, Mugumo-2, Muguka-4, Mutimutiri, Muguka-1, Kira kiiru-2,*

Muguka-3, Mugumo-1, Muguka wa karimi, Muguka-2, Muchuri, Muruti, Muguka-5, Mutamucii, Gitune, Mumbu, Muceke and Mugumo-5. These sub-clusters also divided into several groups and various cultivars were clustered together.

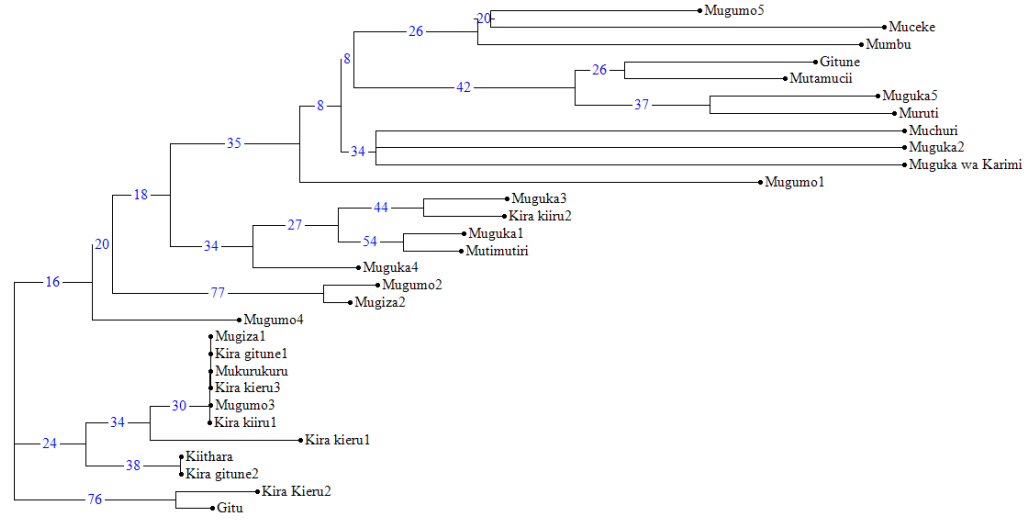

Figure 4: A neighbour joining tree showing the genetic relationships among the 30 selected *C. edulis* samples based on the 5 SSR markers.

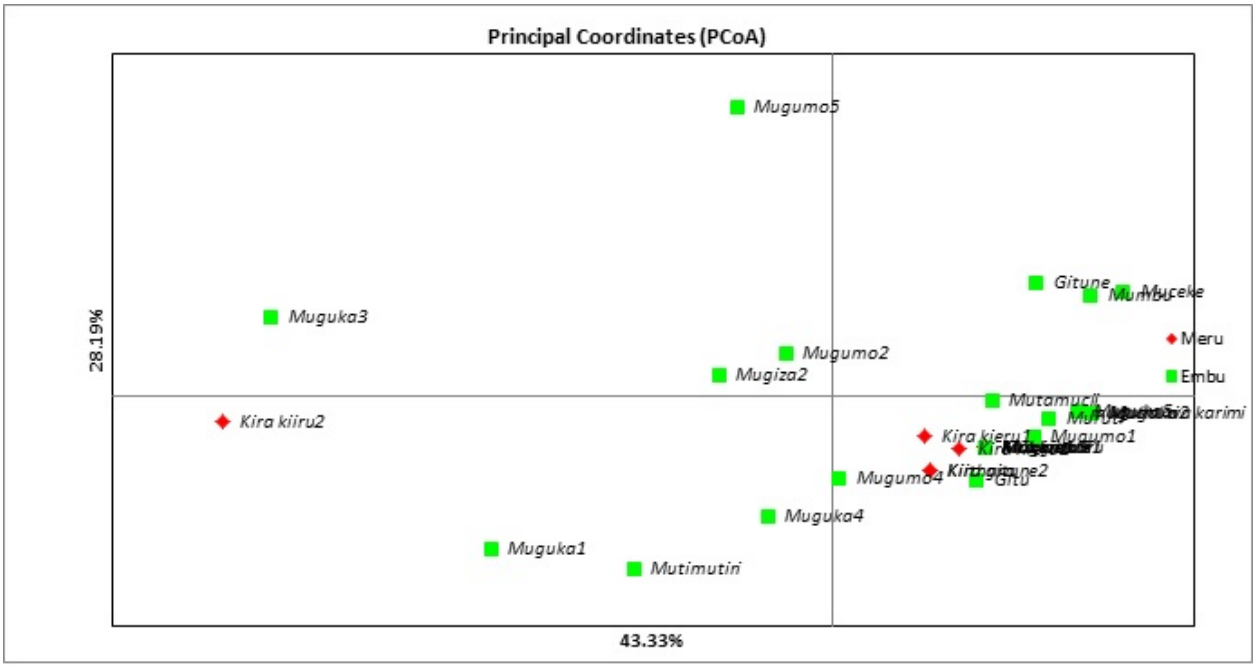

Figure 5: Principal Coordinates Analysis of the *C. edulis* Cultivars based on 5 SSR markers.

Principal coordinates analysis (PCoA)

Principal coordinate analysis was done to visualize the genetic dissimilarities between cultivars following which the two-dimensional plot was drawn. As the plot shows, the first principal coordinate accounts for 43.33% of the total variation while the second coordinate accounts for 28.19% of the variation (Figure 5). The scatter plot grouped the 30 khat samples into four parts. The first quadrant comprised of 4 cultivars *Muguka*-3, *Mugumo*-5, *Muguka wa karimi* from Embu county, and *Kira kieru*-2 from Meru county. Quadrant 2 had 3 cultivars *Gitu*, *Muceke* and *Mumbu* from Embu Counties. Quadrants 3 comprised of *Kithara*, *Muchuri* from Meru County and

Mutimutiri, *Muguka*-4 and *Mugumo*-3 from Embu County. Quadrant 4 comprised the most cultivars that clustered together (Figure 4).

Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) determines genetic variations within and among populations. Ninety three percent (93%) (P<0.001) of genetic variation was distributed within population while 7% (P<0.001) was distributed among populations (Table 4).

Source of Variation	Df	SS	MSD	%Variation	P-value
Among population	2	9.733	4.87	7	
Within population	27	75.9	2.81	93	<0.001
Total	29	85.63	7.68	100	

Table 4: Analysis of Molecular Variance (AMOVA)

Discussion

Genetic variation occurs within and among individual species as well as higher taxonomic groups [32-34]. Factors that contribute to genetic variation include mutations, interactions with the environment, fitness selection and genetic drift [35-39]. Different types of mutations accumulate over time which serves as a source of polymorphism within a species. Useful genetic variations are inheritable and discernible either phenotypically or genetically [35,38,39]. Genetic diversity in plant species is essential for efficient utilization of plant genetic resources. Geographical isolation of populations may cause its genomic drift away from other populations of the same species [40,35]. Variations within a population can be determined through an assessment of gene diversity and allelic richness [40]. Traditionally, morphological characterization has been used in identifying species but over the years, DNA profiling is commonly being used as it is more reliable due to several limitations of morphological data [36,37,39,41].

The current study describes genetic variability of *C. edulis* in Kenya. A total of 5 SSR markers developed by [42] were used to characterize khat cultivars. PIC values were used to determine levels of polymorphism which revealed the allele diversity and variation between loci. Three categories of the PIC values and their informative levels have been highlighted by [42]. The PIC values take into account the number of alleles and their distribution, thus determining the strength of the markers [43]. The PIC values range from 0 to 1 with values that are greater than 0.5 being highly informative. The PIC values ranging from 0.25 to 0.5 show a reasonably informative marker while those with values less than 0.25 are slightly informative [20,21,43] Based on this, markers CE 37 and CE 39 were reasonably informative. The 5 SSR markers generated polymorphic alleles revealing considerable genetic diversity among the various khat genotypes used in this study. The mean PIC value observed in this study was 0.2475. Marker CE 37 had PIC value of 0.2878, which was lower than 0.89 as observed by [44] in 23 khat genotypes.

The attained gene diversity had an average of 0.2888 and ranged from 0.2296 to 0.3344. This shows that these results were close to what was reported by [45] in Yemen on first comparative study of khat genotypes. It showed between 0.202 and 0.172 on a genotype Abyadh and close to results obtained by [42] 0.40 and 0.29 for Ethiopia and Kenyan khat population respectively. The different gene diversity attained could be attributed to the use of different set of primers and khat cultivars under study.

The number of alleles had an average of 2.4 alleles, this was lower than what was observed by [42], which ranged between 2 and 16 on evaluation of microsatellite using pyrosequencing. The allele frequencies ranged from 78.3% to 86.6%. However, they were higher than what was obtained by [46] who obtained 18.21% in pepper. These differences could be due to different markers used. It could also be caused by variation in the nucleotide sequences of flanking regions causing null allele which prevents the primer-template DNA annealing during amplification [42,47].

The grouping of 30 khat samples in a phylogenetic tree into 3 main clusters: A, B and C is an indication of genetic diversity. In cluster A, the two cultivars (*Gitu* and *Kira kieru*-2) were grouped together, at a bootstrap value of 76%. *Gitu* was characterised as a shrub from Embu while *Kiira kieru*-2, as a tree from Meru county. The cluster showed a high level of relatedness amongst these cultivars. This can be attributed to the existence of common ancestry [48]. In cluster B, there were 2 sub-clusters which divided into two groups. Most of the cultivars clustered together regardless of their geographical origin. This shows that there was no much differentiation between the sub-populations. For example, *Mugiza*-1, *Mugumo*-3 and *Mukurukuru* from Embu County and *Kiira gitune*-1, *Kiira kieru*-3, and *Kiira kiiru*-1 from Meru were grouped together indicating similarity of 100%. This is regardless of the cultivars being collected from varying geographic regions and different agronomic practices. This shows that they were similar cultivars despite the different names given by the locals and growth habitats Therefore, there is a possible existence of a common gene pool.

Cluster C segregated into several sub-clusters at a bootstrap value of 16% and consisted of 19 cultivars, each clustering into different groups. The cultivars in this cluster comprised both cultivars collected from different geographical regions with different local names. Despite the different given names, these cultivars grouped in the same cluster. The many sub clusters dividing from the main clusters suggests that there is high level of genetic variability in Mt. Kenya region khat cultivars. Populations from different geographical regions and growth types in Mt. Kenya region showed genetic differentiation. This result concurs with the results obtained by [45] in genotyping of khat in Yemen using RAPD markers. His study revealed that populations from different geographical regions and growth habits in Yemen had clear genetic differentiation.

Genetic dissimilarity in khat cultivars ranged from 1.0000 to 0.0000. The highest dissimilarity of 100 % was in cultivars Muguka-2, *Kira kieru*-1, *Muchuri*, *Muguka wa karimi*, *Mugumo*-1, *Kira kiru*-1, *Muguka*-2, *Mugumo*-1, *Mutimutiri*, *Mugiza*-2 and *Muguka*-1. This indicated a wide variation among the cultivars. Dissimilarity matrix of 0.000% was observed in genotypes Kira gitune-1, *Mugiza*-1, *Mugumo*-3, *Kithara, Kiira kieru*-3, *Mugumo*-3, *Mugiza*-1, *Mukurukuru* and *Kira kiru*-1, which showed that they had a common ancestry. The genetic distance of 0.000 corresponded with that found in wheat genotypes such as *Balaka vs Aghrani* and *Triticale vs* BAW-1036 [49].

Unweighted neighbour joining tree and PCoA clustering pattern gives similarity that help to analyse the dissimilarity index among cultivars. Cultivars that cluster in a one quadrant are closely related. A two-dimensional scatter plot involving all 30 khat samples showed that

the first two PCoA axes accounted for 43.33% and 28.19% of the genetic variation among populations. The obtained coordinates showed that coordinate 1 had the highest variability followed by coordinate 2 that had the next greatest variability. Therefore, the cultivars that are located further to one another have less similarity than those that are closer. Lower coordinates of 29.43% and 19.89% in coordinates 1 and 2 respectively in 12 rice genotypes using 8 SSR markers was obtained [48].

Euclidean distance matrix [50] was used to perform Analysis of Molecular Variance (AMOVA) within and among 30 khat individuals then variations in the population were compared. Percentage variations showed that there was genetic diversity among the Meru and Embu khat cultivars. The results indicated that 93% of the total variation was within the populations, while 7% was among populations. It is reported a closely related result that observed 97.3% of total variation in Eritrean barley using SSR markers [51]. It is reported similar huge differences in percentage variations between and among a group of khat genotypes studied using SSR markers [42].

Gene flow or mutations could be the origin of high genetic variation within the sample population [52,34]. On the other hand, sharing of the same SSR profiles among the cultivars could account for the relatively low genetic variation among these khat cultivars. Also common ancestry of cultivars could contribute to low variability despite the fact that they are grown in different countries [34].

Conclusion

Thirty Khat genotype showed genetic diversity using SSR markers that was within the range obtained in other studies. In addition the study re affirms the value of SSR Markers in the use of genetic diversity. Finally the study showed that the khat population from different Meru and Embu Counties did not cluster based on their geographical origin and the different local names given.

Recommendations

The SSR markers used in this study may be useful to construct a genome database for khat breeding programmes and characterization of khat cultivars.

The study could help in conservation of genetically distinct *C. edulis* cultivars collected in Embu and Meru cultivars.

Suggestions for further research

Genotyping by sequencing should be conducted on these khat cultivars based on geographical region to give more details on genetic diversity.

Conflict of Interest

The authors declare no conflict of interests.

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