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Genetic Diversity of Jute Mallow (*Corchorus spp.*) Accessions Based on ISSR Markers

Munguatosha Ngomuo[1,2*], Tsvetelina Stoilova[3], Tileye Feyissa[1,4], Patrick A. Ndakidemi[1]

[1]School of Life Sciences, Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania
[2]Tanzania Official Seed Certification Institute (TOSCI), Arusha, Tanzania
[3]AVRDC—World Vegetable Centre, Arusha, Tanzania
[4]Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia
Email: *ngomuo2004@gmail.com

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Abstract

Jute mallow is a nutritious leafy vegetable. The leaves are rich in proteins, vitamins and essential amino acids. Molecular characterization of Jute mallow with focus on improvement of leaf yield is scarcely reported. In the present study, inter sequence simple repeats (ISSR) molecular markers were employed to assess genetic diversity and relationships of 83 accessions of Jute mallow from different parts of Africa and Asia conserved at the World Vegetable Center East and Southern Africa. A total of 89 bands were amplified by 8 ISSR primers. Number of polymorphic bands per primer ranged from 2 to 6 with an average of 2.75 bands per primer. Polymorphic information content (PIC) values ranged from 0.390 to 0.760 with average of 0.53. Average Nei's gene diversity (*h*) and Shannon's information index (*I*) were 0.335 and 0.494 respectively. The highest pairwise genetic distance was 0.431 observed in a population from East Africa accessions. PC1 and PC2 axis explained 21.69% and 11.66% of the total variation respectively. UPGMA cluster analysis grouped the accessions into six main clusters at genetic similarity coefficient of 0.53 as standard value for classification. These results have important implications for jute mallow breeding and conservation.

Keywords

Corchorus spp., Genetic Diversity, ISSRs, Jute Mallow, Leafy Vegetable

1. Introduction

Jute mallow (*Corchorus spp.*) is a traditional leaf vegetable that is used in many households in Africa, Middle East and Latin America as both vegetable and medicinal plant. It is an annual herb belonging to a family Malvaceae which is

comprised of 50 - 60 species which are distributed in the tropics, subtropics and warm temperate regions of the world [1] [2] [3]. The leaves are well known as emollient, diuretic, tonic and purifying body [4] [5]. The leaves contain on average 85 - 87 g H_20, 5.6 g protein, 0.7 g oil, 5 g carbohydrate, 1.5 g fiber, 250 - 266 mg Ca, 4.8 mg Fe, 1.5 mg vitamin A, 0.1 mg thiamine, 0.3 mg riboflavin, 1.5 mg nicotinamide and 53 - 100 mg ascorbic acid per 100 g [6]. It also contains high amount of all essential amino acids and antioxidants needed for a good health [7] [8] [9]. Merha green and Merha red are the genotypes of jute mallow with high nutrional content, they are rich in β-carotene content, 125.4 and 127.5 $\mu g \cdot g^{-1}$ respectively [10]. Most of jute mallow genotypes have reasonable leaf yield, the accession with notable high leaf yield is TOT 7866 from Taiwan region [11].

Though jute mallow has significant contribution to food security; its cultivation in many parts of Africa is very limited. The crop grows as volunteer crop in farmer's fields and the leaves are collected during rainfall season and dried and stored for use during dry seasons [11]. Jute mallow is still classified as neglected and underutilized crop; it is neglected by researchers and national agricultural development policies [12] [13]. Thus, there are few improved varieties of jute mallow in Africa and those which are grown by farmers are locally obtained from germplasm maintained by farmers [14].

In order to promote the use of jute mallow, it is crucial to make availability of improved genotypes a priority. To achieve this goal, the information on the genetic diversity of the available germplasm is very important [13] [15].

Morphological data coupled with the use of appropriate molecular markers are more reliable and informative of genetic diversity of any species [16]. Moreover, molecular markers are not affected by environment and they have been used successfully in different plant species for genetic characterization of germplasm at different levels [17].

The use of molecular markers to study genetic diversity of jute has been reported by several authors. Recently, various molecular markers such as random amplified polymorphic DNA (RAPD) [17] [16], simple sequence repeats (SSR) [18] [19] [20] [21] [22], amplified fragment length polymorphism (AFLP) [23] [12], sequence-related amplified polymorphisms (SRAP) [24] [25] and sequence tagged microsatellite sites (STMS) [26] have been used to detect genetic diversity in different germplasms of jute mallow. Most of these studies focused on jute mallow as a fiber crop and the major emphasis were on improvement of fiber related traits. The results of these studies indicated high diversity between the species but low diversity within the species. Only few studies have been conducted with focus on jute mallow as vegetable with emphasis on improvement of leaf yield. Also in many studies conducted, the materials used were from Asia where jute mallow is grown for fiber. Collections from East, West and Southern Africa largely remain unexploited for genetic diversity particularly with traits related to leaf yield which is the main use of the crop in Africa.

ISSR markers are significant molecular markers possessing different genome

coverage [27]. ISSR use microsatellite sequences as primers to amplify genomic regions flanked by microsatellite repeats [16]. ISSR markers are simple and cost effective to use with high degree of reproducibility [28].

In view of the above, the present study was conducted to assess genetic diversity of accessions of Jute mallow present in the World Vegetable Center by using ISSR markers.

2. Materials and Methods

2.1. Plant Materials

Seeds of 83 accessions of Jute mallow were sown in plastic trays and after 30 days leaf samples were collected from the seedlings for DNA extraction. The Jute mallow collection represent one of the different types of traditional vegetables collected from farmers' fields and preserved *exsitu* for breeding, other research activities and farmers purposes. The list of these accessions is presented in **Table 1**.

Table 1. List of *Corchorus spp.* accessions used in the molecular characterization.

SN	Accession Name	Country/Area of Origin	SN	Accession Name	Country/Area of Origin
1	ALV MN 059	Unknown	42	TOT 4413	Bangladesh
2	AZIGA	Cameroon	43	TOT 4429	Bangladesh
3	BAFIA	Cameroon	44	TOT 4500	Bangladesh
4	CAM EX CO	Cameroon	45	TOT 4519	Bangladesh
5	CAM MULA	Cameroon	46	TOT 4541	Bangladesh
6	ES	Tanzania	47	TOT 4623	Bangladesh
7	EX CHAMALAWI	Malawi	48	TOT 4624	Bangladesh
8	EX ZIMBABWE	Zimbabwe	49	TOT 4669	Bangladesh
9	GKK 10	Malawi	50	TOT 4701	Bangladesh
10	GKK 25	Malawi	51	SUD - 2	Sudan
11	HS	Tanzania	52	TOT 4712	Bangladesh
12	IP-10	Kenya	53	TOT 4713	Bangladesh
13	IP-13	Kenya	54	TOT 4721	Bangladesh
14	IP-2	Kenya	55	TOT 4800	Vietnam
15	IP-5	Kenya	56	TOT 4876	Japan
16	IP-4	Kenya	57	TOT 4885	Japan
17	MIX	Tanzania	58	TOT 5876	Japan
18	ML-JM-1	Malawi	59	TOT 5877	Japan
19	ML-JM-10	Malawi	60	TOT 5999	Taiwan region
20	ML-JM-12	Malawi	61	TOT 6278	Vietnam
21	ML-JM-13	Malawi	62	TOT 6370	Unknown

Continued

22	ML-JM-14	Malawi	63	TOT 6425	Uganda
23	ML-JM-2	Malawi	64	TOT 6426	Kenya
24	ML-JM-3	Malawi	65	TOT 6427	Kenya
25	ML-JM-4	Malawi	66	TOT 6430	Cameroon
26	SUD 1	Sudan	67	TOT 6667	Philippines
27	SUD 3	Sudan	68	TOT 6669	Philippines
28	SUD 4	Sudan	69	TOT 6730	Unknown
29	T0T 4067	Vietnam	70	TOT 6749	Unknown
30	TOT 0124	Malaysia	71	TOT 7865	Unknown
31	TOT 3499	Vietnam	72	TOT 7866	Unknown
32	TOT 4064	Vietnam	73	TOT 7974	Bangladesh
33	TOT 4097	Tanzania	74	TOT 7977	Bangladesh
34	TOT 4140	Vietnam	75	TOT 7979	Bangladesh
35	KIPUMBULIKO	Unknown	76	TOT 7980	Bangladesh
36	TOT 4157	Vietnam	77	TOT 8532	Unknown
37	TOT 4235	Bangladesh	78	TOT 9736	Unknown
38	TOT 4312	Bangladesh	79	UG	Uganda
39	TOT 4316	Bangladesh	80	TZA 3002	Tanzania
40	TOT 4352	Bangladesh	81	TZA 3070	Tanzania
41	TZA 681	Tanzania	82	UG-JM-1	Uganda
83	UG-JM-13	Uganda			

2.2. Molecular Analysis

DNA extraction was done by using modified Cetyltrimethly ammonium bromide (CTAB) method according to [29]. The DNA was purified by RNase treatment followed by Sodium acetate and ethanol. The quality and concentration of DNA was checked on 0.8% agarose gel by comparing with 100 kb ladder. Fifteen ISSR primers namely $(GA)_6GG$, $(CAC)_3$, $(GAG)_3GC$, $CAC(TCC)_5$, $TGTA(CA)_7$, $TAC(CA)_7$, $(AG)_8T$, $CGTC(AC)_7$ and $(AG)_8CT$. Others include $(CAG)_6$, $(CAG)_{10}$, $(CGG)_6$, $(CTT)_6$, $(TTG)_{10}$ and GATA were used in this study.

2.3. PCR and Electrophoresis of PCR Products

After initial screening of the 15 ISSR primers, 8 primers with good and clear banding pattern were used for analysis of genetic diversity (Table 2). The PCR was performed in a 10 µl reaction mixture as follows. A 2 µl of 50 ng DNA template was used, 2 µl of primer (Inqaba Biotech, South Africa), 0.5 µl of dNTPs, 2.5 µl of 10× one taq standard buffer, 0.93 µl of nuclease free water. The reaction mixture was loaded in a 96 well plate initially denatured at 94˚C for 5 min;

Table 2. ISSR primers used in this study and their amplification results.

Primer Number	Repeat Motif	Number of polymorphic Bands	Number of amplified Bands	PIC
ISSR 4	$(GA)_6GG$	2	6	0.5
ISSR 5	$(CAG)_3GC$	2	5	0.39
ISSR 7	(GAC)TCC5	6	17	0.76
ISSR 8	$(AG)_8G$	3	18	0.586
ISSR 9	$(GATA)_8$	2	9	0.493
ISSR 11	$(AG)_8T$	2	10	0.48
ISSR 14	$TGTA(CA)_7$	2	9	0.494
ISSR 15	$(CTT)_6$	3	15	0.567
Average Per primer		2.75	11.13	0.53

followed by 35 cycles of 94˚C for 1 min. Annealing temperature was according to primer for 1 min and 72˚C for 1 min followed by final extension at 72˚C for 5 min. The amplified products were separated on 1.5% agarose gels stained with EZ-Vision (Amresco, fountain parkway solon OH USA) in 0.5 TBE (Tris Borate Ethylenediaminetetraacetic acid) buffer. 100 base pair ladder was loaded with the samples. The gels were viewed in a Biorad Gel Doc EZ imager

2.4. Data Analysis

ISSR amplified bands in the gel were manually scored as present (1) or absent (0). Only the consistently and clear bands were scored and used to create 1/0 matrix. This matrix was then used to assess the genetic diversity of *Corchorus spp*. accessions. Polymorphism information content was calculated for each band according to [30] by using the formula;

$$\mathrm{PIC} = 1 - \sum (\mathrm{Pi})^2$$

where Pi is the frequency of the i^{th} band phenotype detected. Nei's pairwise genetic distance, Nei's gene diversity and Shannon's information index (*I*) were calculated using computer program POPGENE version 1.32 [31]. The obtained matrix was also used to calculate principal coordinate analysis (PCoA) by using PAST software version 1.93 and to perform cluster analysis and construct the unweighted pair group method with arithmetic average (UPGMA) dendrogram using NTsys - pc 2.1 software.

3. Results

3.1. ISSR Polymorphism

Of the 15 primers used 8 primers showed good and clear banding pattern. The number of polymorphic bands per primer ranged from 2 to 6 with an average of 2.75 bands per primer. Regarding the average number of bands amplified, 11.13

bands were amplified per primer and a total of 89 bands for all primers. Total number of bands amplified per primer ranged from 5 - 18 bands. Polymorphic information content (PIC) values ranged from 0.390 in primer $(CAG)_3GC$ to 0.760 in primer (GAC) TCC5. The primer sets with PIC value > 0.5, were classified as highly informative. Three primers, (GAC) TCC5, $(AG)_8G$ and $(CTT)_6$ were highly informative (Table 2).

3.2. Genetic Diversity

Table 3 shows Nei's gene diversity (*h*) and Shannon's information index (*I*) for seven populations from different parts of the world. The Nei's gene diversity ranged from 0.164 in population 1 from East Africa to 0.417 in population 2 from East Asia with an average of 0.335 across all populations. Thus diversity between the populations is low. Similarly Shannon's information index ranged from 0.245 in population 1 from East Africa to 0.605 in population 2, from East Asia with average of 0.494 across all the populations.

Nei's measure of original genetic distance is summarized and presented in Table 4. The highest pairwise genetic distance (0.431) was observed between

Table 3. Nei's gene diversity and Shannon's information index for different populations of *Corchorus spp.* Population 1—East Africa, 2—East Asia, 3—North Africa, 4—South Africa, 5—South East Asia, 6—Unknown, 7—West Africa.

Population	Number of Individuals	Nei's gene diversity (*h*)	Shannon's information index (*I*)
1	18	0.1636	0.2447
2	16	0.4167	0.6045
3	4	0.3403	0.4977
4	11	0.3949	0.5769
5	20	0.3278	0.4966
6	9	0.2936	0.4490
7	5	0.4089	0.5877

Table 4. Neis' measure of original genetic distance in *Corchorus spp.* Populations 1 - 7 are as listed in Table 3.

Populations	1	2	3	4	5	6	7
1	****						
2	0.1908	****					
3	0.2685	0.1135	****				
4	0.2180	0.0216	0.1264	****			
5	0.2812	0.0445	0.1517	0.0223	****		
6	0.4308	0.0671	0.1760	0.0557	0.0368	****	
7	0.2638	0.0554	0.1423	0.0486	0.0775	0.0701	****

population 1 from East Africa and population 6 from unknown. The lowest pairwise genetic distance 0.0216 was recorded between population 2 from East Asia and population 4 from South Africa. Population from East Africa had a highest pairwise genetic distance as compared with other populations, this was also observed in population from North Africa. Other populations with lowest pairwise genetic distance (0.0223) was between population 4 from South Africa and population 5 from South East Asia.

Principal coordinate analysis results from the SSR markers for the 83 accessions are presented in Figure 1. PC1 and PC2 axis explained 21.69% and 11.66 % of total variation respectively. Accessions from East Africa were grouped in the right side of both positive and negative Y-axis in the first quadrant and fourth quadrant. Also accessions from East Asia and South East Asia were grouped with accessions from East Africa. The second quadrant contained mixed accessions but mainly from East and South East Asia. The unknown accessions were mainly found in second quadrant and third quadrant. The highly different accessions in their clustering pattern were four accessions from East Asia which clustered far from the rest in each quadrant. Two accessions from West Africa also clustered separately in the third quadrant as well as South African accessions in the fourth and second quadrant.

3.3. Cluster Analysis

UPGMA tree showed six main clusters at genetic similarity coefficient of 0.53 as standard value for classification within the collection of 83 accessions from this germplasm (Figure 2). The first cluster contained 5 accessions, three from Asia and one from South Africa and the remaining from unknown. The second cluster

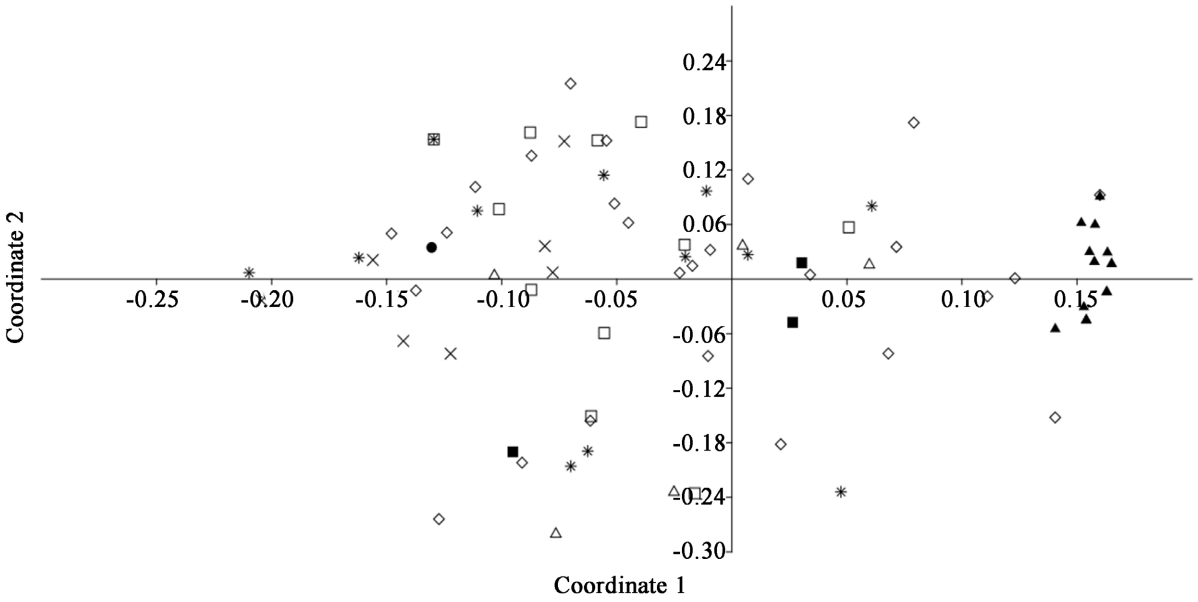

Figure 1. Scatter plot of a PCoA showing the distribution of ISSR genetic diversity in *Corchorus spp.* accessions. The populations are presented in different symbols based on their origin. ◊—East Asia, ▲—East Africa, □—South East Asia, ▪—North Africa, ○—South East Asia, Δ—West Africa, *—South Africa, X—Unknow.

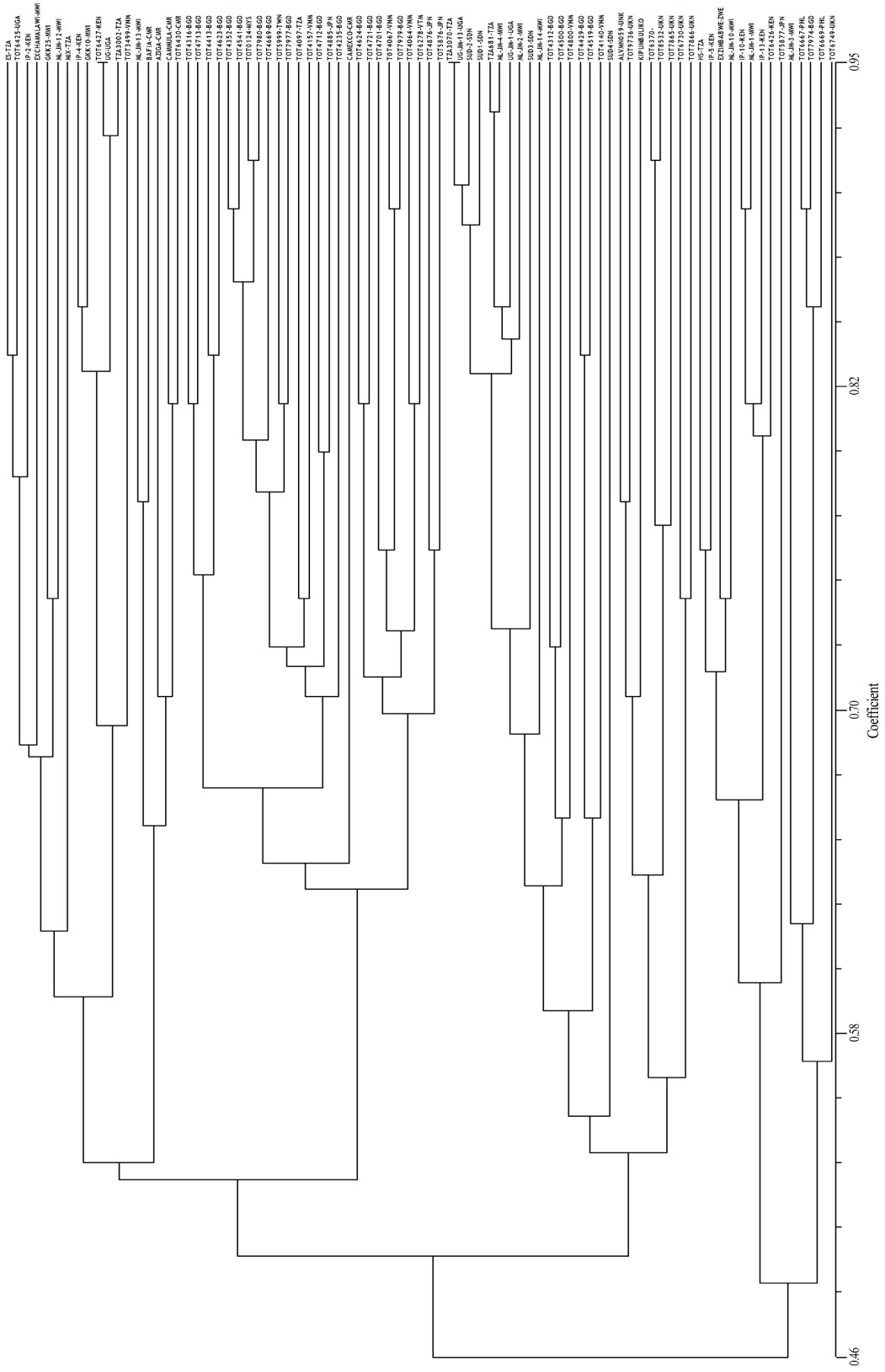

Figure 2. Dendrogram constructed from cluster analysis based on UPGMA using the SM coefficient option for 83 accessions of *Corchorus spp*. Country of origin of each individual is given with 3—digit international codes after accession name.

contained 10 accessions, six from East Africa, and three from South Africa and one from East Asia. Cluster three contained 24 accessions, 8 accessions from unknown origin were grouped in this cluster. Four (4) accessions from North Africa and six from East and South Africa as well as six accessions from East Asia were found in this cluster. Cluster four was formed by 26 accessions mainly from East and South East Asia (24 accessions). The remaining two accessions were from East and South Africa. The accessions from West Africa were grouped in cluster five and one accession from South Africa. Cluster six contained 13 accessions mainly from East and Southern Africa; only one accession was from East Asia.

4. Discussion

Assessment of genetic diversity within cultivated crops has important implications in breeding for improvement and conservation of genetic resources. Molecular markers can be employed as a tool to reveal the diversity within crop species. In this study ISSR markers were used to assess the diversity of 83 accessions of indigenous vegetable Jute mallow.

In our study, number of polymorphic bands ranged from 2 - 6 with an average of 2.75 bands per primer. The number of bands amplified ranged from 5 to 18 bands. [15] reported a relatively high range of amplified fragments which ranged from 12 to 21 by using four ISSR primers of Jute mallow. High PIC values in our study clearly indicated usefulness and applicability of the markers used in establishing the diversity within the studied accessions. A PIC value of 0.34 from primer $(CCT)_6$ is reported as highest when polymorphism was considered across all species by [15]. This is contrary report to our observations where the highest PIC value was 0.76 from primer (GAC) TCC5. This was the most informative primer compared with the remaining primers in our study.

Highest Nei's gene diversity (0.42) was observed in population from East Asia and West Africa. High number of accessions from East Asia was used in this study and that could be a reason for high diversity observed. Relatively higher genetic diversity was detected in African populations by [22] by using AFLP markers. This is similar to our study where the overall genetic diversity detected in African populations was higher except for a population from East Africa. Low gene diversity was recorded in East African accessions indicating high similarity of the accessions in the region. In all these observations, Shannon's information index values were relatively higher than Nei's gene diversity. Apart from gene diversity based on different origins; average gene diversity (h) for complete set of all accessions was 0.34 and Shannon's information index was 0.49. This shows that there is relatively high level of genetic variation among these accessions. Similar results were reported in *N. nimmoniana*, an endangered medicinal plant in India (h = 0.3; I = 0.44) [32]. [33] reported a gene diversity of 0.29 in goat's rue accessions by using ISSR markers.

Highest pairwise genetic distance was observed between the unknown acces-

sions and the East African accessions. This indicated that East African accessions were more distinguished from the unknown accessions and the rest of other accessions. These results corroborated with the Nei's gene diversity recorded in this study. We recorded higher genetic distances in populations from East and North Africa than from the rest of other population under the present study. The remaining populations were relatively similar. This similarity may be due to sharing of genetic materials between different regions or possibly due common parents especially for accessions from East Asia and Southeast Asia where there is long history of domestication and breeding of Jute mallow [34].

In PCoA where PC1 and PC2 explained 33.4%, accessions from East Africa were distinctly found in the first and fourth quadrant separated from other accessions. This pattern of clustering is also supported by the results of Nei's pairwise genetic distance reported in this study that showed highest distance between these accessions and the rest. There was no clearly defined pattern of clustering for the remaining accessions as they overlapped in different quadrants of the PCoA. Accessions such as Cameroon Ex. Co, TOT 4623, TOT 4624, GKK 10, ES and ML-JM-10 displayed highest diversity across all four quadrants. In cluster analysis six clusters were obtained at genetic similarity coefficient of 0.53, however the clustering of geographically closer accessions was not clearly reflected in dendrogram except for cluster 4 which contained accessions from Asia. This showed that the association between genetic similarity and geographical location was insignificant. Similar results were reported in goat's rue [33] and in Azuki bean [35]. Although there was no clearly defined clustering pattern reflecting geographical distribution of the accessions in the six clusters generated, generally accessions from Africa and Asia were grouped separately. This could be attributed to species differences where most of Asian accessions were from *C. capsularis* where as those from Africa were mostly from *C. olitorius*.

5. Conclusion

Our results indicated the presence of great genetic diversity among jute mallow collection. Genetic variation among this germplasm as revealed by ISSR markers could be useful in selection of parental lines that can be crossed to generate populations that are suitable for breeding. The findings of this study can also be used in conservation of this underutilized vegetable.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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