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Genetic diversity of grapevine (Vitis vinifera L.) from Tanzania as revealed by ISSR markers

Andekelile Mwamahonje[1], Deusdedit Kilambo[2], Leon Mrosso[3] and Tileye Feyissa[1]

[1]Department of Sustainable Agriculture and Biodiversity Conservation and Ecosystem Management, School of Life Science and Bio-engineering, The Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania.

[2]Tanzania Coffee Research Institute, P.O. Box 3004, Kilimanjaro, Tanzania

[3]Makutupora Agricultural Research Institute, P.O. Box 1676, Dodoma, Tanzania

ABSTRACT

Grapevine (V*itis vinifera*L.) is a perennial horticultural crop which is used worldwide for production of wine, table grapes, dried fruits and grape juice. In spite of its importance as a cash crop, the purity of each variety and genetic diversity of this crop has not been identified. The study therefore, was conducted to assess the purity and genetic diversity among and within 10 varieties of grapevines represented by a total of 43 individual plants. By using nine ISSR primers, a total of 56 polymorphic bands were generated. Within variety diversity based on polymorphic bands ranged from 26.79% for Ruby seedless to 73.21% for Alphonce lavallee with a mean of 55.18 %, Nei's genetic diversity of 0.089–0.308 with a mean of 0.202, Shannon information index of 0.137–0.445 with a mean of 0.302 and analysis of molecular variation (AMOVA) of 76.767% within varieties were detected. With all diversity parameters, the highest diversity was obtained from Regina, Alphonce lavallee, Syrah and Makutupora white varieties, whereas the lowest was from Ruby seedless. AMOVA showed a 23.23% between varieties variability was less than that of within varieties variation. Varieties differentiation with Fixation index (FST) was 0.23. From Jaccard's pairwise similarity coefficient, Makutupora red and Chancellor were most related varieties exhibiting 0.976 meanwhile Queen of vineyard and Ruby seedless were most distantly related varieties with similarity of 0.408. Alphonce lavallee and Makutupora white varieties exhibited the highest genetic diversity. Therefore, these varieties should be considered for further improvement of this species.

Indexing terms/Keywords

Genetic distance; Genetic diversity; Grapevine; ISSR marker; Polymorphism.

Academic Discipline and Sub-Disciplines

The relevant academic disciplines for this journal is Plant genetic resource

SUBJECT CLASSIFICATION

The subject is Agriculture classification

TYPE (METHOD/APPROACH)

The research type is molecular genetic analysis based on DNA extraction and amplification using ISSR-marker

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INTRODUCTION

Grapevine is a perennial horticultural crop which is used worldwide for production of wine, table grapes; dried fruits and grape juice (Hassan et al., 2011). It has approximately 60 species and 10,000 grapevine cultivars worldwide (Emmanuel et al., 2013). The distribution of the grapevines has been reported to be high in Europe, North America and Asia (Ken and Robert, 2014). In Tanzania, grapevine is produced in Central Zone mainly in Dodoma region (Mwamahonje et al., 2015). However, its varieties and local grape cultivars are grown marginally often endangered and/or not officially registered (Schneider et al., 2001). Despite large number of clonally propagated accessions are maintained, the grapevine germplasm collections for crop breeding lack important information on genetic diversity, population structure as well as proper phenotypic assessments (Rao et al., 2014). Problems related to cultivar identification due to intermixing and lack of checking the purity have been reported within germplasm, such that one cultivar grown for different name, others are cultivated under similar name in other geographical location, in addition, there is high intermixing among grapevine genotypes (Tangolar et al., 2009). The research activities that could enhance use of existing collections for crop breeding have not been as frequent as the conservation activities because of lack of information concerning genetic variation of grapes as well as long period in field experiments (Emmanuel et al., 2013). This has led to drastic reduction of grapevine genetic diversity for decades and the major cultivars focus wine production in the region to remain few. Therefore, the use of molecular markers for grapevine identification has been shown to supplement ampelography (Dhanorkar et al., 2005). The uses of markers have proven the valid tool for characterization and finding of synonymies within grapevines because they are not affected by the environmental condition and their interpretation is more realistic (Almadanim et al., 2007; Pinto et al., 2003). Some of molecular markers which have been useful for characterization of grapevines include; Random Amplified Polymorphic DNA (RAPD), Simple sequence repeats (SSRs) and Inter Simple Sequence Repeat (ISSR) (Kocsis et al., 2005). ISSR markers have been reported to be easy, rapid and consistent, no need of prior sequence information (Moreno et al., 1998). It does not only play an important function of analyses intra varietal variations in grapevines but also identifies cultivars (Hanorkar et al., 2005). Research is needed to be carried out for tracing their origin and genetic variation and population structure through time. A proper identification of representative ancient grapevine varieties is necessary and can make easy access to genetic diversity available for collections (Guo et al., 2013). This ultimately enables development of improved varieties with different traits including plant disease resistance. This study therefore aimed to utilizes ISSR marker for genome-wide germplasm characterization to assess genetic diversity within and among the grapevine varieties.

MATERIALS AND METHODS

Plant materials

Forty three individual plants of ten grapevine varieties (Queen of vineyards, Halili belyji, Regina, Black rose, Makutupora red, Syrah, Chancellor, Ruby seedless, Alphonce lavallee, and Makutupora white) were collected from Makutupora Agricultural Research Institute in Dodoma (Longitude:35º, 46.093'E and Latitude: 05º, 58.669'S) (Altitude: 1080m). Three to five plants per variety were sampled making a total of 43 samples, three leaves from each plant were sampled; young leaves from each plant was collected and packed in the envelope. All samples were frozen in ice box at the time of collection. The samples were transported to Mikocheni Agricultural Research Institute in Dar-es-salaam and then stored at -80°C before genomic DNA analysis.

DNA extraction

About 200-300 mg fresh leaves from each sample were ground to fine powder with mortar and pestle and the DNA was extracted using the CTAB method described by Piccolo et al. (2012) with minor modifications. The quality of isolated DNA was checked by gel electrophoresis using 1 % agarose gel and later the concentration was determined by nanodrop 1000 Spectrophotometer.

ISSR-PCR amplification condition and electrophoresis

Twenty ISSR primers were screened using a total of twenty individuals by random selection of two individuals from each variety, and nine primers that produced clear, reproducible and polymorphic band pattern were selected for further study using 43 individuals of the 10 populations (Table 2). PCR reactions were performed in a 25 µL reaction volume [1.7 µL of 10X Taq buffer (with $MgCl_2$), 3 µL of 100 mMdNTPs, 1 µL (50 ng) of genomic DNA, 1µL 10 pM primer, 0.3 µL of Taq DNA polymerase (1 U), 18 µL of sterile water] using Eppendorf Master Cycler (Eppendorf, USA). PCR was performed by using following thermal profile: 94°C for 5 minutes of initial denaturation, 94°C for 30 seconds, 45 seconds at a primer-specific annealing temperature (between 50–55°C) depending on the primers used,72°C for 2 minutes (35 cycles); final extension at 72°C for 10 minutes. The ISSR-PCR products were then electrophoresed on 1.5 % agarose gel in 1 X TAE buffer at constant voltage of 150 V for 0.75-1 h. The gel was then stained with ethidium bromide and visualized under UV trans illuminator and documented using gel documentation system. Band size was estimated using1kb plus ladder.

Data analyses

Polymorphic fragments (bands) were scored manually as binary data; 1 for present, 0 for absent and 999 for missing data. Various softwares were used for analysis of binary data matrix. Percentage of polymorphic bands (PPB), Nei's (1973) gene diversity (h), Shannon– Weaver diversity index (I) Lewontin (1972) were estimated using POPGENE Version 1.32 (Yeh et al., 1999) under the assumption of Hardy–Weinberg equilibrium. Two comparable estimators: Nei's gene diversity

(h) and Shannon's information indices (I) were used to calculate genetic diversity for each variety. Jaccard's similarity coefficient (Jaccard 1908) was used to estimate similarity between pairs of varieties from NTSYS- pc version 2.02 (Rohlf, 2000). An analysis of molecular variation (AMOVA) was used to estimate genetic variance within and among each population using Areliquin version 3.01 (Excoffier et al*.,* 2006). Cluster analysis was performed to construct dendrograms with both Unweighted Pair Group Method with Arithmetic averages (UPGMA) tree using NTSYS- pc version 2.02 and neighbor joining (NJ) tree (Saitou and Nei, 1987) using Free Tree 0.9.1.50 (Pavlicek et al., 1999) using Jaccard's coefficient similarity. Two- dimensional principal coordinate analysis (PCoA) was also used to reveal patterns of variation among individual samples based on Jaccard's similarity coefficient that was drawn using PAST software version 1.18 (Hammer et al,. 2001).

RESULTS

ISSR band variation and level of polymorphism

A total of 56 polymorphic bands were amplified by nine primers with an average of 6.22 bands per primer having ISSR fragment size ranged from 300-3500 base pair. Percentage of Polymorphic bands (PPB %) was 100% for both primers used at variety level. The primer 856 generated only four polymorphic bands while other two primers 815 and 890 generated 8 polymorphic bands (Table 1).The highest Nei's gene diversity (0.38) and Shannon information index (0.57) were displayed by primer 889. In contrast, primer 856 showed the least Nei's gene diversity and Shannon information index with 0.22 and 0.35 values, respectively. The mean Nei's gene diversity and Shannon information index for all primers were 0.31 and 0.47, respectively (Table 1). The largest base pair (3500 bp) was recorded from two individuals (42-43) of Makutupora white (Fig. 1).

Table 1. ISSR Primers with their scored band polymorphism and level of gene diversity

			Scored band			Diversity	
Primer	Sequence 5' 3'	Band size (pb)	Total bands	NPB	PPB (%)	h+SD	I+SD
807	(AG)8 T	300-2000	6	6	100	0.302±0.128	0.471± 0.152
811	(GA)8 C	300-1500	6	6	100	0.378± 0.102	0.561± 0.116
815	(CT)8 G	300-3000	8	8	100	0.233± 0.182	0.367± 0.244
855	(AC)7 YT	400-1500	5	5	100	0.243± 0.153	0.390± 0.201
856	(AC)7 YA	400-1500	4	4	100	0.223± 0.206	0.353± 0.269
857	(AC)8YG	400-2000	7	7	100	0.298± 0.132	0.464± 0.166
888	BDB(CA)7	300-1500	7	7	100	0.359± 0.128	0.537± 0.149
889	DBD(AC)7	300-1500	5	5	100	0.381± 0.093	0.566± 0.107
890	VHV(GT)7	300-1500	8	8	100	0.357± 0.112	0.537± 0.131
Average			6	6	100	0.308±0.137	0.472±0.170
All primers			56	56	100		

NPB = Number of polymorphic band, PPB = Percentage of polymorphic band, h = Nei's (1973) gene diversity, I = Shannon's Information Index.

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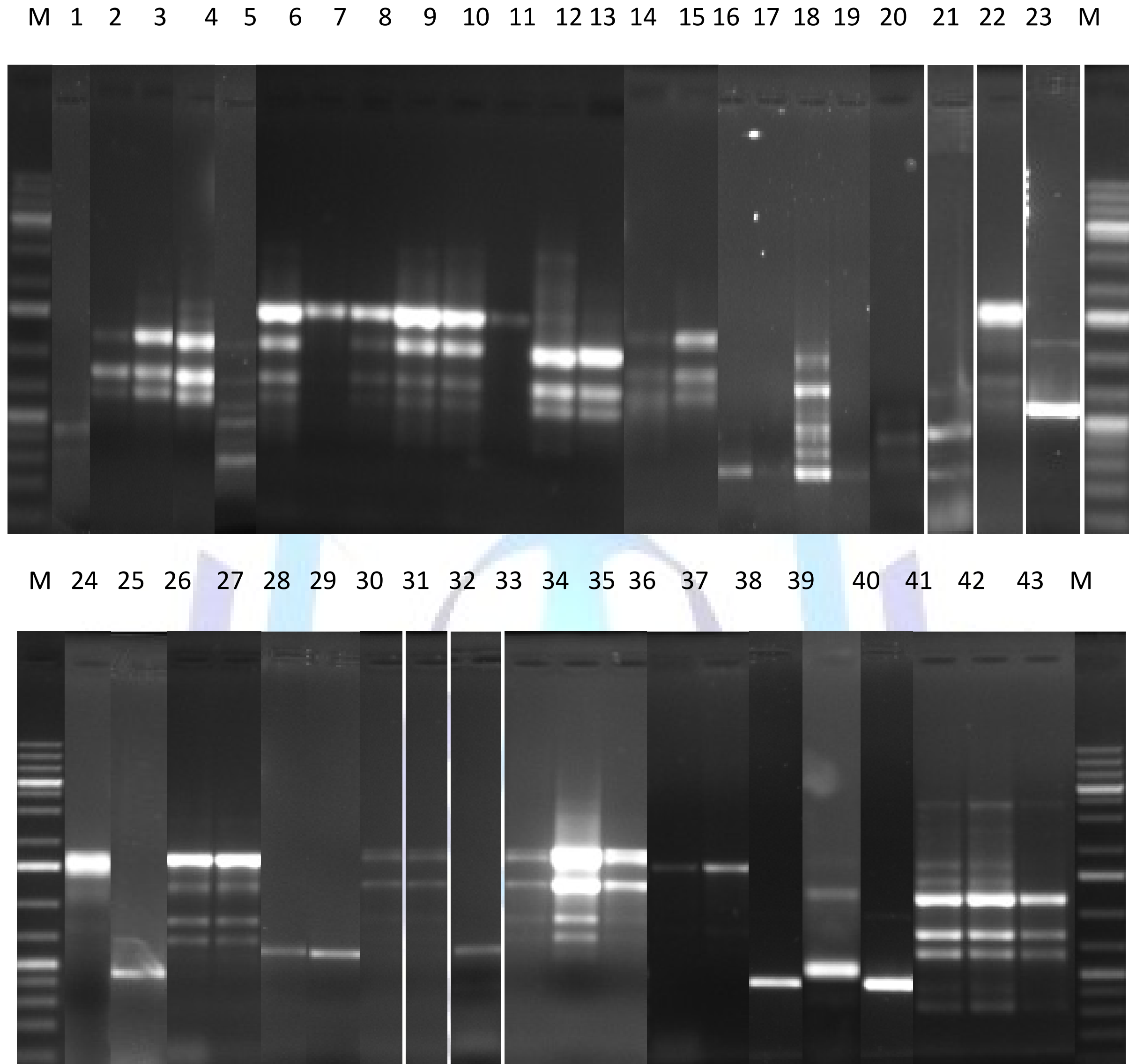

Fig. 1 ISSR fingerprints generated using primer 815 of 1.5% agarose gel by using 1 kb plus ladder (75-5000 bp), the number of wells from 2-4 represents individuals of varieies Black rose,5-7 Regina, 8-12 Queen of vineyard, 13-17 Alphoncelavallee, 18-22 Makutupora red, 23,24,24,25,27 Chancellor, 29-33 Halilibelyji, 34-37 Syrah, 38-40 Ruby seedless and 41-45 MakutuporaMakutupora white. Wells number1, 26, 28 and 46 represent 1 kb plus ladder

Population genetic diversity

Within-variety Percentage of polymorphic band ranged from 26.79% for Ruby seedless to 73.21% for varieties Aluphoncelavallee, Regina and Syrah with a mean of 55.18 % respectively, indicating that Aluphoncelavallee, Regina and Syrah had the highest genetic diversity while Ruby seedless exhibited the lowest. Gene diversity was ranged from 0.09 for Ruby seedless to 0.31 for Aluphoncelavallee with mean of 0.20, and the same patterns has been observed for the Shannon information index which ranged from 0.14 for Ruby seedless to 0.45 for Aluphoncelavallee with a mean value of 0.30. The grape varieties of Makutupora red, Queen of vineyards, Chancellor, Black rose, Syrah, Regina, Halilibelyji and Makutupora white showed gene diversity value which ranged from 0.11 to 0.29. The least percentage of polymorphic band (26.79%), gene diversity (0.09) and Shannon diversity index (0.14) were exhibited by Ruby seedless population. Generally, grape populations Makutupora white and Aluphoncelavallee showed the highest gene diversity (0.29 and 0.31), PPB (71.43 and 73.21 %), and Shannon index (0.43 and 0.45), respectively. Although samples of Makutupora white and Makutupora red were local populations; Makutupora white exhibited higher gene diversity with value of 0.29 (Table 2).

Table 2. Measures of analyses of gene diversity, population differentiation and gene flow estimate in each and overall grapevine varieties

Variety	NPB	PPB%	h+SD	I+SD	Gst	Nm
Black rose	27	48.21	0.192± 0.209	0.282±0.302	_	_
Regina	41	73.21	0.241± 0.157	0.373±0.235	_	_
Queen of vineyard	16	28.57	0.132± 0.215	0.186±0.302	_	_
Alphonce lavallee	41	73.21	0.308± 0.205	0.445±0.287	_	_
Makutupora red	19	33.93	0.111± 0.174	0.169± 0.254	_	_
Chancellor	30	53.57	0.163± 0.164	0.256± 0.251	_	_
Halili belyji	39	69.64	0.269± 0.188	0.399± 0.273	_	_
Syrah	41	73.21	0.226± 0.178	0.349± 0.249	_	_
Ruby seedless	15	26.79	0.089± 0.153	0.137± 0.232	_	_
Makutupora white	40	71.43	0.293± 0.203	0.426± 0.286	_	_
Mean	31	55.18	0.202±0.185	0.302±0.267	0.404	0.737

**Gst- Genetic differentiation coefficient, Nm=estimate of gene flow from Gst. Nm= 0.5(I – Gst)/Gst, h = Nei's (1973) gene diversity, I = Shannon's Information Index, NPB = Number of polymorphic band, PPB = Percentage of polymorphic band*

Varietal genetic divergence

The overall, Jaccard similarity coefficient that ranged from 0.408 to 0.976 was recorded. The pairwise comparison of Jaccard value showed that Makutupora red and Chancellor as well as Chancellor and Halilibelyji were closest varieties with similarity coefficient of 0.976 and 0.973 respectively. The distantly related varieties were Queen of vineyard and Ruby seedless with coefficient dissimilarity of 0.408 (Table 3).

Table 3. Jaccard pairwise similarity between varieties

V ID	1	2	3	4	5	6	7	8	9	10
	****	0.8114	0.7126	0.8759	0.7652	0.7812	0.8354	0.8431	0.7720	0.8220
		****	0.4996	0.7887	0.9549	0.9722	0.9625	0.9261	0.9534	0.8742
			****	0.7788	0.4380	0.4540	0.5633	0.5593	0.4077	0.6432
				****	0.7648	0.7803	0.8384	0.8181	0.7291	0.8171
					****	0.9757	0.9554	0.9236	0.9485	0.8713
						****	0.9726	0.9368	0.9621	0.8713
							****	0.9466	0.9450	0.9027
								****	0.8992	0.8774
									****	0.8512
										****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)Key: 1- Black rose, 2- Regina, 3- Queen of vineyard, 4- Alphonce lavallee, 5- Makutupora red, 6- Chancellor, 7- Halili belyji, 8- Syrah, 9- Ruby seedless, 10- Makutupora white, ID- Identification and V- Variety

Analysis of molecular variance (AMOVA) indicated a total of 76.77% within varieties and 23.23% among varieties variation with 0.23 fixation index (FST) indicating about 23 % genetic differentiation among varieties independent of the marker used (P≤ 0.05) (Table 4).

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Table 4. Analysis of molecular variance (AMOVA) among the 10 grape vine varieties

Source of variation	Sum of squares	Variance components	Percentage of variation	Fixation Index (FST)	P value
Among varieties	175.318	2.6863	23.23325	0.23233	0.05
Within varieties	264.5	8.876	76.76675		
Total	439.818	11.5623			

Principal coordinate analysis (PCoA)

Principal coordinate analysis (PCoA) grouping was performed using the first three coordinates having Eigen values of 4.66, 2.14 and 1.65, which accounted for 12.30%, 4.37% and 4.37% variation, respectively. On the first principal coordinate, varieties having relatively large variation both within and among them were grouped together. Most individuals from the Queen of vineyards and Makutupora white varieties formed a close pattern on the first coordinate towards negative side. However, there were intermixed individuals with other varieties. The individuals from Chancellor, Ruby seedless, Halilibelyji and Makutupora white were dispersly clustered on first coordinate towards positive Eigen values. However, they were intermixed. The individuals from varieties Makutupora red, Syrah and Chancellor were dispersed on the second principal coordinate. In the second principal coordinate, individuals of Regina, Alphonse lavallee and Makutupora white were intermixed (Fig. 2).

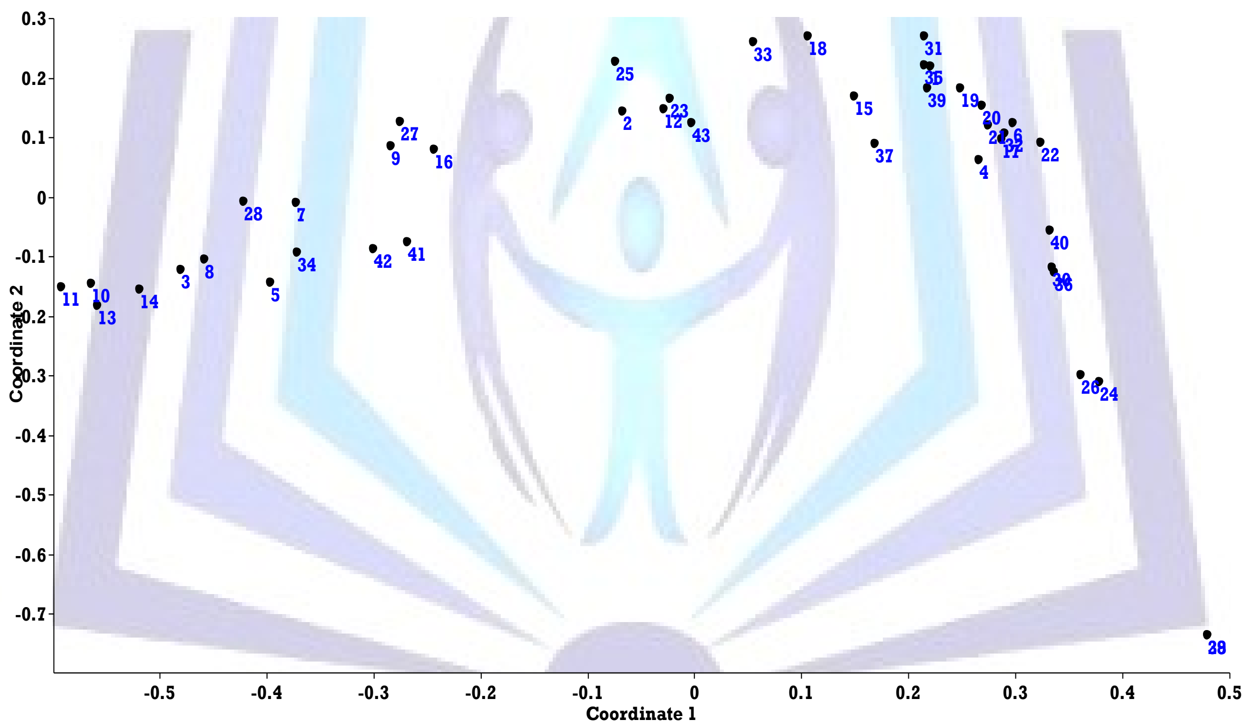

Fig 2. Two-dimensional principal coordinate analyses of 43 individuals of 10 grapevine varieties assessed with ISSR.

Key: 1-3- Black rose, 4-6-Regina, 7-11 Queen of vineyards, 12-16- Alphonce lavallee,17-21-Makutupora red,22-26 Chancellor, 27-30- Halili belyji, 31-35- Syrah36-38-, Ruby seedless, 39-43-Makutupora white

Cluster analysis

Clustering based on NJ tree resulted in two main clusters ignoring four outliers of Chancellor (22, 24, 26), one of Alphonce lavallee (15) and Regina (6). In the outlier cluster, one individual of Regina formed a very small separate cluster. The second cluster grouped into two sub-clusters, the first sub-cluster contained four individuals of Alphonce lavallee, Makutupora red, Halili belyji and Ruby seedless population. In the second sub-clusters individuals of varieties Blackrose, Regina, Queen of vineyards, Alphonce lavallee, Makutupora red, Chancellor, Halili belyji, Syrah, Ruby seedless and Makutupora red tend to form separate groups averaged by individuals from the same variety intermixed with other

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individuals from other varieties (Fig. 3). Most of the individuals from the variety Queen of vineyard and Chancellor maintained within clusters with little intermixing of other individuals. The highest intermixing was from individuals of variety Ruby seedless indicating large variation with other varieties.

Fig 3.NJ tree based analysis of 43 individuals of grapevine varieties using Jaccard's similarity coefficient.Key: 1-3- Black rose, 4-6-Regina, 7-11 Queen of vineyards, 12-16- Alphonse lavallee,17-21- Makutupora red,22-26 Chancellor, 27-30- Halili belyji, 31-35- Syrah36-38-Ruby seedless, 39-43- Makutupora white.

UPGMA based dendrogram for the 10 grape varieties generated two major clusters that further formed sub-clusters (Fig. 4). The first sub-cluster contained populations of Regina, Halili belyji, Chancellor and Makutupora red. The second sub-cluster contained populations of Syrah and Makutupora white. The third sub-cluster contained populations Queen of vineyard and Alphonce lavallee. The fourth sub- cluster contained populations Black rose and Ruby seedless (Fig. 4).

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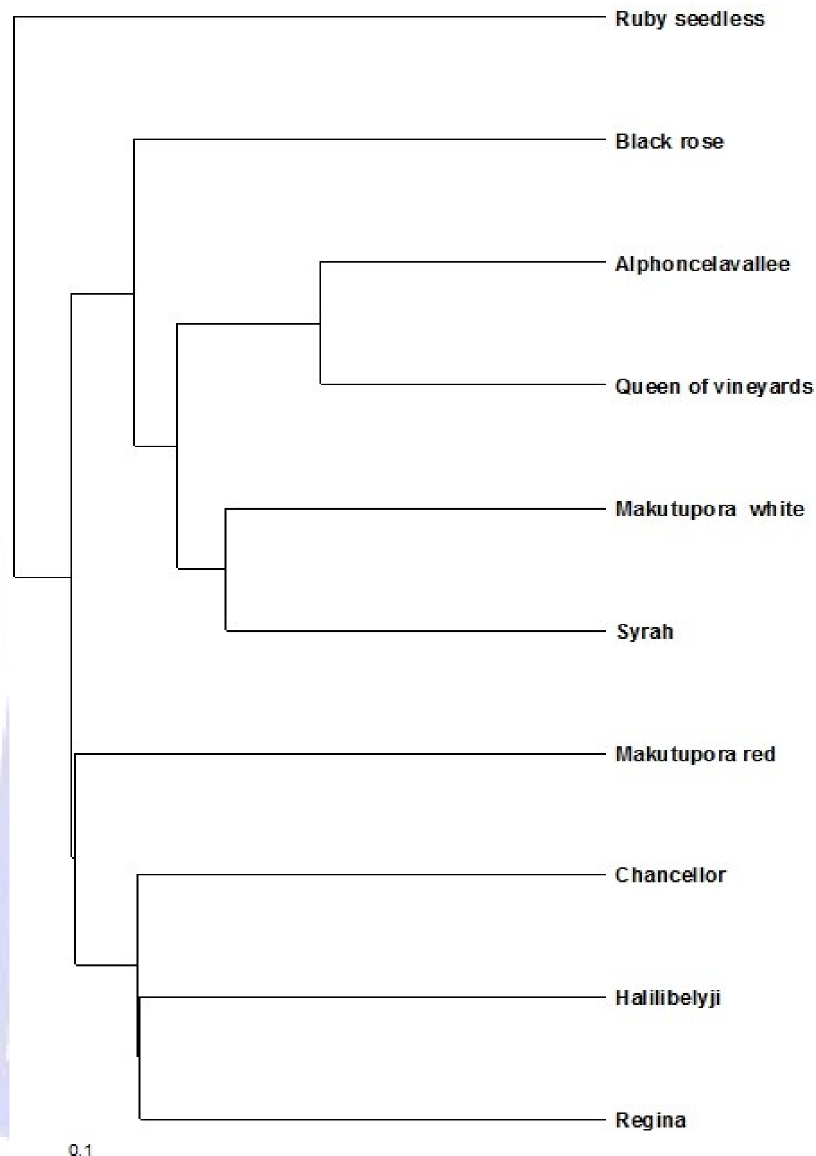

Fig 4.PhylogramUPGMA based dendrogram for 10 grapevine populations on Jaccard similarity coefficient

DISCUSSION

Genetic diversity

The present study reports molecular genetic diversity parameters for Tanzanian grapevine. Twenty ISSR primers were used to evaluate genetic diversity of grapevine varieties. A total of 56 polymorphic bands were generated. The primer 815 (CT)8 G and VHV(GT)7 amplified the highest number of polymorphic bands. The efficiency of ISSR to show 100% polymorphism per primer could be due to good screening of primers, the close results were documented by Asadiar et al., (2012);Dhanorkaret al., (2014) that ISSR markers can be used in population genetic studies as they effectively detect very low levels of genetic variation. The present study was contrasted with Hassan et al., (2012) who reported an average of polymorphism 53.93 % of all primers used this could be caused by low efficient of primers used for amplification. The mean Nei's and Shannon information index for all primers were 0.20 and 0.30 respectively, indicating the massive genetic diversity at population level which could be due to long primers and good annealing temperature used in DNA amplification and therefore, high polymorphism indicating high genetic variation among primers tested (Zeinali et al., 2012; Dhanorkaret al., 2014).

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Genetic differentiation and structure of grapevine

Genetic diversity estimators, Percentage of polymorphic band, gene diversity (h), and Shannon's diversity (I) were the highest for Alphonce lavallee (73.21 %, 0.308, 0.445) this could probably be due to effects on gene variation associated with environmental changes which is totally different from the origin (France) of this variety characterized by temperate climate, in addition the high diversity may be due to depression of genes available in Alphonce lavallee due to probably

hybrid of this variety. In contrast with Seyedimoradi et al., (2012) reported the highest genetic diversity of 77% with an average of 64.5% across all varieties this could be due to the fact that some genetically related cultivars are morphologically very similar and difficult to distinguish visually which may easily misplaced with other variety (Asadiar et al., 2012; Mwamahonje et al., 2015). In our study we found genetic diversity within available grapevine varieties and confirmed previous analyses suggesting that grapevine is a very diverse species (Martinez et al. 2006; Ibanez et al., 2009). In addition, other studies reported the highest genetic diversity of 0.99 with percentage polymorphic bands (PPB) of 77.31% which is close with our results with small variation this could be due to mixed cropping among varieties which phenotypically most look like and therefore causing high possibility of genetic diversity within varieties (Seyedimoradi et al., 2012; Fan et al., 2015). The lowest percentages of polymorphic bands, gene diversity and diversity index were revealed by Ruby seedless (26.79, 0.089 and 0.137) respectively, showing that individuals of Ruby seedless could be uniform, low intermixing and high purity of planting materials used. Therefore, high polymorphism in our study could possibly be due to intermixing of different varieties on the same plot, furthermore, difficulties in identification of grapevine and their cuttings due to resemblances phenotypically and therefore easily intermixing one variety and another during planting without knowing. The low genetic diversity in Ruby seedless variety could also be due to existence of uniformity due to vegetative propagation (Choudhary et al., 2014; This et al., 2006). The present study showed that Ruby seedless variety is distantly related to Queen of vineyard by 0.408, Makutupora red from Tanzania and Chancellor from France, showed closest genetic similarity of 0.956. From all varieties studied, Makutupora red and Chancellor showed closest genetic similarity irrespective of the longest geographical distance between them. Hence, our study showed that there is no strong correlation between geographic distance and genetic diversity (Seyedimoradi et al., 2012; Zeinali et al., 2012). AMOVA indicated that 76.77% of total variation was accounted for within variety variation. The estimate of population differentiation of grapevine using FST was 0.23 indicating 23 % of the genetic variation could be due to differences among varieties.

PCoA, NJ and UPGMA showed comparable results with an indication of the strong clusters in majority of individuals of the studied varieties. In our study, the overall, Jaccard similarity coefficient ranged from 0.408 to 0.976. The pairwise comparison of Jaccard value showed that Makutupora red and Chancellor are the closest. The distantly related populations were Queen of vineyard and Ruby seedless with coefficient similarity of 0.408. This is in agreement with the results reported by Doulati-Banehet al., (2009); Jiang et al., (2009) and Zeinali et al., (2012) on cultivars of *V. vinifera*. High level of observed polymorphism reflects ability of the ISSR technique to effectively distinguish populations among *V. vinifera*. Previous studies on genetic diversity of grapevine *V. vinifera* have detected low level of polymorphism using RAPD marker (Herrera et al., 2002). In contrast, high polymorphism was reported using RAPD marker (Lima et al.,2006). The parameters also revealed highly intermixed individuals possibly due to admixture that may be resulted from gene flow, migration of people from place to place, short and long distance marketing of cuttings.

The UPGMA-based dendrogram of the 10 grapevine varieties formed two major clusters with further sub-clusters. The highest within-cluster similarity was observed between Makutupora red and Chancellor, which are different in geographical origin, similar pattern of cluster distribution was reported by Asadiaret al., (2012). Loss of originality of the specific population could be caused by clonal variation associated with vegetative propagation (Fan et al., 2015). Makutupora white and Syrah clustered were closely related and the same was observed for Alphonce lavallee and Queen of vineyard. Black rose and Ruby seedless were at least closely related and other results reported by Sabir et al., (2009).

Implications for conservation and use

The knowledge on genetic diversity of crop facilitates the efficient protection and use of genetic resources. Based on our study, no genetic information study using molecular markers has ever been conducted on grapevine in Tanzania. The present study showed the existence of high genetic diversity in some varieties. However, there is allelic loss of local varieties which ultimately, might lead to complete loss of the populations. Conservation should be done to protect the existing genetic diversity of local and improved grapevine varieties. Small variation on genetic diversity and diversity index was observed between local and improved varieties of which, the highest and the lowest was from Alphonce lavallee (0.308, 0.445) and Ruby seedless (0.089, 0.137) varieties respectively. The varieties with high genetic diversity could be of interest because of their importance (Dallakyan et al., 2015). The local varieties of Makutupora red and Makutupora white had genetic diversity and diversity index of 0.111, 0.293, and 0.169 and 0.426 respectively. The high diversity of Makutupora white compared to Makutupora red could be due to mutation or poor vegetative propagation as reported by This et al., (2006). Uniqueness of Makutupora red to maintain its originality and high preference by local community in Tanzania might be due to high yield, disease and drought tolerance as well as monocropping. There is need of conserving the present grapevine varieties in order to keep available for a long time use. The varieties with high and low genetic diversity should be conserved to maintain their originality.

CONCLUSION AND RECOMMENDATION

Based on identification of individuals within and among varieties tested high genetic variability and divergence have been identified. Genetic diversity is important to provide information on gene pool for future use and to prevent the loss of these

genetic resources which are useful in breeding programs. ISSR markers are reliable and efficient for the investigation of genetic diversity among and within grapevine varieties. Though, to be sure with these results, we recommend further genetic diversity studies to be done using more varieties, primers and other marker systems. The varieties which showed high genetic diversity should be well conserved in addition, more research should be done for confirmation of high diversity existed within grapevine varieties. However, we further recommend that even the varieties which showed low genetic diversity should be conserved because they might have some peculiar traits which may be useful in crop

improvement. In addition, sequencing and characterization of useful markers might be important for improved selections of traits of breeder interest and could improve this crop through marker assisted selection.

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