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Genetic diversity assessment of farmers' and improved potato (*Solanum tuberosum*) cultivars from Eritrea using simple sequence repeat (SSR) markers

Biniam Mesfin Ghebreslassie[1,2]*, S. Mwangi Githiri[2], Tadesse Mehari[1], Remmy W. Kasili[3], Marc Ghislain[4] and Eric Magembe[4]

[1]Department of Horticulture, Hamelmalo Agricultural College, Keren, Eritrea.
[2]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[3]Institute of Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[4]International Potato Center (IPC) P.O. Box 25171, Nairobi 00603, Kenya.

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Sixty three potato clones (51 farmers' and 12 varieties) from Eritrea, 18 and 12 varieties from Kenya and Rwanda, respectively were characterized using 12 highly polymorphic simple sequence repeat (SSR) markers. The study was designed to assess the genetic diversity and varietal distinctness among the different samples. In total, 91 alleles ranging between 2 (STM1053) to 13 (STM0031) alleles per marker were scored. All but 97.8 SSR markers were highly polymorphic with an average PIC value of 0.87 (0.51 to 0.98). All of the 51 farmers' cultivars were clearly distinct from each other. Samples from Eritrea showed the highest genetic diversity as explained by the diversity index (h). The principal coordinate analysis (PCoA) revealed that the local farmers' Eritrean samples are different from the Kenyan, Rwandese and even the imported varieties. Genetic distance analysis generated three clusters correlating with the PCoA findings. Cluster I consisted of 45 samples with 6 sub-clusters; Cluster II consisted of 29 samples with a majority (26) from Eritrea while cluster III consisted of 19 samples. Potato materials from Eritrea appeared to cluster separately from the other samples, which reflects a contribution from the Tuberosum germplasm prominent in temperate regions, unlike from the Andigenum germplasm for Kenyan and Rwandan potato materials. Most of the Eritrean samples in cluster I are farmers' cultivars with intermediate maturity, good performance and better tuber quality characteristics. Cluster II contains mainly the imported variety from Eritrea characterized by late emergence and late maturity. The Kenyan and Rwandese were grouped mainly in Cluster III. In summary, the farmers' cultivars are distinct from the Kenyan and Rwandese materials and represent more genetic diversity than the varieties imported into Eritrea. This finding is of interest to national breeding program to use the farmer's materials as source of genetic variation for traits of interest.

Key words: Potato, simple sequence repeat (SSR), principal coordinate analysis (PCoA), cluster analysis, Eritrea, multivariate.

INTRODUCTION

Potato *(Solanum tuberosum* L*)* is the fourth most important food crop in the world, after maize, wheat, and rice. The crop plays a significant role in human nutrition worldwide, where more than 320 million tons of potatoes

are produced annually on 20 million hectares of land (Poczai et al., 2010). Being one of the major food staple crops in the Eastern African countries, potato is rated among the choice crops for food security and income generation for resource-poor farmers (Kyamanywa et al., 2011).

Likewise, potato is one of the most important and widely grown vegetable crops in Eritrea. Over 70% of the population lives in rural areas and relies on subsistence agriculture (NEPAD, 2005), where the potato crop plays an important economic role as it is cultivated by small scale and subsistence farmers. The crop is generally believed to have entered into Africa around the turn of 20th century (FAO, 2008). It is also assumed that the Italian colonizers introduced the crop to Eritrea around the same time. During the long tradition of potato cultivation in Eritrea, farmers adopted a number of landraces. We define here a landrace as potatoes produced by farmers under a local name with no known origin. Although farmers are now growing recently introduced varieties from Europe, they still recognize the important values of landraces such as good resistance to various stresses and market value, but these landraces are unfortunately low in yielding (Biniam et al., 2014).

The landraces and imported potatoes varieties have not been genetically characterized, which would help to understand their genetic diversity and distinctness. This knowledge is important to better orient potato breeding and germplasm conservation (Liao and Guo, 2014). For a long time, breeding was largely done based on morphological descriptors (Yada and Tukamuhabwa, 2010) but with the new molecular technologies in breeding programs with more diverse genetic resources, including those bearing known alleles of interests can be utilized (Nováková et al., 2010).

Molecular markers are useful tools for genetic diversity assessment, and classification of genetic materials (Tiwari et al., 2013; Spooner et al., 2007). Several types of molecular markers have been applied for potato genetic characterization with each having its own pros and cons (Nováková et al., 2010). Simple sequence repeat (SSR) markers are particularly useful, since they are highly polymorphic, represent co-dominant markers (Tiwari et al., 2013; Ghislain et al., 2009; de Galarreta et al., 2007; Chimote et al., 2007; Barandalla et al., 2006), have low operational costs, and are highly reproducible (Favoretto et al., 2011; Spooner et al., 2007). To that effect, they have been used extensively in potato and other crops for various breeding and diversity studies (Ghislain et al., 2009; Kandemir et al., 2010). Ghislain et al. (2004) recommended 18 highly informative and user friendly SSRs markers after screening 156 SSRs for their characterization power in potato.

According to Raker and Spooner (2002), the high level of polymorphism and heterozygosity explained by SSR markers in potato make them also useful tools for measuring genetic differences between closely related taxa. Several other studies demonstrated that five or six pairs of SSR markers were sufficient to distinguish many potato cultivars (Zhuk et al., 2008). It was reported by Berg and Hamrick (1997) that a sample size of 30 to 50 using 10 to 20 markers can successfully characterize the genetic diversity in the population. The authors added that adding more individuals or loci will not appreciably change the standard statistics of genetic variability.

This study reports the assessment of genetic diversity and relatedness among farmers's potato and introduced varieties from Eritrea and compares it to other potato germplasm from Kenya and Rwanda using 12 SSR markers. The results will benefit potato farmers, breeders and germplasm conservationists.

MATERIALS AND METHODS

Plant material

A total of 63 potato samples from Eritrea and 30 samples from the International Potato Center (CIP Sub-Saharan regional office) comprising 18 from Kenya and 12 from Rwanda were analyzed. The samples from Eritrea were collected from farms in Zoba Debub (27) and Zoba Maekel (24), with additional varieties from the National Agricultural Research Institute, NARI (12). The samples from Eritrea were grown in pots in a greenhouse located at NARI Halhale, Eritrea while those from CIP were grown in the greenhouse located at the Biosciences eastern and central Africa (BecA) –ILRI hub in Nairobi Kenya. Two weeks after germination, a fresh leaf from each sample was collected for DNA extraction.

DNA extraction and PCR

Extraction of DNA was done from fresh tender leaves with a combination of the modified Cetyl-trimethyl Ammonium Bromide (CTAB) (Semagn, 2014) and the QIAGEN DNeasy mini kit methods. The quantity and quality of the extracted DNA was determined using the Nanodrop® 2000C spectrophotometer and agarose (0.8%) gel electrophoresis stained with GelRedTM (Biotium USA) (25 µl/L). Genomic DNA was normalized to a final concentration of 20 ng/µl after which it was subjected to PCR amplification using a set of 12 fluorescently labelled SSR markers (Table 1) as described by Ghislain et al. (2009). The PCR conditions were set as follows: Denaturation at 94°C for 5 min; 35 cycles consisting of a denaturation at 94°C for 30 s, annealing (55 to 60°C depending on the markers) for 1 min, and extension at 72°C for 1 min and a final extension at 72°C for 20 min.

Quality of the amplified PCR products were determined using 2% agarose gel electrophoresis after 45 min at 70 V, and observed using Syngene bio-imaging gel documentation. High quality amplified PCR products were pooled based on the florescent dye used and analyzed by capillary electrophoresis on ABI PRISM 3730 (Applied Biosciences).

*Corresponding author. E-mail: bmghebreslassie@gmail.com

Table 1. Characteristics of the 12 SSR markers used in the study.

Locus	Motif	Forward	Reverse	Size (bp) range	Dye	Annealing T (ºC)	Chromosome position
STG0016	$(AGA)_n$	AGCTGCTCAGCATCAAGAGA	ACCACCTCAGGCACTTCATC	137-174	PET	56	I
STM5114	$(ACC)_n$	AATGGCTCTCTCTGTATGCT	GCTGTCCCAACTATCTTTGA	297-325	VIC	55	II
STM1053	$(TA)_n$ $(ATC)_n$	TCTCCCCATCTTAATGTTTC	CAACACAGCATACAGATCATC	170-196	6-FAM	55	III
STI0012	$(ATT)_n$	GAAGCGACTTCCAAAATCAGA	AAAGGGAGGAATAGAAACCAAAA	183-234	NED	56	IV
STI0032	$(GGA)_n$	TGGGAAGAATCCTGAAATGG	TGCTCTACCAATTAACGGCA	127-148	PET	60	V
STI0004	$(AAG)_n$	GCTGCTAAACACTCAAGCAGAA	CAACTACAAGATTCCATCCACAG	83- 126	PET	56	VI
STM0031	$(AC)_n$ ... $(AC)_n$ (GCAC) $(AC)_n$ $(GCAC)_n$	CATACGCACGCACGTACAC	TTCAACCTATCATTTTGTGAGTCG	168-211	NED	60	VII
STM1104	$(TCT)_n$	TGATTCTCTTGCCTACTGTAATCG	CAAAGTGGTGTGAAGCTGTGA	178-199	VIC	60	VIII
STM1052	$(AT)_n$ GT $(AT)_n$ $(GT)_n$	CAATTTCGTTTTTTCATGTGACAC	ATGGCGTAATTTGATTTAATACGTAA	214-263	NED	55	IX
STM1106	$(ATT)_n$	TCCAGCTGATTGGTTAGGTTG	ATGCGAATCTACTCGTCATGG	151-214	VIC	60	X
STM0037	$(TC)_n$ $(AC)_n$ AA $(AC)_n$ $(AT)_n$	AATTTAACTTAGAAGATTAGTCTC	ATTTGGTTGGGTATGATA	87-133	6-FAM	55	XI
STI0030	$(ATT)_n$	TTGACCCTCCAACTATAGATTCTTC	TGACAACTTTAAAGCATATGTCAGC	94- 137	6-FAM	56	XII

Data analysis

Fragment analysis from the raw data generated on the ABI PRISM 3730 was done using the GeneMapper v4.1 to determine and score allele peaks. Most genetic analysis software is designed for diploid organisms and therefore, their use for ploidy crops like potato required conversion of co-dominant SSR markers (up to 4 alleles per locus) into dominant markers (presence and absence of each allele) as described by Kubik et al. (2009). Data was converted into binary (0/1) results using the ALS binary software. AlleloBin was used to determine the exact allele call size. The quality index from the AlleloBin was interpreted as follows: 0.00 to 0.30: no inspection required; 0.31 to 0.40: binning likely good; 0.41 to 0.45: binning or sizing poor; > 0.45: binning and sizing unacceptable (Idury and Cardon, 1997). GenAlex version 6.4 (Peakall and Smouse, 2012) was used to calculate genetic distances matrix among the populations which further yielded the Principal Coordinate Analysis (PCoA) to display graphic distribution of populations. GenAlex was also used to calculate Analysis of Molecular Variance (AMOVA) to compute the differences of variance among the populations and unbiased genetic identity among populations based on 999 permutations. DARwin 6.0 (Perrier and Jacquemoud, 2006) was used to construct a cluster tree by estimating dissimilarity indices based on the binary data (simple allele matching). The genetic similarity matrix of the populations was calculated using Jaccard coefficient after which a dendrogram was generated using Unweighted Pair-Group Method using Arithmetic averages (UPGMA) based on the estimates of genetic similarity. Power-Marker version 3.25 (Liu and Muse, 2005) was used to calculate Polymorphic Information Content (PIC) to estimate the power of each of the markers in explaining variation among the populations as well as to generate PCoA at the population level.

RESULTS

Allele profile

Analysis of the data using AlleloBin yielded a total of 91 amplified alleles, of which 33 alleles (36%) were rare (≤5%) allele. The number of allele per locus ranged between 2 (STM1053) to 13 (STM0031) with an average of 8 alleles per marker (Table 2). The quality index of the markers ranged from 0.089 (STM1053) to 0.5 (STM0032) with an average of 0.322. There was a moderately negative correlation between the motif size (SSR repeats length) and the allele number amplified (r = -0.31); and PIC (r = -0.37). There was also a moderate positive correlation between the allele number scored and PIC value (0.58). On average, 78% of the 93 potato samples shared a common major allele at any given locus ranging from 46 (STM0031) to 100% (STM1053 and STI0030) common alleles at each locus. The SSR markers used were highly informative and polymorphic as observed from their PIC value (Table 2). The lowest PIC value was 0.51 (STM1053) while highest was 0.98 (STM0037). All of the landraces were found to be distinct from one another.

Potato germplasm structure

Mean number of observed and expected heterozygosity was calculated using the data set (93 clones by 12 SSR markers) to estimate the structure of the various populations (Table 3). Samples from Eritrea showed highest genetic diversity as explained by the diversity index (h). Moreover, highest number of different (private) alleles was observed from Eritrean samples, Zoba Maekel (1.45). The observed genetic diversity ranged between 0.19 (Kenya and Rwanda) to 0.21 (Zoba Debub). The mean expected diversity for the total of samples was 0.21 indicating about

Table 2. SSR marker allele analysis.

Marker	Total no of allele	Gene diversity	PIC	Repeat length	Quality index	Abundant allele (%)	Rare allele (<=5%)
STG0016	9	0.94	0.93	3	0.166	160 (69)	145 148 166 184
STM5114	6	0.89	0.88	3	0.255	321 (79)	318 324
STM1053	2	0.58	0.51	3	0.089	195 (100)	None
STI0012	7	0.91	0.91	3	0.152	195 (62)	213
STI0032	5	0.95	0.95	3	0.501	135 (73)	144
STI0004	10	0.87	0.86	3	0.386	103 (73)	91 100 115 118 124 130
STM0031	13	0.93	0.93	2	0.444	164 (46)	150 184 190 230
STM1104	6	0.87	0.86	3	0.413	194 (76)	191 200 203 206
STM1052	7	0.95	0.94	2	0.456	232 (54)	None
STM1106	6	0.81	0.80	3	0.130	181 (76)	184 217
STM0037	8	0.98	0.98	2	0.335	94 (98)	104 106
STI0030	12	0.94	0.94	3	0.344	109 (100)	85 91 94 100 106 121 136
Mean	8	0.89	0.87	-	0.322	-	-

Table 3. Populations estimation based on number of different alleles, polymorphism (%), and diversity level.

Population	N	Na (SE)	%P	uh (SE)	h (SE)
Zoba Debub	27	1.33 (0.10)	65.9	0.22 (0.02)	0.21 (0.02)
Zoba Maekel	24	1.45 (0.09)	72.5	0.21 (0.02)	0.20 (0.02)
NARI	12	1.12 (0.11)	56.0	0.22 (0.02)	0.20 (0.02)
Kenya	18	1.32 (0.10)	65.9	0.20 (0.02)	0.19 (0.02)
Rwanda	12	0.28 (0.10)	62.6	0.21 (0.02)	0.19 (0.02)
Mean	-	1.30	64.6	0.21	0.20
SE	-	0.05	2.7	0.01	0.01

N = sample number; Na = number of different alleles; %P = polymorphic percentage; uh = unbiased expected diversity; h = observed diversity; SE = standard error.

21% of the samples were expected to be heterozygous at a given locus. There was slight increase in the value of Nei's unbiased estimate of expected genetic diversity (uh) as compared to the observed diversity (h) across all markers for each population.

Principal Coordinate analysis (PCoA)

Analysis of PCoA showed that the first 3 axes explained a cumulative 27.7% of the variation among the populations which grouped them into three clusters (Figure 1).

The Eritrean (Maekel and Debub) samples were widely scattered in all the four coordinates. This can be ascribed to the fact that potato seed tubers are imported to the country from different sources with no standard importation system. Most of the Kenyan and a few Rwandese samples were grouped together. The PCoA analysis provided an alternative way to view the genetic similarity and diversity among and within the populations. PCoA analysis of only the Eritrean population indicated that there was no clear distinction between the samples of different origins but samples from NARI clustered together in the middle (Figure 1).

A separate PCoA test at population level gave a total of 92.5% variation by the first three axes with the first and second axes being 49.3 and 28.7%, respectively. The analysis differentiated the populations distinctly where Kenya and Rwanda clustered together in the fourth quadrant. Zoba Debub and Zoba Maekel clustered together in the second quadrant while NARI grouped separately in the first quadrant (Figure 2).

Analysis of molecular variance

To assess and quantify the diversity level and the genetic

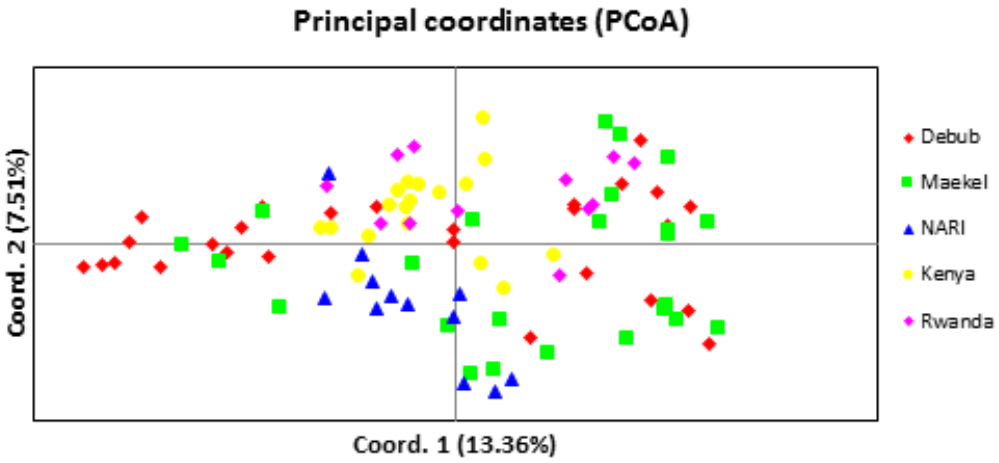

Figure 1. Principal coordinate analysis of the potato samples from Eritrea, Kenya and Rwanda.

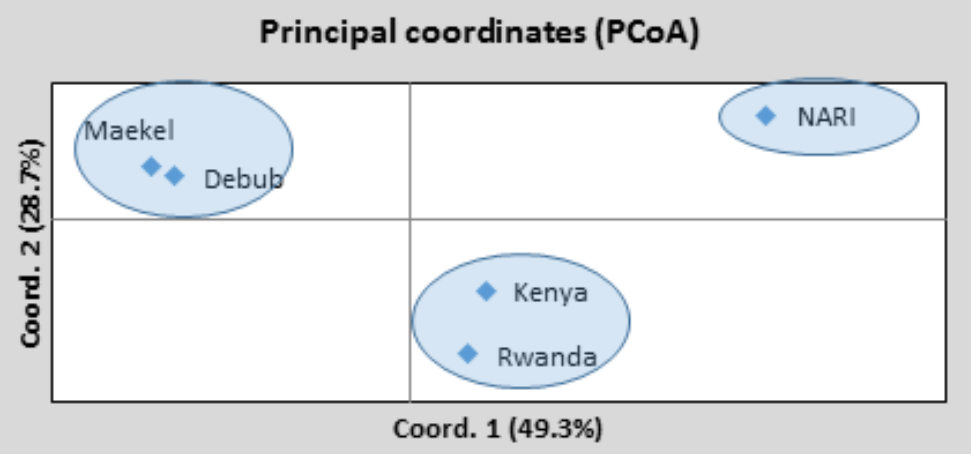

Figure 2. PCoA of the populations based on genetic distance matrix (NeiP) using Coordinate 1 and 2.

Table 4. Pairwise sample matrix of Nei unbiased genetic distance.

Variable	Zoba Debub	Zoba Maekel	NARI	Kenya	Rwanda
Zoba Debub	0.000	-	-	-	-
Zoba Maekel	0.011	0.000	-	-	-
NARI	0.033	0.024	0.000	-	-
Kenya	0.032	0.036	0.036	0.000	-
Rwanda	0.032	0.028	0.048	0.018	0.000

relationship among the 93 samples, an analysis of molecular variance (AMOVA) was done. AMOVA showed that there was a significant variation (p=0.001) among and within populations. About 92% of the variation was observed within population while the remaining 8% variation was observed among population.

The pairwise sample matrix among the potato samples was determined using the unbiased Nei's genetic distance. The genetic distance matrix index among population ranged from 0.011 to 0.048 (Table 4). The results indicate that there is relatively close relationship between the two Eritrean samples Zoba Debub and Zoba

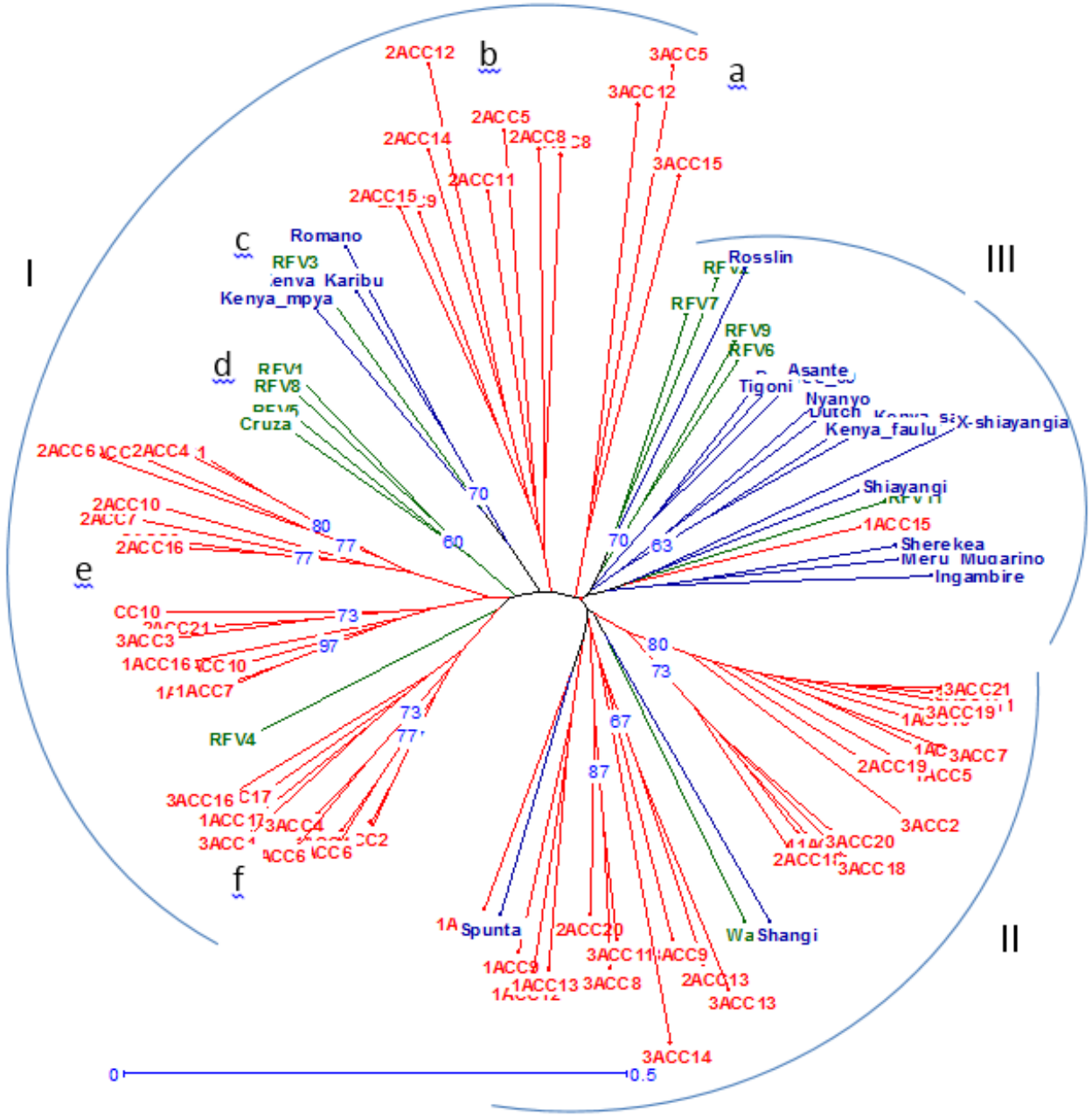

Figure 3. Unrooted UPGMA dendrogram of the 3 potato populations from Eritrea, Kenya and Rwanda on the 93 *Solanum tuberosum* sample. Bootstrap values ≥ 50% from 100 replications are indicated above the nodes.

Maekel as well as between the Kenyan and Rwandese populations.

This conclusion was further supported by the previous PCoA analysis. The highest variation was noted between NARI and Rwandese samples (0.048).

Cluster analysis

The dendrogram tree generated by UPGMA analysis revealed similar findings as those of the PCoA analysis. The samples were clustered into three main distinct clusters with the majority of Kenyan and Rwandese samples grouping together (Figure 3). The largest was Cluster I consisting of 6 sub clusters (a, b, c, d, e, and f). Sub cluster “a” contains 3 samples from Eritrea; while sub cluster “b” had 8 from Eritrea, cluster “c” contains 3 from Kenya and 1 from Rwanda. Sub cluster “d” consists of 4 from Rwanda.

Moreover, sub cluster “e” and “f” consisted of 15 from

Eritrea and 10 from Eritrea and one from Rwanda, respectively. Cluster II consists of 29 samples with a majority (26) being from Eritrea; 2 from Kenya and 1 from Rwanda. Cluster III consists of 19 samples with 13 from Kenya; 5 from Rwanda and 1 from Eritrea. For most of the Eritrean samples, there was no sharp relationship in clustering according to their geographic origin. The tree in Figure 3 shows bootstrap (60 to 97) values at the inner node indicating the shared similarity between the different samples in the specified bootstrap replications. Eritrean samples in Cluster I belong to the intermediate maturity and good performance group with better tuber quality whereas most of the Eritrean samples in Cluster II are from NARI characterized by late emergence and late maturity.

DISCUSSION

The total amount of data point generated by the 12 SSR markers on the Eritrean, Kenyan, and Rwandese populations is within the range of the several studies reported previously. The 12 SSR markers amplified a total of 91 alleles with a mean of 8 allele per locus.

In previous similar allele scoring studies, great variation of results have been reported. Chimote et al. (2007) found a total of 123 amplified alleles using 4 SSR markers with an average of 34 alleles per marker. Moreover, Rocha et al. (2010) reported a total of 136 polymorphic fragments amplified using 20 primers with an average of 6.8 per primer. On average, 2 to 14 alleles per locus were amplified with an average of 6.67 (Muthoni et al., 2014).

Similarly, Favoretto et al. (2011) found a total of 46 alleles amplified using 10 SSR markers. Carputo et al. (2013) reported a total of 46 alleles using 12 SSR markers with an average of 3.8 alleles per locus while Solano et al. (2013) reported an average of 9.16 per locus where 64 alleles were amplified using 7 SSR markers. Recently, Muhinyuza et al. (2015) reported a total of 84 alleles amplified using 13 SSR markers. This high amplification level in potato is ascribed partially to the ploidy level of the crop. Moreover, the wide range of variation in the report by authors is associated to the different SSR markers used.

Similar to other studies, we observed higher polymorphism for short repeat motifs. Previously, Madhusudhana et al. (2012) reported significant negative correlation between repeat motif and repeat number ($r = -0.44$); allele number ($r = -0.39$) and PIC values ($r = -0.38$). On the other hand, Solano et al. (2013) reported a positive correlation between allele number and SSR motif length while Sajib et al. (2012) reported that there were no correlations between the number of allele detected and the number of SSR repeats present in a particular locus. However, de Flamingh et al. (2014) suggests that complexity, rather than repeat length alone, influence amplification success. Our results coincide with the majority that the shorter the motif length the more alleles are amplified. In addition, Muthoni et al. (2014) and Muhinyuza et al. (2015) reported positive and strong correlations between number of alleles and PIC.

The PIC value of each SSR markers was within the range of several reports with an average of 0.87. PIC value describes the discriminatory power of the markers between the samples. Moreover, according to Muhinyuza et al. (2015) PIC effectively demonstrates the power of SSR markers in measuring genetic variation among potato cultivars. The value reported by several studies in potato varied depending on the SSR marker used and samples tested. In the current study, the PIC value ranged between 0.51 to 0.98. Our finding is slightly lower than the report by Lioa and Gua (2014), but relatively higher than reports of Ghislain et al. (2006), Rocha et al. (2010), Muthoni et al. (2014), Favorreto et al. (2011), Solano et al. (2013), D'hoop et al. (2010) and Muhinyuza et al. (2015) in potato.

The PCoA analysis showed that there was no distinct relationship between the samples and their geographic origins within the Eritrean materials. This result can be ascribed to the fact that samples are freely moving from Zoba Debub to Zoba Maekel by farmers and thus the same germplasm is available everywhere. This result is in contrast with those of Solano et al. (2013) who reported that samples were clustered in accordance to their geographical origin in Chile. The later may illustrate the difference in how farmers obtain their seed, which in Chile is largely through specialized seed producers.

The study clustering analysis generated three groups of potato samples pertaining to different populations. This result is supported by the PCoA analysis discussed previously. Cluster I was mainly dominated by the Eritrean landraces with distinct characteristics from the newly introduced varieties. The latter are mainly grouped in Cluster II, whereas the majority of Kenyan and half of the Rwandese are found in cluster III. The relatively low unbiased Nei genetic distance between the Eritrean samples (Zoba Debub and Zoba Maekel) could be attributed to the free seed movement within the country.

Previously, Liao and Guo (2014) reported that among 85 potato cultivars from Yunnan China studied using 24 SSR markers there was relatively low genetic diversity as explained by the genetic similarity matrix. Earlier, it was also reported by Gebhardt et al. (2004) that high genetic similarity was noted as a result of narrow genetic base in European cultivated potatoes. The Eritrean populations were found to be different from the Kenyan and Rwandese. This result was also supported by the PCoA analysis and Nei unbiased genetic distance matrix.

Conclusion

Genetic diversity assessment is essential for the

characterization of the distinctiveness of field and germplasm collection samples, which can help to identify new parents for breeding programs as well as the most interesting samples for conservation of useful germplasm. SSR markers provided a clear molecular characterization and genetic diversity assessment between potato samples. The samples from Eritrea showed some degree of distinctness from the samples of Kenya and Rwanda.

However, Eritrean landraces were markedly more genetically diverse than the new introduced varieties confirming the observations by farmers. The taxonomic origin of the Eritrean potato germplasm is not known, but this study results seem to indicate that it could be more of a Tuberosum origin from European germplasm rather than an Andigenum origin from CIP breeding materials released in the tropical zone (Kenya and Rwanda). The study main conclusion is that the potato Eritrean landrace germplasm presents more genetic variation than newly introduced varieties, and can therefore be exploited for potato breeding.

Conflict of interests

The authors have not declared any conflict of interests.

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