Original Research Article

Analysis of Genetic Variability of Potato Mutants Using Simple Sequence Repeat (SSR) Markers

ABSTRACT

Aims: Potato (*Solanum tuberosum* L.) is ranked fourth amongst the world's major stable food crop and second in Kenya after maize and thus plays a vital role in food and nutrition security and sustainable development. Despite its importance, potato production in Kenya is still low due to abiotic and biotic constraints. Traditional breeding and improvement have been difficult due to the narrow genetic diversity of the crop owed to the complex tetrasomic inheritance patterns. Induced mutation has been used to generate genetic variations in potato from which desired putative mutants are selected. In most cases the level of genetic variability is not known.The objective of this study was to determine the genetic variability of potato mutants using SSR markers.

Study design: The study involved three potato varieties; Asante, Kenya Mpya and Kenya Sherekea which are high yielding commercially grown varieties in Kenya..

Place and Duration of Study: Sample: They were irradiated at different dose rates of gamma rays at Co^{60} source at FAO/IAEA laboratories, Seiberdorf Austria in 2014. A total of 163 mutants were advanced to M1V4 generation at the University of Eldoret between 2015 and 2018.

Methodology: Genomic DNA was extracted to assess the diversity with 20 SSRs markers.

Results: All the 20 SSR primers were polymorphic with 6-19 bands amplified per primer and marker STM5127 showed the highest allele number (19) using PowerMarker software. The STRUCTURE analysis suggested that the potato mutants were clustered into six sub-populations based on the unweighted pair-group method of arithmetic averages (UPGMA) thus, the accessions were divided into three major clusters. Analysis of molecular variance (AMOVA) indicated that 8.6% of total molecular variance was attributed to diversity among sub-populations, while 91.4% of variance was associated with differences within sub-populations.

Conclusion: This study represents the most comprehensive investigation of the genetic diversity and population structure of potato mutants, and provides valuable information for genetic improvement, and systematic utilization.

Keywords: Variability, Mutants, Potato, food security, gamma rays

1. INTRODUCTION

The cultivated potato (*Solanum tuberosum* L.) is the second most important staple food in Kenya after maize in terms of consumption and production and the world's fourth major food crop after wheat, rice and maize in terms of production and area covered (1, 2, 3). In Kenya, potato is mostly grown by small scale farmers as source of food, employment and cash crop, therefore plays an important role in food security and provides high energy, protein as well as substantial amount of vitamins and minerals (3).

Plant breeding requires genetic variation of useful traits for crop improvement. In potato, most often the desired variation is lacking due to preferences of few elite local traditional cultivars for potato improvement in most parts of the world (4). The narrow genetic diversity in cultivars has led to an increased vulnerability to new abiotic and biotic stressors, especially those directly and indirectly arising from changing climatic conditions making it difficult for yield improvement to be achieved (5).

Traditional improvement has been difficult due to the narrow genetic diversity of the crop owed to the complex tetrasomic inheritance patterns. Mutagenic agents such as ionizing radiation and certain chemicals can be used to induce mutations and generate genetic variations from which desired mutants may be selected. The use of induced mutations is highly effective in enhancing genetic variability and this technique has been used to develop improved cultivars (6). Knowledge of levels of genetic variation is required if farmers are to continue using these cultivars and also form part of the conservation strategy DNA fingerprinting techniques (markers) are available for genetic identification in plant breeding and germplasm management and have been used successfully in other crops. Simple sequence repeat (SSR) markers have been extensively used in potato and other crops for various breeding and diversity studies (7, 8, 9, 10,11). The SSRs are highly polymorphic, co-dominant markers (12, 13), low operational costs, and are highly reproducible (14). The study therefore was to characterize potato mutants using SSR markers.

2. MATERIAL AND METHODS

2.1 Plant Materials

A total of 160 potato mutants at M1V4 generation tubers generated from three potato varieties (Asante, Kenya Mpaya and Kenya Sherekea) that were irradiated from at different dose rates of gamma rays at Co^{60} source at Seiberdorf laboratories in Vienna, Austria at 0, 3, 6, 9, 12 and 15 gray (Gy) for Asante, 0, 5, 6, 10 and 15 Gy for Kenya Mpya and 0, 3, 5, 10, 12, 15, 20, and 30 Gy for Kenya Sherekea (15, 15, 17). The tubers were planted at the University of Eldoret, Biotechnology Green House Research facility to raise plants to be used for DNA extraction.

2.2 DNA Extraction and Polymerase Chain Reaction (PCR)

Approximately 0.2 - 0.25 g per genotype/clone tissues were ground in a mortar and pestle and placed in a 1.5-millimeter (ml) Eppendorf® tube containing 600 µl of extraction buffer [0.1 M of Tris-Hydrochloric acid (Tris-HCl) pH 8.0, 0.05 M of (w/v) Ethylene diaminetetraacetate (EDTA), 0.5 M Sodium chloride (NaCl), 1 % of Polyvinylpirrodine (PVP), 0.07 % β mercaptoethanol and 20% (0.7 µl) of (w/v) sodium dodecyl sulphate (SDS) added separately]. The mixture was incubated at 65 °C for 15 minutes with agitation every 5 minutes. Then the samples were placed at room temperature for 5 minutes followed by addition of 350 µl of ice-cold 5M potassium acetate then incubated at -20 °C for 20 minutes. The samples were then centrifuged for 15 minutes at 13,000 revolutions per minute (rpm) at room temperature. The supernatant was transferred to another tube and then 480 µl of ice-cold isopropanol was added and mixed gently. The mixture was kept at -20 °C for one hour and then centrifuged for 15 minutes at 13,000 rpm. The pellet was left to dry at room temperature by inverting the tubes on paper towels until all isopropanol droplets disappeared from the walls of the tubes. The supernatant was removed and the pellet was washed with 700 µl of 70 % ethanol and the pellet dried at room temperature followed by a brief centrifugation of 5 minutes at 13,000 rpm. The above process from the addition of ice-cold isopropanol was done twice and the incubation was done for 20 minutes at -20 °C. The pellet was then air dried and later resuspended in 50 µl of Tri-EDTA (TE) 10:1 mM buffer and then incubated at -4 °C.

2.3 DNA Quantification

DNA quantity and quality of each accession was determined by running samples on 1 % (w/v) agarose gels for 1 hour at 80 volts diluted in 100 ml 1X TAE buffer and 900 ml of distilled water. A standard undigested lambda DNA with a range variation of 10, 20, 50, 80 and 100 ng was used as a comparison to determine the DNA concentration of the potato accessions by comparing band sizes and intensities. The gel was stained in ethidium bromide (10mg/ml) for 30 minutes and later de-stained in distilled water for 20 minutes before viewing under ultraviolet transilluminator. Between 0.5 µg and 1µg of high quality DNA was obtained and was diluted to 0.01 µg/µl with deionized distilled water for PCR amplification.

2.4 PCR Amplification

A subset of 20 SSR markers (Table 1) were selected from a data base of SSRs applied in previous studies based on their high polymorphic content and broad coverage of the potato genome (18, 19, 20, 12, 21, 7, 9).

Table 1: Description of the 20 SSR loci used to characterize 163 potato mutants

Locus	Forward/ Reverse	Ta (°C)	Range Size (bp)	Chr Pos
STG0016	AGCTGCTCAGCATCAAGAGA	56	135-175	I
	ACCACCTCAGGCACTTCATC			
STM5114	AATGGCTCTCTCTGTATGCT	55	295-325	II
	GCTGTCCCAACTATCTTTGA			
STM1053	TCTCCCCATCTTAATGTTTC	55	170-195	III
	CAACACAGCATACAGATCATC			
STI0012	GAAGCGACTTCCAAAATCAGA	56	180-235	IV
	AAAGGGAGGAATAGAAACCAAAA			
STI0032	TGGGAAGAATCCTGAAATGG	60	125-150	V
	TGCTCTACCAATTAACGGCA			
STI0004	GCTGCTAAACACTCAAGCAGAA	56	80-130	VI
	CAACTACAAGATTCCATCCACAG			
STM0031	CATACGCACGCACGTACAC	60	110-210	VII
	TTCAACCTATCATTTTGTGAGTCG			
STM1104	TGATTCTCTTGCCTACTGTAATCG	60	180-200	VIII
	CAAAGTGGTGTGAAGCTGTGA			
STM1052	CAATTTCGTTTTTTCATGTGACAC	55	220-255	IX
	ATGGCGTAATTTGATTTAATACGTAA			
STM1106	TCCAGCTGATTGGTTAGGTTG	60	165-200	X
	ATGCGAATCTACTCGTCATGG			
STM0037	AATTTAACTTAGAAGATTAGTCTC	55	85-108	XI
	ATTTGGTTGGGTATGATA			
STI0030	TTGACCCTCCAACTATAGATTCTTC	56	73-122	XII
	TGACAACTTTAAAGCATATGTCAGC			
STM2013	TTCGGAATTACCCTCTGCC	55	160-185	VII
	AAAAAAAGAACGCGCACG			
STM1049	CTACCAGTTTGTTGATTGTGGTG	57	136-212	I
	AGGGACTTTAATTTGTTGGACG			
STM5127	TTCAAGAATAGGCAAAACCA	55	255-305	I
	CTTTTTCTGACTGAGTTGCCTC			
STI0046	CAGAGGATGCTGATGGACCT	55	196-230	XI
	GGAGCAGTTGAGGGCTTCTT			
STI0036	GGACTGGCTGACCATGAACT	55	130-160	II
	TTACAGGAAATGCAAACTTCG			
STI0023	GCGAATGACAGGACAAGAGG	55	80-220	X
	TGCCACTGCTACCATAACCA			
STWAX-2	CCCATAATACTGTCGATGAGCA	53	230-260	VIII
	GAATGTAGGGAAACATGCATGA			
STPoAc58	CAGAGGATGCTGATGGACCT	57	240-255	V
	GGAGCAGTTGAGGGCTTCTT			

Ta (°C) = Annealing temperature in degree centigrade, Chr Pos= Chromosome position (Source: 18; 9)

The PCR reactions were performed in a Mastercycler (Eppendorf®) using in a final volume of 20 µl Bioneer AccuPower® containing 4 µl pre-mix (1U Top DNA, 250 µM each dNTP, 10 mM Tris-HCl pH 9.0, 30 mM KCl, 1.5 mM $MgCl_2$, stabilizer and tracking dye), 0.5 ng/µl of each forward and reverse primer, 0.5 ng of DNA template, and 6 µl of double distilled water (ddH2O). The PCR cycles consisted of initial denaturation at 95 °C for 5 minutes, followed by 35 cycles at 95 °C for 30 seconds, annealing at 45 or 60 °C (depending on primer) for 1 minute, extension at 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. The DNA fragments were separated on 2 % agarose gel run at 100 V for 3 hr using 1 M TAE buffer. The DNA fragments in gel was visualized by staining at 0.5 ug/mg ethidium bromide for 30 minutes and rinsed with distilled water for 20 minutes, visualized and photographed using UV transilluminator at 312 nm. Allele sizes were scored using a 100 bp molecular size ladder.

2.5 Statistical analysis

The PowerMarker software package (22) was used to calculate the following summary statistics; percentage of polymorphic loci, mean number of alleles per polymorphic locus, observed heterozygosity (HO), expected heterozygosity (HE) and polymorphic information content (PIC). The genetic variance within and among populations was analyzed using the ARLEQUIN 3.01 software by analysis of molecular variance (AMOVA) (23).

2.6 Population structure analysis

The population structure analysis of the entire germplasm was carried out based on Bayesian model (24) as implemented in the STRUCTURE program version 2.3.4 (Pritchard *et al.,* 2010). The number runs for K values ranging from 1 to 10 were performed with a burn-in length of 100,000 followed by 1,000,000 Monte Carlo Markov Chain (MCMC) interactions using admixture model. The number of subpopulations was determined using the Delta *K* (Δ*K*) *ad hoc* method proposed by (25) and implemented in the online tool Structure Harvester (26) to estimate the most likely *K* in each set of potato mutants. The number of sub-populations (ΔK) was determined using the ad-hoc statistical method, based on the rate of change in the log probability of data between successive K values (25). Mutants were assigned to a subpopulation if the probability of membership was greater than 70 % (27). If membership was ≤70 %, the mutants were allocated to the mixed subpopulation.

2.7 Molecular phylogenetic and principal coordinate analyses

Phylogenetic trees were produced using genotyping data with 20 SSR markers using the hierarchical clustering method based on the dissimilarity matrix calculated with Manhattan index, as implemented in the DARwin software (version 6.0.9) (Perrier & Jacquemoud-Collet, 2006). The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA) based on the estimates of genetic similarity of the potato mutants and controls. Principal coordinate analyses were also performed with DARwin 6.0.9 (28).

3. RESULTS

All the 20 SSR primers gave polymorphic bands, marker STM5127 (Figure 1) gave the highest allele number and gene diversity of 19 and 0.81 respectively. Marker STWAX-2 gave the highest allele frequency of 0.95 and the lowest allele number of 6. STI0032 marker had the highest heterozygosity of 0.82 while STPoAc58 marker had the highest polymorphic information content (PIC) of 0.89.

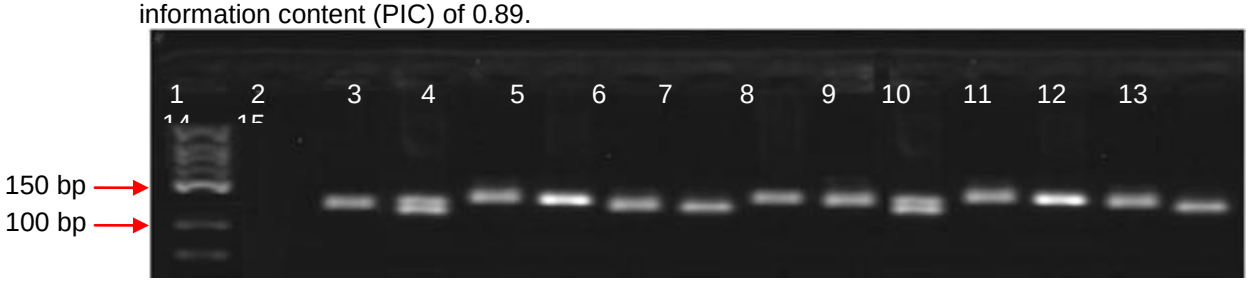

Figure 1: SSR markers profile of Kenya Mpya potato mutants generated by primer STM5127; *(1 = ladder, 2 = water, 3 to 15 are mutants from M1 to M12); bp – base pair*

A total number of 211 alleles (average of eleven alleles) were generated with Asante generating 69 alleles (average of three alleles), Mpya was 75 alleles (average of four alleles) and Sherekea with 67 alleles (average of three alleles) (Table 2).

Table 2: Summary of statistical analysis of genetic diversity across all the 160 potato mutants and 3 parent genotypes based on 20 microsatellite loci

Marker	Allele Frequency	N_a	Allelic composition			Gene Diversity	Heterozygosity	PIC
			Private	Rare	Common			
STG0016	0.86	12	1	2	9	0.26	0.81	0.79
STM5114	0.49	9	3	2	4	0.63	0.61	0.56
STM1053	0.48	7	2	1	4	0.67	0.57	0.61
STI0012	0.42	9	4	3	2	0.69	0.76	0.64
STI0032	0.45	10	4	4	2	0.66	0.82	0.59
STI0004	0.69	12	1	5	6	0.06	0.55	0.59
STM0031	0.79	8	4	2	2	0.06	0.45	0.59
STM1104	0.39	13	5	4	4	0.01	0.6	0.81
STM1052	0.45	11	2	4	5	0.65	0.34	0.57
STM1106	0.58	6	1	2	3	0.5	0.25	0.85
STM0037	0.42	9	4	2	3	0.68	0.56	0.61
STI0030	0.73	10	3	4	3	0.42	0.61	0.7
STM2013	0.96	8	3	2	3	0.08	0.64	0.79
STM1049	0.29	17	5	6	6	0.76	0.59	0.73
STM5127	0.34	19	9	4	6	0.81	0.68	0.79
STI046	0.63	11	4	4	3	0.48	0.67	0.74
STI0036	0.53	9	1	4	4	0.52	0.32	0.77
STI0023	0.5	16	4	7	5	0.68	0.58	0.65
STWAX-2	0.97	6	1	3	2	0.06	0.24	0.58
STPoAc58	0.95	9	3	4	2	0.09	0.65	0.89
Overall Mean	0.6	11				0.44	0.57	0.69
Asante Mean	0.68	3				0.22	0.06	0.24
Mpya Mean	0.78	4				0.45	0.11	0.19
Sherekea Mean	0.8	3				0.23	0.16	0.18

4.2: Analysis of Molecular Variance (AMOVA)

A population diversity analysis was performed to explore the genetic variations among and within groups of the potato mutants (Table 3). AMOVA revealed that the diversity within populations of Asante, Kenya Mpya, Kenya Sherekea and all the three combined (160) potato mutant clones and the 3 parents were 83 %, 79 %, 87.4 % and 91.4 % respectively. The Kenya Sherekea mutants were the most diverse within populations with 87.4 %. The

minimum diversity attributed to genetic differentiation among groups of Asante, Kenya Mpya, Kenya Sherekea and all the three combined (160) potato mutant clones and parents were 17 %, 21 %, 12.6 % and 6.2 % respectively (Table 3).

Table 3: Analysis of Molecular Variance (AMOVA) based on SSR markers for each population of potato mutants

Population	Sources of variations	Variance components	Percentage of variation
Asante	Among Populations	96	17
	Within Individuals	1160	83
	Total	**1184**	**100**
Kenya Mpya	Among Populations	106	21
	Within Individuals	964	79
	Total	**1140**	**100**
Kenya Sherekea	Among Populations	68	12.6
	Within Individuals	679	87.4
	Total	**986**	**100**
Combined	Among Populations	24	6.2
	Among sub-populations	66	2.4
	Within Individuals	128	91.4
	Total	**245**	**100**

4.4.3: Population structure analysis

The Bayesian clustering method with admixed model indicated that 160 mutant accessions and 3 parents of Asante (72), Kenya Mpya (46) and Kenya Sherekea (42) were clustered into six genetic groups (K = 6) with estimated membership probability threshold (q) values of between 0.34 and 0.60 (Figure 2 and 3). The estimated membership probability threshold (q) indicated that 146 (89.6 %) of the 163 individuals, while the remaining 17 (10.4 %) potato mutants were assigned into an admix group. The accessions in admix group presented the probability of belonging to more than one subgroup, demonstrating that these accessions shared some degree of genetic information with some other groups. The SP2 was the largest group and which accounted for 52 % and 46% of the mutants, respectively. The figures 2 and 3 shows the estimated population structure based on Delta K (ΔK) reaches its maximum value following the *ad-hoc* method and subpopulation clusters (K) that are represented by different colors, respectively.

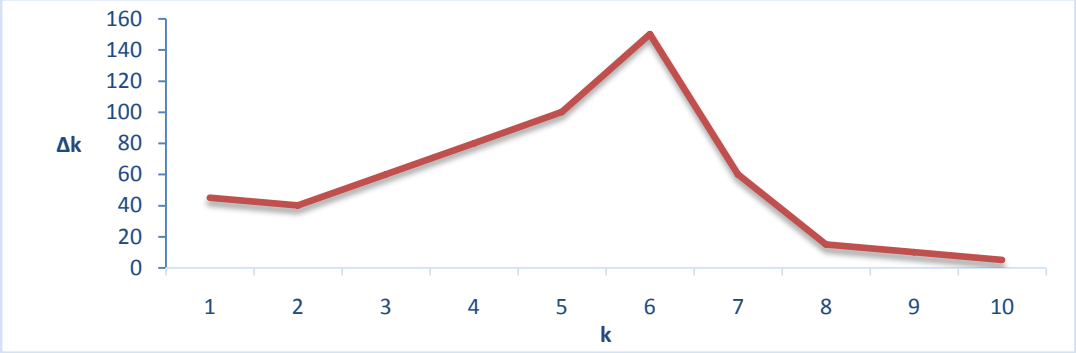

Figure 2: STRUCTURE estimation of the number of subgroups for the K values ranging from 1 to 10, by delta K (ΔK) values of the 160 potato mutants and 3 parents

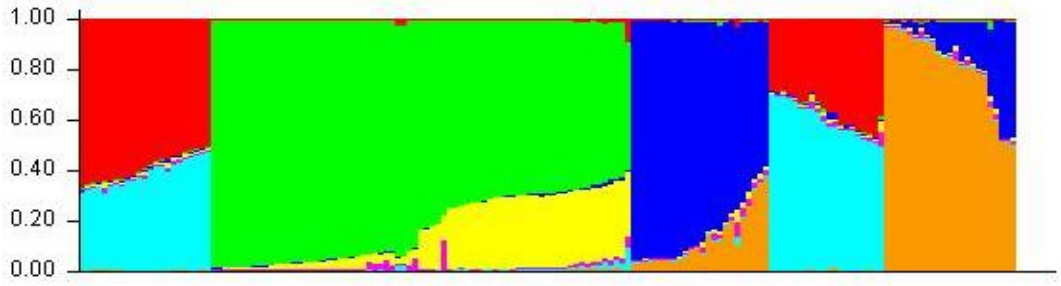

Figure 3: Population structure of 160 potato (Asante, Kenya Mpya and Kenya Sherekea) mutants and 3 parents obtained with the STRUCTURE program based on SSR markers for K = 6. Each colour represents one subpopulation and the length of the colored segment shows the estimated membership proportion of each sample to designed group.

4.4: Molecular phylogenetic analysis

The dendrogram based on UPGMA cluster analysis constructed from SSR markers in DARwin software package among potato mutants were grouped into three groups (Figure 4). Group I was the most diverse group consisting of four subgroups (SG) A, B, C and D while group II group III consisted of similarly equal number of potato mutants (Figure 4). These makes a total of six subgroups as revealed by population structure (Figure 2 and 3). The dendrogram showed that group I consisted of 74 % of the total mutant population while group II and III consisted of 15 % and 11 % respectively.

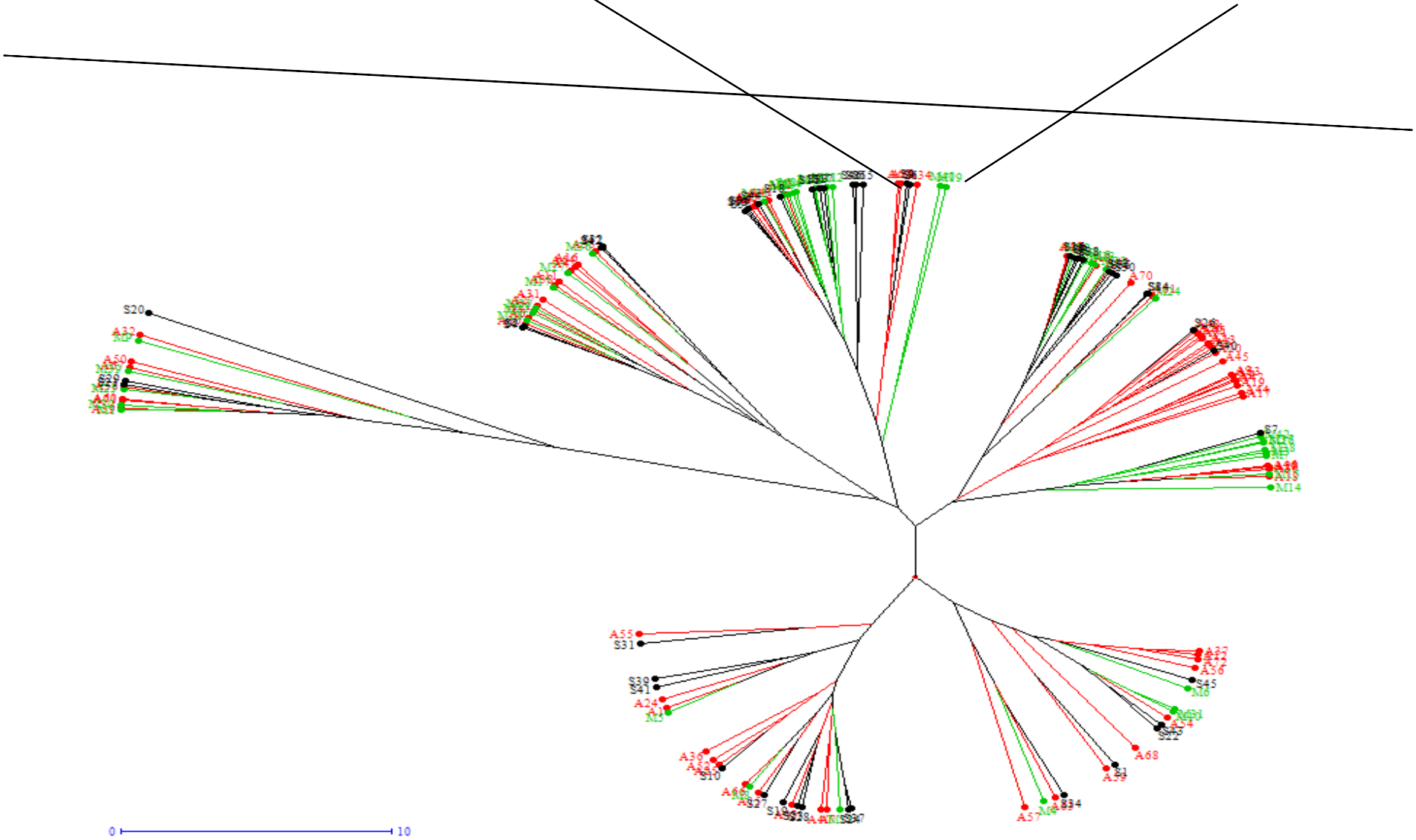

Figure 4: Unrooted tree using Unweighted pair-group method of arithmetic averages (UPGMA) illustrating the genetic relationship among 160 potato mutants (Asante – red, Kenya Mpya – green and Kenya Sherekea – black) based on 20 SSR markers

4.5: Principal co-ordinate analysis

A further molecular analysis using SSR data in Figure 5 shows the scatter plot that splits the potato mutants in three main clusters defined by the first two coordinates. The principal co-ordinate analysis resembled more strongly the dendrogram obtained with SSR data analysis (Figure 4) with mutants contributing more to the first principal component analysis. The first and second principal components of the potato mutants comprised 54.2 % and 18.6 % respectively, accounting to 72.8 % of the total variation (Figure 5).

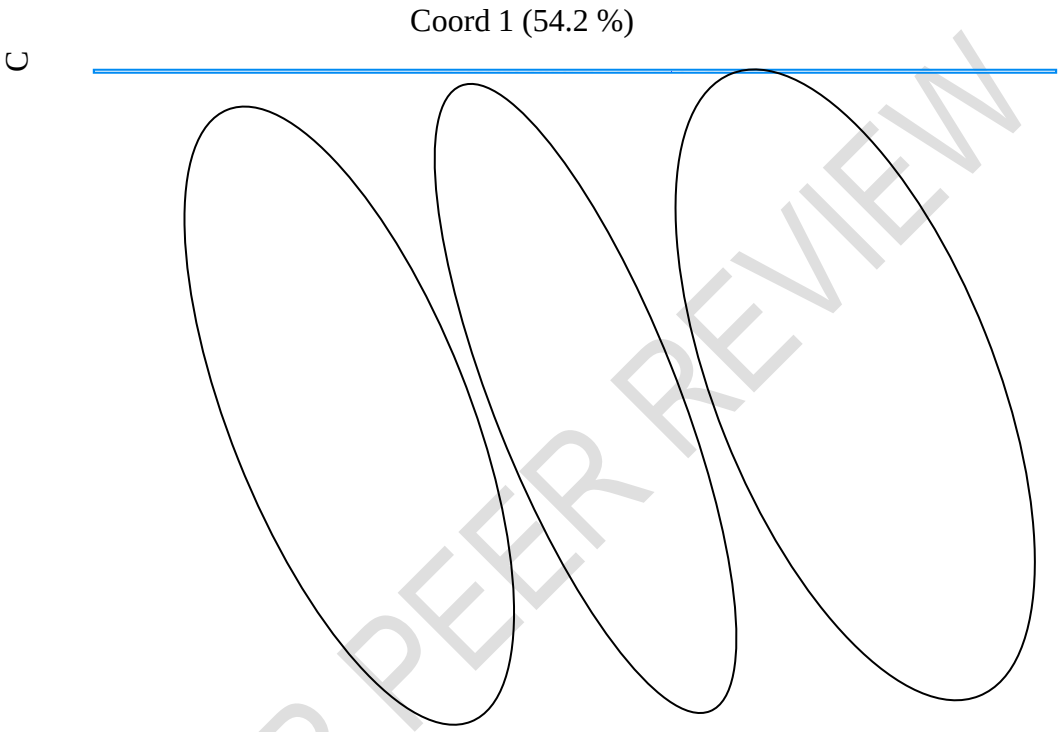

Figure 5: A scatter plot of 160 potato mutants and 3 parents based on first and second components of principal coordinate analysis using 20 SSR data (Red represent Asante mutants, Green represent Kenya Mpya mutants and Black represent Kenya Sherekea mutants.

4. DISCUSSION

The SSRs studies showed there was significant genetic variability among the 160 potato mutants and 3 parents studied generating a total number of 211 alleles with a range of 6 to 19 alleles per marker. This could be due to the fact that microsatellites are often valuable for only directly related germplasm sources and some significant distortion in genetic similarity estimates can arise due to amplification of moderately divergent cross species (29). In addition, differences in laboratory procedures may have also led to the reason for the diverse results reported by these studies is mainly associated with the origin and sources of potato collections or genotypes studied (30), as well as the marker type applied and the appropriate platform used for resolution of amplified products (31). These results are within

the range of several studies in potato previously reported by 10,9, 32, 33, 7, 13, 14, and *21)*. The markers used in the present study had diverse allele numbers compared to previous studies (7, 34, 9).

The polymorphic information content (PIC) values were moderately high with an average 0.69 in most of the SSR markers used in this study. The individual potato mutant populations (Asante (0.24), Kenya Mpya (0.19) and Kenya Sherekea (0.18) generated low average PIC values. The results observed in the present study could be due to the fact that the potato mutant populations were generated from gamma (induced) irradiation from three parental genotypes and each mutant population are directly related to their parents. Furthermore, it could be due to the fact that SSRs are generally useful only for closely related germplasm sources and the effects of mutation as well as the amplification of moderately divergent cross species can bring about significant distortion in genetic similarity estimates (Peakall *et al.,* 1998). Similarly, high PIC values have been reported by several authors in potatoes; (7) ranged from 0.839 to 0.208 with an average of 0.649; Biniam *et al.,* (2016) reported a mean of 0.87 with a range of 0.51 to 0.98; (35) also reported a range from 0.9857 to 0.9897 with the average value as 0.9871; (10) reported a range of 0.57 to 0.93 with a mean value of 0.85 among others (18, 12, 19, 21).

In the present study, the average allele frequency (0.6), gene diversity (0.44) and heterozygosity (0.69) values were found to be moderate to high. The observation in this present study could be due to a result of narrow genetic base of potato mutants created as a result of induced mutation. Liao and Guo (33) reported low genetic diversity as explained by the genetic similarity matrix among 85 potato cultivars from Yunnan, China, studied using 24 SSR markers. Similarly, (36) also detected lower genetic diversity using 19 SSR markers to fingerprint 41 local potato cultivars from 10 locations of Tenerife Island. (10) reported low allele frequency in 53 conserved potato genotypes in Ethiopia using 12 SSRs markers. Other previous studies in potato populations have reported moderate to high genetic diversity using SSR markers in their individual countries; Europe (37), Argentina (38), Canada (39), Turkey (40),Kenya (7), China (33), Rwanda (32), Eritrea (9) and Ethiopia (10).

The Bayesian clustering method generated different clusters in the potato mutants with Asante Δk = 5, Kenya Mpya Δk = 4, Kenya Sherekea Δk = 4 and combined for all potato mutants Δk = 6. The results from the three individual potato mutants (Asante, Kenya Mpya and Kenya Sherekea) showed that the clustering were relatively based on the dosage rates applied that grouped the potato mutants into different Δk populations. According to (41), the susceptibility of seed and vegetatively propagated crops to physical and chemical mutagens varies between and within species. Similar studies reported that the potato genotype showed different responses with increasing doses of gamma rays progressively inhibited the growth of stem cuttings (42).

The dendrogram and the principal co-ordinate analysis (PCoA) revealed three cluster groups of potato mutants with group (I) generating the largest and diverse mutants from the three mutant populations compared to group II and III. The variation suggests that mutation which is a random phenomenon might have occurred resulting in variations within the mutants (43). The genetic similarity and dissimilarity observed between and within the different clusters of mutant populations could possibly due to resemblance and differences in ancestry. The selection of primers is critical to the accuracy of potato cultivar identification since it affects the results depending on different primer(s) combinations employed. Liao and Guo, (33) reported that a cultivar could be in a cluster with a few cultivars in a dendrogram, and possibly cluster with other cultivars in another dendrogram based on different primer combinations.

Analysis of Molecular Variance (AMOVA) revealed higher percent (91.4 %) genetic diversity within populations. The increased genetic variability effects within individual genotype could be as a result of induced mutation which could also reduce the diversity among populations. The high genetic diversity within individuals in the potato populations in the study could imply that the crop is primarily propagated vegetatively by tubers and mini-tubers have common alleles due to directional selection done on agronomic plant traits with true seed propagation being mainly for breeding purposes as suggested by (44). Other researchers have reported analogous findings; Gwandu *et al.,* (2012) (97 %) on sweet potato virus disease resistance variation, (13) (93 %) within the Indian Andigena potato core collection, (9) (92 %) in 93 potato genotypes, (10) (96 %) in 53 conserved potato genotypes. Outcrossing crops like maize, as well as potato have reported most of the variation observed within rather than between populations (45).

The analysis of Molecular Variance (AMOVA) revealed high genetic diversity within the potato mutant populations (91.4 %) as a result of induced mutation. These results were also corroborated by principal co-ordinate analysis (PCoA) and the dendrogram which gave three clusters, however, the dendrogram analyses consisted of sub-clusters in group I which supported the STRUCTURE analysis giving six affiliate clusters. (46) also reported comparable results on genetic diversity and population structure of Chinese natural bermudagrass (*Cynodon dactylon* (L.) Pers.) germplasm based on SRAP markers. (11) as well reported similar findings on genetic diversity and association mapping in the Colombian Central Collection of *Solanum tuberosum* L. Andigenum group using Single Nucleotide Polymorphisms (SNPs) markers. Similarly, (47) reported that principal co-ordinate analysis (PCoA) and cluster analysis yielded the same grouping of similar accessions.

5. CONCLUSION

The SSR data was successfully used to group potato mutants into six distinct groups. The SSR marker STM5127 gave the highest number of alleles (19) and gene diversity (0.81). The UPGMA dendrogram revealed that mutants from the same parent were generally, but not entirely, clustered into the same cluster. Comparison of the UPGMA dendrogram and the Bayesian STRUCTURE analysis showed general agreement between the population subdivisions and the genetic relationships among accessions.

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