Genetic Characterisation and Diversity Assessment of Potato Genotypes Using SSR Markers

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Abstract

Information on diversity of genetic materials is vital for choosing parents in a breeding program. The objective of the study was to determine the pattern and level of genetic diversity among the selected 20 tetraploid potato genotypes using 16 SSR markers to identify suitable parents for breeding purposes. The microsatellites showed considerable variation among genotypes and sixty four alleles were amplified by the 16 primer pairs. The number of polymorphic alleles per locus ranged from 2 to 8 with an average of 3.9. The highest number of null alleles was observed was six for genotype Nakpot1. The overall size of the amplified product varied from 48 bp (marker STI0023) to 309 bp (marker STM5121). PIC values ranged from 0.0948 to 0.7832, with an average of 0.4307 per locus. Heterozygosity values ranged from 0.0997 to 0.805 with an average of 0.466919. Significant positive linear correlations were observed between PIC values and number of alleles ($r = 0.905$); and heterozygosity and number of alleles ($r = 0.8659$) at $p < 0.001$. Cluster analysis separated the genotypes into three different groups. The genetic distance between clones ranged from 1 to 5.7. Cruza had the highest genetic distance while the shortest genetic distance was observed between 396026.103 and 396034.104. The microsatellites used in this study provided useful information regarding the variability of the tested genotypes and their selection for breeding purposes.

Keywords: genetic variation, polymorphism, clustering, heterozygosity, microsatellites, Uganda

1. Introduction

The cultivated potato, *Solanum tuberosum*, is a highly heterozygous autotetraploid species ($2n = 4x = 48$), with a genome size of 844 Mb (Muthoni et al., 2012). It is primarily outcrossing and experiences severe inbreeding depression (Park et al., 2009). The crop displays tetrasomic inheritance which results into increased number of progeny classes and allelic dosage (Hirut, 2015).

Selection of parental materials and understanding of appropriate parents to be used for a particular mating design are key in breeding (Acquaah, 2007). A number of approaches have been used by breeders to select the best parents and cross- combinations. These include: combining ability effects, use of mid-parent values, progeny tests, estimated breeding values, and genetic diversity (Gopal, 2015). However, to obtain reliable results with the intricacies of potato genetics and inheritance pattern, various methods should be combined to aid in the selection of parents (Sharma and Nandineni, 2014). While a narrow genetic base would result into inbreeding depression as a consequence of accumulation of deleterious alleles in a population (Gopal, 2014), high level of genetic diversity among potato genotypes possessing different desirable traits is important for crop improvement. This is because selection of parents based on genetic diversity will maximize heterozygosity, broaden the genetic base and produce heterotic progenies (Sun et al., 2003).

Diversity assessment can be achieved through the use of phenotypic information, pedigree, biochemical and molecular markers (Govindaraj et al., 2015). The use of molecular markers is the most reliable method of assessing genetic diversity because they are: stable, independent of the environment and not affected by the developmental

stage of the plant, pleiotropic, and epistatic effects. Different molecular markers have been used to estimate genetic diversity in plants and animals. These comprise of- random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) among others. With RAPDs, the primers are commercially available and do not require prior information of the target DNA sequence. However, this type of marker does not easily demonstrate Mendelian inheritance of the loci and unable to differentiate between homozygotes and heterozygote. The key strength of RFLP markers is that they are codominant markers and relatively easy to score because of the large size difference. The major shortcoming of these markers is that they either require sequence information or probes. This makes it difficult and time-consuming to develop markers for species lacking known molecular information (Liu & Cordes, 2004). AFLPs are reliable but expensive, labour intensive with long assays (Tiwari et al., 2013).

Microsatellites are therefore the most commonly used markers due to their strong molecular approach to genetic diversity studies. Microsatellites are highly polymorphic, abundant, co-dominant and can be used to detect heterozygosity. They are simple to use, provide high genetic information and are highly reproducible (Muthoni et al., 2014). Additionally, the SSRs have the capacity to reflect ploidy status with the high heterozygosity of the tetraploid potatoes. It is against this back ground that SSR markers were used in this study to determine the genetic relationships among 20 potato clones with variable resistance to late blight.

2. Materials and Methods

2.1 Plant Materials

The test materials comprised of twenty potato genotypes. These were eleven commercial varieties from the national potato programme, six farmer varieties and three CIP clones of which two were released as varieties in Uganda and two crosses from the national potato research centre with variable resistance to late blight (Table 1).

Table 1. List of the twenty potato genotypes and their sources

Entry	Clones	Source	Entry	Clones	Source
1	Cruza	NARO	11	NKRI59.41	NARO
2	Kabera	Farmer	12	Petero	Farmer
3	Kachpot1	NARO	13	Rwangume	NARO
4	Kimuri	Farmer	14	Rwashaki	NARO
5	Kinigi	Rwanda	15	Rutuku	NARO
6	Mabondo	Farmer	16	Shutama	Farmer
7	Mbubamagara	Farmer	17	Victoria	NARO
8	Nakpot1	NARO	18	396026.103	CIP
9	Nakpot5	NARO	19	396034.103	CIP
10	NKRK19.10	NARO	20	396038.107	CIP

Note. CIP = International Potato Centre, NARO = National Agricultural Research Organisation.

2.2 DNA Extraction and SSR Amplification

Fresh young leaves were picked from one month old plants in the field for DNA extraction. DNA was obtained using fast technology for analysis of nucleic acids (Whatman FTA cards) following modified protocols of FTA paper technology (Mbogori et al., 2006). One FTA classic card measures 750×130 mm and each was labelled prior to the day of sampling. Ten plants were sampled from each clone and one leaf per plant. Each sampled leaf was immediately placed on the FTA card and pressed using a pair of pliers until both sides of the FTA paper were with the sap. Ethanol 70% was used to clean the pliers between samples to prevent cross contamination. The FTA card was then dried at room temperature for 2-5 hours, after which packed and sent to INCOTEC South Africa (pty) Ltd. for laboratory analysis. DNA was extracted from FTA cards and sixteen microsatellite markers were used. These were: STM2013, STM1104, STM1049, STM0037, STI0012, STI0023, STI0030, STI0036, STI0032, STWAX-2, STI046, STI031, STM0030, STM1031, STM5121, STM0019a and STPoA58.

2.3 Data Analysis

PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 313oxl automatic sequencer (Applied Biosystems, Johannesburg, South Africa). The analysis was performed using GeneMapper

4.1. The SSR marker alleles were scored for presence or absence the band for all the 20 potato genotypes. Each amplified fragment was considered as one locus. The GGT 2.0 program (Van Berloo, 2008) was used to calculate the Euclidian distances between bulked samples and the matrix of the genetic distances was used to create a UPGMA dendogram. The genetic similarity matrix of potato genotypes was calculated using the Jaccard's coefficient (Anderberg, 1973). Genetic diversity parameters, such as the total number of alleles per locus, expected heterozygosity and polymorphic information content (PIC) were determined. The PIC, which is a measure of allelic diversity, was calculated, based on the equation: $PIC = 1 - \Sigma \, (pi^2)$, where pi is the frequency of ith allele in the accessions. Pearson's correlation coefficients showing pair-wise association between PIC, He and number of alleles were calculated using Genstat statistical package, 14th edition (Payne et al., 2011).

3. Results

3.1 Allelic Information

The twenty potato genotypes used in this study were uniquely differentiated by the 16 SSR markers (Table 2). Markers differed significantly in their ability to establish variability amongst the clones. Particular markers generated several alleles while others produced a few. The 16 SSR primers identified a total of 64 alleles among the test clones. The number of polymorphic alleles scored across SSR loci ranged from 2 to 8 with an average of 3.9. The number of polymorphic alleles above average was obtained from only 50% of the markers. Null alleles were observed for some markers and the genotype with the highest frequency recorded was NAKPOT1. With this genotype 38% (6) of the markers failed to yield detectable amplification. This was followed by genotype Mbubamagara with 31% of null alleles. The overall size of the amplified product varied from 48 bp (marker STI0023) to 309 bp (marker STM5121).

Heterozygosity values (He), the measure of allelic diversity at a locus ranged from 0.099 to 0.805 with an average of 0.467. The level of polymorphism among the potato clones was evaluated by calculating PIC values for each of the 16 SSR loci which differed significantly from locus to another. These ranged from 0.095 for markers STI002 to 0.783 for STI031, with an average value of 0.431 per locus (Table 3). However, markers STI0023, STM1049, STM0037 and STM5121 had a very limited ability to detect differences among the potato genotypes as shown by their low PIC values. The marker with the highest PIC value (0.805) was STI031. There was significant positive linear correlations between PIC values and number of alleles; and heterozygosity and number of alleles at the SSR locus ($r = 0.905$ and $r = 0.866$; $p < 0.001$) respectively (Table 2).

Table 2. Correlation coefficients showing the relationship between polymorphic information content, heterozygosity and number of alleles

	He	Number of alleles
He	-	
Number of alleles	0.844[****]	-
PIC	0.994[***]	0.891[***]

Note. [***] = significant at P ≤ 0.001, PIC = polymorphic information content (PIC), He: heterozygosity.

3.2 Cluster Analysis of Potato Clones

The dendrogram was constructed using UPGMA clustering algorithm based on SSR data matrices and grouped the potato clones into three major clusters (Figure 1). The first cluster consisted of ten clones seven of which are commonly grown varieties bred by the International Potato Center (CIP) and released in Uganda. The two lines in this cluster (NKRK19.10 and NKRN59.41) are crosses from potato genotypes (Rutuku × Kackpot1 and Rutuku × NakPot5) respectively. The second cluster consisted of seven clones, four of them (Kabera, Shutama, Mbumbamagara and Kimuli) are farmers' varieties while the rest were from population B3C2 obtained from CIP. And the third cluster consisted of Kachpot1, Mabondo and NakPot1. Shutama was the least genetically related to other clones (1.9) followed by Kabera, Kachpot1 and Mbumbamagara. The most closely related varieties were 396026.103 and 396034.104. Varieties Shutama and Mbumbamagara, are taken to be the same by farmers but given different names in the potato growing districts of Kisoro and Kabale respectively. However, the results of this study revealed a genetic difference between the two with genetic distances of 1.9 and 1.6 respectively albeit belonging to the same cluster. There was a close relationship between the genetic clustering and the phenotypic characteristics of the studied genotypes. For example, 57% of the susceptible genotypes belonged to cluster two and all these take between 70-90 days to maturity. Additionally, 86% of the moderately late blight resistant and

high yielding genotypes were in cluster one. The genetic distance between clones ranged from 1 to 5.7 (Table 4). The highest range was found with Cruza indicating its less relatedness to other clones studied. The shortest genetic distance between 396026.103 and 396034.104 (1.0) could be due to the fact that both are from population B2C3 and possibly selections from a single cross.

Table 3. SSR repeat types and primer sequences, allelic information, heterozygosity and PIC values of 16 SSR loci used for genotyping

No	Marker name	Repeat	Primer sequences(5'-3') Forward-Reverse	Allele size (range bp)	No of alleles	He	PIC	PGI Kit
1	STM2013	(TCTA)6	TTCGGAATTACCCTCTGCC AAAAAAAGAACGCGCACG	164-166	2	0.420	0.338	No
2	STM1104	(TCT)5	TGATTCTCTTGCCTACTGTAATCG CAAAGTGGTGTGAAGCTGTGA	185-189	4	0.565	0.509	Yes
3	STM1049	(ATA)6	CTACCAGTTTGTTGATTGTGGTG AGGGACTTTAATTTGTTGGACG	201-210	3	0.277	0.257	No
4	STM0037	(TC)5(AC)6AA (AC)7(AT)4	AATTTAACTTAGAAGATTAGTC TCATTTGGTTGGGTATGATA	89-96	3	0.265	0.247	Yes
5	STI0012	(ATT)n	GAAGCGACTTCCAAAATCAGAA AAGGGAGGAATAGAAACCAAAA	185-191	3	0.602	0.531	Yes
6	STI0023	(CAG)n	GCGAATGACAGGACAAGAGG TGCCACTGCTACCATAACCA	48-49	2	0.099	0.095	No
7	STI0030	(ATT)n	TTGACCCTCCAACTATAGATTCTTC TGACAACTTTAAAGCATATGTCAGC	53-54	2	0.388	0.313	Yes
8	STI0036	(AC)n(TC)imp	GGACTGGCTGACCATGAACT TTACAGGAAATGCAAACTTCG	134-147	4	0.582	0.544	No
9	STI0032	(GGA)n	TGGGAAGAATCCTGAAATGG TGCTCTACCAATTAACGGCA	128-144	6	0.760	0.723	Yes
10	STWAX-2	(ACTC)n	CCCATAATACTGTCGATGAGCA GAATGTAGGGAAACATGCATGA	239-243	4	0.427	0.393	No
11	STI046	(GAT)n	CAGAGGATGCTGATGGACCT GGAGCAGTTGAGGGCTTCTT	200-218	7	0.776	0.746	No
12	STI031	(TCA)n	CAGAGGATGCTGATGGACCT GGAGCAGTTGAGGGCTTCTT	140-156	8	0.805	0.783	No
13	STM0030	Compound (GT/GC)(GT)8	AGAGATCGATGTAAAACAC GTGTGGCATTTTGATGGATT	153-178	5	0.549	0.506	No
14	STM5121	(TGT)n	CACCGGAATAAGCGGATCT TCTTCCCTTCCATTTGTCA	306-309	2	0.124	0.117	Yes
15	STM0019a	(AT)7(GT)10(AT)4 (GT)5(GC)4(GT)4	AATAGGTGTACTGACTCTCAATG TTGAAGTAAAAGTCCTAGTATGTG	210-225	5	0.504	0.478	Yes
16	STPoA58	(TA)13	TTGATGAAAGGAATGCAGCTTGTG ACGTTAAAGAAGTGAGAGTACGAC	246-250	3	0.328	0.313	Yes

Table 4. Jaccard's similarity matrix for 20 potato genotypes analysed using 17 SSR markers

	CR	KB	K1	KL	KG	MD	MB	N1	N5	19.1	9.41	PT	RG	RS	RT	SH	VC	6.1	4.1	8.1
Cruza																				
Kabera	4.7																			
Kachpot1	5.5	4.8																		
Kimuli	4.3	3.6	3.9																	
Kinigi	2.8	5.1	4.7	4.0																
Mabondo	5.7	5.4	2.9	4.6	5.0															
Mbumbamagara	4.5	4.2	3.7	3.0	4.4	4.1														
Nakpot1	4.5	4.8	3.4	4.1	4.2	2.7	3.6													
Nakpot5	3.7	3.5	4.5	3.5	3.9	4.9	3.7	4.4												
NKRK19.10	4.5	3.6	4.7	4.1	4.4	5.1	4.6	4.9	2.6											
NKRI59.41	3.1	4.2	5.3	4.8	3.6	5.1	4.6	4.0	2.9	3.6										
Petero	3.1	4.0	4.0	3.5	3.2	4.4	4.0	4.3	3.5	3.8	3.9									
Rwangume	3.8	4.3	5.2	4.4	4.2	5.1	4.1	4.6	3.4	3.6	3.5	3.6								
Rwashaki	3.2	4.1	3.8	3.8	3.6	3.9	3.5	3.8	3.3	3.4	3.2	1.9	2.5							
Rutuku	3.8	3.8	4.3	3.1	3.5	5.0	4.1	4.4	2.5	3.2	3.9	3.2	2.6	3.1						
Shutama	4.2	3.6	4.6	3.9	4.0	5.3	3.8	4.7	3.5	4.3	4.0	4.4	5.3	4.7	4.4					
Victoria	3.0	3.8	5.2	4.2	2.7	5.2	4.2	4.3	3.3	3.9	2.3	3.5	3.9	3.6	4.0	3.0				
396026.103	5.1	3.3	5.1	4.0	4.8	5.2	4.8	5.2	3.3	3.1	4.1	4.1	3.8	4.0	3.5	4.3	3.8			
396034.103	5.3	3.3	5.2	4.2	5.1	5.3	4.7	5.3	3.5	3.6	4.2	4.2	3.9	4.1	3.7	4.2	4.0	1.0		
396038.107	4.4	3.2	4.4	1.9	4.5	4.8	3.6	4.4	2.9	3.4	4.1	3.8	4.6	3.8	3.5	3.8	3.9	3.8	3.9	

Note. CR = Cruza; KB = Kabera; K1 = Kachpot1; KL = Kimuli; KG = Kinigi; MD = Mabondo; MB = Mbumbamagara; N1 = Nakpot1; N5 = Nakpot5; 19.1 = NKRK19.10; 9.41 = NKRI59.41; PT = Petero; RG = Rwangume; RS = Rwashaki; RT = Rutuku; SH = Shutama; VC = Victoria, 6.1 = 396026.103, 4.1 = 396034.103; 8.1 = 396038.107.
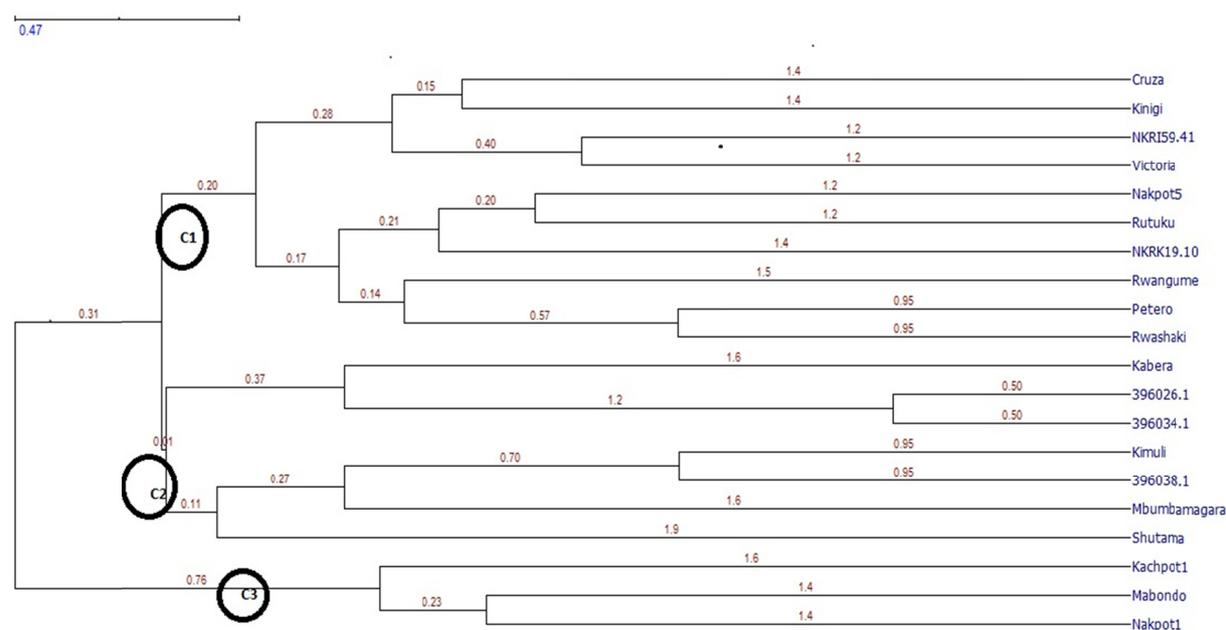
Figure 1. Dendogram showing genetic relationship among 20 potato clones obtained using 16 SSR markers generated by UPGMA. The three clusters identified are C1, C2 and C3

4. Discussion

The 64 number of alleles revealed by microsatellites in this present study and the number of alleles per locus (range 2 to 8) was low compared to earlier studies. For instance, Sharma and Nandineni (2014) obtained 139 alleles while alleles per locus ranged from 6 to 11 with 44 potato genotypes using 17 SSR markers. Ghislain et al. (2009) detected 137 alleles using 24 SSR markers on 742 potato genotypes with the range of 3 to 9 alleles per locus. In both studies more potato genotypes were used with almost the same primers. However, Muthoni et al. (2014) identified 160 alleles and the SSR loci ranged from 2 to 14 with 20 potato clones using 24 SSR markers. In this study more alleles were identified with relatively the same number of markers and genotypes. On the other hand, Solano et al. (2103) recorded 64 alleles using seven SSRs and 40 potato clones while Muhinyuza et al. (2015) observed 84 alleles with 13 SSR markers and 18 genotypes. It can thus be observed that other than the number of markers and genotypes used, other factors contribute to the total number of alleles detected. The lower total number of alleles in this study can be attributed to the high percentage of null alleles in some genotypes where several markers failed to produce detectable variation.

However, several markers performed differently in the current study compared to previous ones. For instance ST1046 (0.776), relatively higher PIC values were observed by Rocha (2010); Muthoni et al. (2014); and Muhinyuza et al. (2015) with values of 0.970, 0.836 and 0.842 respectively. Some markers used in this study had extremely low PIC values from those found in previous studies that is ST10023 had 0.795 and 0.813; STM5121 had 0.374; STM1049 had 0.784 while STM0037 had 0.683 (Muthoni et al., 2014; Ghislain et al., 2009). In other studies, STI0030 had 0.76 (Hirut, 2015) while STI0036 had 0.839 (Muthoni et al., 2014). The low PIC values in this study could be explained by the fact that most of the genotypes used were not so closely related yet microsatellites are particularly useful for closely related genetic materials. Furthermore, the differing laboratory procedures could have resulted into the observed variances in PIC values.

Significant variation of genetic distance among genotypes showed the presence of genetic diversity among them. Genotypes from CIP tended to be clustered together suggesting a common ancestry. Farmer's varieties can be used as parents in the breeding programme given their less relatedness while clones 396026.103 and 396034.103 may not be crossed as this may result into reduced genetic variation and in breeding depression in their progenies. Cruza is an old variety with the highest genetic distance; less likely to be related to other genotypes and thus can be used as parent in the breeding program.

5. Conclusion

The study determined the pattern and level of genetic diversity among the selected 20 tetraploid potato genotypes using 16 SSR markers to identify suitable parents for breeding purposes. The microsatellites were useful and revealed considerable genetic variation among genotypes which can be exploited for possible crop improvement. The genotypes were clustered into three and the following were selected for use in the crop improvement programme: Kinigi, Rwangume and NKRI59.41 from cluster 1; 396034.103, 396038.107 and Kimuri from cluster 2; and Nakpot1 from cluster 3.

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