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Assessment of genetic relationship of promising potato genotypes grown in Rwanda using SSR markers

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Abstract

Evaluation of genetic relationship and divergence is important for an efficient choice of parents for breeding and strategic conservation. The objective of this study was to determine genetic relationship among Rwandan potato genotypes using thirteen selected polymorphic simple sequence repeat (SSR) markers to identify suitable parents for breeding. The thirteen SSR primers identified 84 alleles across all genotypes. The number of alleles per locus ranged from 3 to 10 with an average was 6.5. The polymorphic information content (PIC) of loci ranged from 0.51 to 0.85 with an average of 0.71. Heterozygosity (He) varied from 0.59 to 0.86 with an average of 0.75. Significant positive correlations were detected between PIC and He (r= 0.99), PIC and number of alleles (r=0.76) and, He and number of alleles (r=0.80). The genetic distance between cultivars ranged from 0.44 to 0.93 and the average was 0.68. Overall the SSR analysis provided five different genetic clusters of the potato cultivars useful for breeding.

Keywords: Polymorphic information content, potato, Rwanda, SSR markers.
Abbreviations: PIC= polymorphic information content; SSR= simple sequence repeats.

Introduction

Potato (*Solanum tuberosum* L., 2n=4x=48) is a food security crop globally and ranks third after wheat and rice (Haverkort et al., 2009). In Rwanda, potato is the second major food crop after cassava (FAOSTAT, 2013) and its importance is expanding (ISAR, 2008). Lack of high yielding and late blight disease resistant varieties are among the major limiting factors to potato productivity in Rwanda (FAO, 2008; ISAR, 2008). Current potato breeding activities are aimed at developing new high yielding cultivars with pest and disease resistance (Tähtjärv et al., 2013). Genetic analysis using phenotypic or molecular makers helps to determine the variations present among genetic resources for breeding and strategic conservation. Genetic diversity analysis in potatoes is therefore required to identify complementary and unrelated parents to limit genetic depression and to ensure genetic variation for sustained potato improvement (Tarn et al., 1992, Spooner et al., 2007). Potato is a highly heterozygous crop and commercially grown through vegetative reproduction or tubers (Bradshaw, 2007). Microsatellites or simple sequence repeats (SSR) DNA markers have been used in determining potato genetic diversity, genetic structure, and classification (Spooner et al., 2007); tracing germplasm migrations (Rios et al., 2007); fingerprinting (Provan et al., 1996; Schneider and Douches, 1997; Moisan-Thiery et al., 2005); genetic linkage mapping (Feingold et al., 2005); establishment of core collections (Ghislain et al., 2006) and investigations of duplicate collections across gene banks (Del Rio et al., 2006). The SSR markers are currently the most powerful tools to study genetic relationships because they are easy to handle, inherited in a co-domimant fashion, multiallelic and highly polymorphic even among closely related cultivars, due to mutations causing variations in the number of repeating units (Spooner et al., 2005). Potato breeders use various methods in selecting the best parents for making crosses and to select progenies from recombined parents such as the use of pedigree information, phenotypic performance for specific traits, adaptability and yield stability, and designed crosses using various mating designs. Further, genetic distance estimates using molecular markers are helpful to identify the best parents for new pedigrees (Acquaah, 2007). The national potato research program of Rwanda based at Rwanda Agriculture Board, Northern division at Musanze routinely identifies suitable clones with high yield and disease resistance using local and introduced genetic resources from the international potato centre (CIP) (Muhinyuza et al., 2014). Consequently, eighteen potato genotypes were recently selected showing genetic complementarities for yield and late bight resistance useful in the development of farmers-preferred potato varieties in the country. These genotypes were systematically characterized using phenotypic traits indicating their suitability and genetic differences for breeding (Muhinyuza et al. 2014). In light of this, the objective of this study was to determine the genetic relationship among the eighteen selected Rwandan potato genotypes to identify unrelated parents for a breeding programme and genetic conservation.

Results

The 13 SSR primers identified 84 alleles across all the 18 potato genotypes. The number of alleles per locus ranged from 3 to 10 and the average was 6.5 (Table 2). The PIC estimated for all loci ranged from 0.85 (STM 0037) to 0.51

Table 1. List and sources of potato genotypes used in the study.

No	Genotypes	Source	Population	Year of release	Yield (t ha^{-1})	RAUDPC (%)
1	391047.34	CIP	B3C1	Not yet released	37.4	24.1
2	393077.54	CIP	B3C1	Not yet released	33.5	29.6
3	393371.58	CIP	B3C1	Not yet released	50.9	21.6
4	393637.171	CIP	-	Not yet released	19.8	37.6
5	396033.102	CIP	B2C2	Not yet released	27.3	38.2
6	395111.19	CIP	-	Not yet released	34.4	14.3
7	395112.36	CIP	B3C2	Not yet released	25.2	34.5
8	393280.57	CIP	B3C1	Not yet released	23.6	9.3
9	393385.39	CIP	-	Not yet released	27.2	30.1
10	396026.103	CIP	B3C2	Not yet released	26.9	33.9
11	393280.82	CIP	B3C2	Not yet released	34.2	12.5
12	396036.201	CIP	-	Not yet released	30.1	33.5
13	381381.13	CIP	-	Not yet released	33.4	29.8
14	Gikungu	Rwanda		1992	17.1	11.1
15	Kigega	Rwanda		1992	33.9	18.8
16	Kirundo	Rwanda		1983	28.6	27.6
17	Nderera	Rwanda		1992	21.5	25.4
18	Ngunda	Rwanda		1992	33	19.4

CIP=International potato center; RAUDPC = Relative area under the disease progress curve. source: Muhinyuza et al., 2014

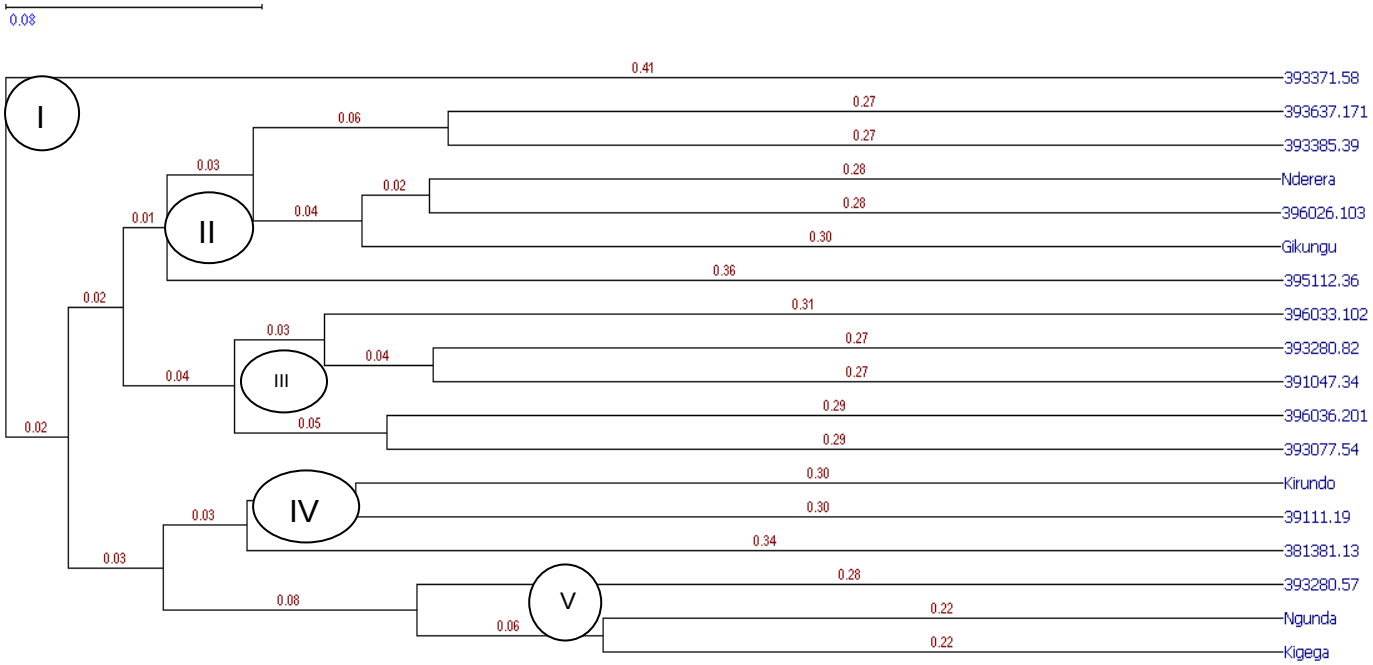

Fig1. Dendrogram showing genetic relationship among 18 potato genotypes using 13 SSR markers generated by UPGMA. The five clusters among the genotypes are denoted from I to V.

(STM1049) with an average of 0.71. These results indicated that the selected microsatellites were very informative in genetically distinguishing the test cultivars. Heterozygosity (He) is a measure of allelic diversity at a locus. The expected heterozygosity values varied from 0.59 to 0.86 with an average of 0.75 (Table 2). Significant and positive correlations were found between PIC and He (r=0.99, P<0.001), PIC and number of alleles (r=0.86, P<0.001) and, He and number of alleles (r=0.88, P<0.001) (Table 3). The dendrogram constructed using the UPGMA clustering algorithm based on SSR data matrices grouped the potato cultivars into five major clusters (Fig. 1). Cluster I consisted of clone 393357.58 standing alone. Cluster II composed of six genotypes: four CIP clones (393637.171, 393385.39, 396026.103 and 395112.36) and two local varieties (Nderera, and Gikungu). Cluster III allocated five CIP clones (396033.102, 393280.82, 391047.34, 396036.201 and 393077.54) representing 38.5% the CIP clones. This showed the genetic similarity among potato clones sourced from CIP. Cluster IV included three genotypes: two CIP clones (39111.19 and 381381.13) and one local variety (Kirundo). Cluster V consisted of two local varieties (Ngunda and Kigega) (Fig.1). The genetic distance between cultivars ranged from 0.44 to 0.93 (Table 4). The shortest genetic distance (0.44) was found between Ngunda and Kigega whereas the highest distance at (0.93) was identified between clone 393357.58 and Ngunda. Among the 18 genotypes, clone 393357.58 was the least genetically related to the other genotypes (Fig.1). Overall, results showed that the thirteen microsatellite markers clearly distinguished all the eighteen potato genotypes.

Discussion

Precise identification of genetic relationship and divergence of genetic resources is a useful tool for an efficient choice of

Table 2. SSR fragment size standard for each SSR marker, allelic information, PIC and He values of the 13 SSR loci used to 18 genotypes.

NO	Marker name	Repeat	Primer sequences (5'-3')Forward-Reverse	No of alleles	Allele Size (bp)	PIC	He	PGI Kit
1	STM0030	Compound(GT/GC)(GT)n	AGAGATCGATGTAAAACACGT GTGGCATTTTGATGGATT	8	140-185	0.7977	0.8218	Yes
2	STM1104	(TCT)n	TGATTCTCTTGCCTACTGTAATCG CAAAGTGGTGTGAAGCTGTGA	6	177-201	0.6713	0.7161	Yes
3	STI0023	(CAG)n	GCGAATGACAGGACAAGAGG TGCCACTGCTACCATAACCA	6	160-220	0.6780	0.7279	No
4	STI0036	(AC)n(TC)imp	GGACTGGCTGACCATGAACT TTACAGGAAATGCAAACTTCG	10	127-157	0.8461	0.8615	No
5	STM5127	(TCT)n	TTCAAGAATAGGCAAAACCA CTTTTTCTGACTGAGTTGCCTC	9	253-298	0.7724	0.8004	Yes
6	STM1052	(AT)nGT(AT)n(GT)n	CAATTTCGTTTTTTCATGTGACAC ATGGCGTAATTTGATTTAATACGTAA	5	220-240	0.5792	0.6154	Yes
7	STM2013	(TCTA)n	TTCGGAATTACCCTCTGCC AAAAAAAGAACGCGCACG	5	155-190	0.7466	0.7822	No
8	STI046	(GAT)n	CAGAGGATGCTGATGGACCT GGAGCAGTTGAGGGCTTCTT	9	195-229	0.8420	0.8582	No
9	STM1049	(ATA)n	CTACCAGTTTGTTGATTGTGGTG AGGGACTTTAATTTGTTGGACG	4	195-215	0.5101	0.5944	No
10	STM0037	(TC)n(AC)nAA(AC)n(AT)n	AATTTAACTTAGAAGATTAGTCTC ATTTGGTTGGGTATGATA	10	80-110	0.847	0.8619	Yes
11	STM1106	(ATT)n	TCCAGCTGATTGGTTAGGTTG ATGCGAATCTACTCGTCATGG	3	170-180	0.5707	0.6465	Yes
12	STI0012	(ATT)n	GAAGCGACTTCCAAAATCAGA AAAGGGAGGAATAGAAACCAAAA	6	182-215	0.7864	0.8120	Yes
13	ST WAX-2	(ACTC)n	CCCATAATACTGTCGATGAGCA GAATGTAGGGAAACATGCATGA	3	235-265	0.5848	0.6593	No
Mean				6.5		0.71	0.75	

PIC= polymorphic information content**,** He: Heterozygosity, bp: base pairs.

Table 3. Correlation coefficients showing pair-wise association between polymorphic information content (PIC), heterozygosity (He) and number of alleles.

	PIC	He
PIC	-	
He	0.99***	-
Number of alleles	0.86***	0.88***

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

Table 4. Jaccard's similarity matrix of 18 potato genotypes analyzed using 13 SSR markers.

Genotypes*	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	Ngunda	Kigega	Kirundo	Nderera	Gikungu
C1																		
C2	0.83																	
C3	0.71	0.86																
C4	0.79	0.86	0.69															
C5	0.83	0.83	0.79	0.92														
C6	0.77	0.54	0.77	0.64	0.82													
C7	0.75	0.75	0.69	0.69	0.73	0.55												
C8	0.85	0.77	0.62	0.75	0.91	0.60	0.70											
C9	0.85	0.77	0.69	0.75	0.92	0.75	0.56	0.64										
C10	0.77	0.77	0.71	0.83	0.64	0.73	0.75	0.75	0.58									
C11	0.85	0.77	0.62	0.75	0.64	0.64	0.64	0.55	0.73	0.55								
C12	0.86	0.86	0.77	0.82	0.67	0.82	0.91	0.73	0.90	0.83	0.55							
C13	0.85	0.83	0.77	0.92	0.73	0.70	0.67	0.78	0.90	0.75	0.73	0.70						
Ngunda	0.93	0.93	0.85	0.91	0.67	0.90	0.80	0.90	0.90	0.82	0.73	0.78	0.67					
Kigega	0.85	0.92	0.69	0.83	0.45	0.82	0.56	0.80	0.78	0.82	0.70	0.70	0.67	0.44				
Kirundo	0.85	0.85	0.86	0.73	0.64	0.64	0.83	0.82	0.92	0.83	0.75	0.60	0.64	0.82	0.83			
Nderera	0.85	0.69	0.86	0.75	0.64	0.55	0.55	0.82	0.82	0.83	0.75	0.70	0.70	0.73	0.64	0.60		
Gikungu	0.85	0.85	0.79	0.67	0.82	0.60	0.64	0.60	0.83	0.82	0.83	0.83	0.70	0.80	0.73	0.64	0.55	

* C1: 391047.34; C2: 393077.54; C3:39371.58; C4:3937.171; C5:3960.102; C6: 395111.19; C7: 395112.36; C8: 39280.57; C9: 393385.39; C10: 396026.103; C11: 39280.82; C12: 396036.201; C13: 381381.13.

parents for breeding and genetic conservation strategies. Further, genetic diversity analysis is useful to estimate genetic distance of germplasm pool and to construct genetic maps. This will assist in minimizing the use of closely related parents in breeding which would otherwise lead to genetic depression and reduced genetic variation. The current study was therefore carried out to establish genetic relationship among selected eighteen potato cultivars to identify appropriate parents for hybridization. In this study microsatellite markers were used for potato genetic identification because of their high genetic information content, high reproducibility, and simplicity to use (Powell et al. 1996; Jones et al. 1997). Moreover, they are appropriate, cost-effective and simple tools for laboratories in developing countries with financial constraints. The results revealed high polymorphism levels among 18 potato cultivars. According to Coombs et al. (2004) and Ghislain et al. (2006), polymorphism level is usually high for potato cultivars because potato is inherently heterozygous and essentially tretrapoid. Significant variation of genetic distance among genotypes indicated the presence of genetic variability among the selected potato cultivars. The mean PIC value determined in the present investigation agrees well with earlier research on the use of SSR markers on potato (Rocha, 2010). PIC demonstrates the usefulness of the SSR loci and their potential to detect differences among the potato cultivars based on their genetic relationships. The ability to measure genetic distances between the potato cultivars that reflect pedigree relationship ensures a more stringent evaluation of the adequacy of marker profile data. The dendrogram generated from the genotypic data grouped CIP clone 393371.58 in a single cluster, making it the least genetically related genotype. They also elucidated the presence of genetic similarity among CIP clones. Two local varieties Ngunda and Kigega showed also genetic similarity among them due probably to closely related parents in breeding. Their crosses would otherwise lead to genetic depression and reduced genetic variation in their progenies. The DNA-based genotyping using simple sequence repeats have been shown to discriminate between tetraploid potato genotypes. There is considerable genetic variability among selected potato genotypes which is useful for potato breeding in Rwanda. The SSR genetic markers were useful and provided five distinct genetic groups enabling breeders to design targeted crosses for hybrid development to exploit heterosis, and maintain genetic diversity.

Materials and Methods

Plant materials

The study used eighteen potato genotypes showing high to medium responses for late blight resistance and high yields; thirteen advanced clones were acquired from CIP and five were local varieties widely grown in Rwanda. The details of the germplasm are described in Table 1.

DNA extraction and genotyping

DNA sampling

DNA samples were collected from four week old plants, using Whatman FTA cards. Samples were collected from fresh young leaves of ten plants per genotype. Each sampled leaf per plant was immediately placed on the FTA card and pressed using a pair of pliers until both sides of the FTA paper were soaked with the sap (Ndunguru et al., 2005). Ethanol (70%) was used to clean the pliers between sampling to prevent cross contamination. The FTA cards were dried at room temperature.

SSR analysis

Samples on the FTA cards from the 18 genotypes were analyzed at the INCOTEC-PROTEIOS laboratory in South Africa (Incotec, SA Pty. Ltd. South Africa). All the samples from each genotype were used in bulked amplification, using DNA extracted from the 10 bulked punches from each FTA card per genotype. Thirteen SSR markers selected from the linkage group of potato and using their high polymorphic information content (PIC) (Ghislain et al., 2004; Feingold et al., 2005; Ghislain et al., 2009; Rocha, 2010) were used in this study. Seven of them belong to the latest potato genetic identity (PGI) kit (Ghislain et al., 2009) while the others were identified from other studies and selected based on high PIC (Ghislain et al., 2004; Feingold et al., 2005; Ghislain et al., 2009; Rocha, 2010). The PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa). The SSR marker alleles were scored for presence or absence of the band for all the 18 potato genotypes. Each amplified fragment was considered as one locus. The genetic similarity matrix of the 18 potato genotypes was calculated using the Jaccard's coefficient (Anderberg, 1973).

Data analysis

Data analysis was performed using GeneMapper 4.1. The program GGT 2.0 (Van Berloo, 2007) was used to calculate the Euclidian distances between bulked samples, the matrix of the genetic distances were used to create an unweighted pair group method with arithmetic mean (UPGMA) dendrogram of the results. The polymorphic information content (PIC), is a measure of allelic diversity and was calculated as $PIC = 1 - \Sigma(pi^2)$, where pi is the frequency of i^{th} allele detected in all individuals of the populations (Nei, 1973; Rafalski et al., 1996). Pearson's correlation coefficients showing pair-wise association between PIC, He and number of alleles were calculated using Genstat statistical package, 14^{th} edition (Payne et al., 2011).

Conclusion

The DNA-based genotyping using simple sequence repeats have been shown to discriminate between tetraploid potato genotypes. Overall the SSR analysis provided five different genetic clusters of the potato genotypes useful for breeding.

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