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Genetic Diversity of Kenyan Potato Germplasm Revealed by Simple Sequence Repeat Markers

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Abstract International potato germplasm (*Solanum tuberosum* L.) has been long introduced to and bred in Kenya and the accumulated Kenyan *Solanum* germplasm is unique in its geographical and climatic ranges of adaptation to tropical highlands. However, little is known about the genetic diversity of these improved *Solanum* accessions. A representative set of 48 potato accessions grouped as farmer varieties, local genotypes and modern varieties was selected and studied using the simple sequence repeat (SSR) technique. Twenty-two SSR primer pairs were applied and 122 polymorphic bands were scored. The frequencies of polymorphic bands ranged from 0.02 to 0.98 and averaged 0.35. The proportion of total SSR variation occurring among four origin categories (International Potato Centre (CIP), Europe, Kenya and Unknown) of accessions was 6.32%; between accessions introduced before and after 1980 4.79%; and among three germplasm classes of accessions 4.36%. Accessions from the CIP displayed more SSR variation than those from Europe. More SSR variation was detected in the accessions introduced/bred after 1980. The modern varieties displayed slightly more diversity than the farmer varieties and local genotypes. Some dominant groups of accessions largely from CIP and Europe were found, but these groups were not distantly separated. Both the genetically most distinct accessions and the possibly genetically related accessions were identified. These results not only demonstrate the considerable genetic variation harbored in the Kenyan potato germplasm, but also are significant for developing effective strategies of acquiring genetically diverse germplasm and for selecting genetically distinct potato materials to widen the Kenyan improved gene pool.

Resumen Desde hace tiempo se ha introducido y mejorado germoplasma internacional de papa (*Solanum tuberosum* L.) en Kenia y el germoplasma acumulado de *Solanum* Keniano es único en sus amplitudes de adaptación geográfica y climática a altiplanos tropicales. No obstante, poco se sabe sobre la diversidad genética de estas introducciones mejoradas de *Solanum*. Se seleccionó y estudió, utilizando la técnica de una secuencia simple de repetición (SSR), un juego representativo de 48 introducciones agrupadas como variedades de los productores, genotipos locales y variedades modernas. Se aplicaron veintidós pares de iniciadores SSR y se registraron 122 bandas polimórficas. Las frecuencias de bandas polimórficas variaron de 0.02 a 0.98 con un promedio de 0.35. La proporción de la variación total de SSR que se presentó entre cuatro categorías por origen (Centro Internacional de la Papa CIP, Europa, Kenia y desconocido) de las introducciones, fue 6.32%; entre las introducciones hechas antes y después de 1980 fue de 4.79; y entre tres clases de accesiones de

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germoplasma, de 4.36%. Las introducciones del CIP mostraron más variación de SSR que las de Europa. Se detectó más variación de SSR en las accesiones introducidas o mejoradas después de 1980. Las variedades modernas mostraron ligeramente más diversidad que las de los productores y que los genotipos locales. Se encontraron algunos grupos dominantes de introducciones, mayormente del CIP y Europa, pero estos grupos no estaban distantes en separación. Se identificaron tanto a las introducciones genéticamente más diversas como a las que posiblemente estaban genéticamente relacionadas. Estos resultados no solo demuestran la considerable variación genética con la que se cuenta en el germoplasma de papa Kenyano, sino que también es significativo para el desarrollo de estrategias efectivas en la adquisición de germoplasma diverso genéticamente y para seleccionar materiales de papa genéticamente distintos para ampliar el acervo genético mejorado de Kenia.

Keywords Potato · SSR · Genetic diversity · Genetic distinctiveness · Germplasm management

Introduction

The cultivated potato, *Solanum tuberosum* L., is an important food and cash crop in Kenya with an annual production of 800,000 tons (Anonymous 2009). Efforts to improve this crop date back to 1880s with many of the introductions originating from Europe. An organized potato breeding programme began in 1943 with the initial objective of selecting materials for late blight (*Phytophthora infestans*) resistance (Todd 1969). In the 1960–1970's, breeding was expanded to select for other traits of importance such as bacterial wilt (*Ralstonia solanacearum*) tolerance and drought tolerance (Todd 1973). The breeding efforts in the 1980's through to the 1990's focused on developing clones with high stable yields; incorporating resistance to late blight, bacterial wilt and viruses; improving adaptability to non-traditional growing areas; and improving storability and culinary quality (Crissman et al. 1993). The current breeding programme is aimed at developing high yielding varieties with tolerance to late blight, wide adaptability, desired cooking and processing qualities (KARI-Tigoni 2007).

The breeding efforts have so far generated 29 officially released cultivars (Crissman et al. 1993; Lung'aho et al. 1998, 2006; KARI-Tigoni 2011). Over the years, farmers also have grown over 60 other varieties that were not formally released and currently about 15 of them are still widely grown (Crissman 1989; Guyton et al. 1994; Kaguongo et al. 2008). The number of international introductions, although with no detailed records, could be up to a thousand. Various donors and partners influenced breeding and germplasm activities in the country and probably the genetic structure of the collection due to the prioritization of certain types of germplasm over others. Prior to 1980 germplasm introductions came mainly from Europe (UK, Germany and Holland). After 1980, most of the germplasm introductions were originated from the International Potato Centre (CIP) in Peru. The germplasm accumulated in the Kenyan potato gene pool is unique for its geographical and climatic ranges of adaptation to tropical highlands includingadaptation to day neutral conditions, moderate temperatures and a 90–120 day growing period. However, many challenges remain for the conservation and utilization of the potato germplasm gathered over the last century. Efforts to conserve existing potato germplasm for future variety development have been limited and have progressed slowly. Some of the earlier released varieties have been lost and are no longer in production or being maintained by the Kenya Potato programme. Because of the dearth in knowledge on genetic diversity and genetic relationships of these germplasm accessions, their use in potato breeding is limited.

Characterization of plant germplasm using molecular techniques has played an increasingly important role in the management and utilization of plant genetic resources (Karp 2002). It has also enhanced plant breeding in selection of diverse parents to widen the breeding gene pool (Fu 2006). Previous efforts to characterize potato germplasm have included the use of allozymes (Ortiz and Huaman 2001), restriction fragment polymorphisms (RFLP) (Powell et al. 1991), random amplified polymorphic DNA (RAPD) (Hosaka et al. 1994; Demeke et al. 1996; del Rio et al. 1997), amplified fragment length polymorphisms (AFLP) (Milbourne et al. 1997; Kim et al. 1998), inter simple sequence repeats (ISSR) (Bornet et al. 2002), and simple sequence repeats (SSR) markers (Ghislain et al. 2004, 2006; Braun and Wenzel 2005; Feingold et al. 2005; Ispizúa et al. 2007; Fu et al. 2009). In addition to providing useful information for understanding the genetic diversity and structure of various potato gene pools established in different geographical regions for effective management of germplasm, these characterizations have facilitated potato breeding by identifying released cultivars and determining their genetic relationships. The existing gene pools of cultivated potato are reported to be genetically narrow (Hawkes 1978), especially in North American *Solanum* gene pool that is mainly derived from a small number of parental lines (Love 1999; Simko et al. 2004, 2006). It would therefore be of interest to perform diversity assessments of the potato gene pools established from different geographical regions.

With the goal of facilitating future activities associated with germplasm conservation and breeding in Kenya, we collected 48 representative potato accessions and studied their genetic diversity, structure, association, and distinctiveness using 22 M13-tailed SSR markers.

Materials and Methods

Plant Materials

The potato germplasm used in this study consisted of 48 representative accessions that have been adapted to Kenya (Table 1). They included the 14 most important farmer materials widely grown across the country (designated as farmer varieties), the 12 advanced genotypes of the potato collection at the Kenya Agricultural Research Institute (KARI) -Tigoni (designated as local genotypes), and 22 officially released varieties held by the national potato programme (designated as modern varieties). Seven of the 29 officially released varieties were not included in the study, as six have since gone out of production and are not maintained by the potato programme at KARI-Tigoni and one did not have a disease free stock available for the study. The details of the selected accessions are given in Table 1, along with their identifier code, accession name, parentage, and other important characteristics (classification, tuber skin color, breeding period and origin). Based on these characteristics, the accessions were grouped to assess their genetic structures. The plants used were obtained from sprout cuttings derived from disease free tubers grown in a glasshouse.

DNA Extraction and SSR Analysis

Two newly expanded leaves (approximately 100 mg) from the plantlets were used to extract DNA following a modified CTAB method of Mace et al. (2003). DNA quantity and quality were assessed on 0.8% (w/v) agarose gel with lambda DNA as a concentration standard.

Based on polymorphism and genome coverage (Veilleux et al. 1995; Kawchuk et al. 1996; Milbourne et al. 1998; Ghislain et al. 2004, 2009; Feingold et al. 2005), 11 genomic and 11 EST derived SSR primer pairs were selected for this assessment (Table 2). The M13-tailed primer method (Schuelke 2000) was applied to label amplicons. The forward primers were synthesized by adding an M13-forward primer sequence (5'CACGACGTTG TAAAACGAC3') at the 5'end of each primer. All polymerase chain reactions (PCR) were performed in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA). PCRs were performed in 5 µl reaction volume with final concentrations of 5 ng DNA, 2 mM MgCl2, 0.12 mM of dNTPs, 1X PCR buffer, 0.012 pM of M13-tailed forward primer, 0.192 pM of M13-forward primer labeled with either 6-Fam or Vic or Ned or Pet (Applied Biosystems), 0.192 pM of reverse primers and 0.1 U of *Taq* DNA polymerase (AmpliTaq Gold®, Applied Biosystems, USA). The cycling conditions consisted of three steps: 1) an initial denaturation at 94°C for 15 min (to activate *Taq* DNA polymerase); 2) followed by 10 cycles of denaturation at 94°C for 15 s, annealing at 61°C for 20 s (temperature reduced by 1°C for each cycle) and extension at 72°C for 30 s; and 3) 40 cycles of denaturation at 94°C for 10 s, annealing at 54°C for 20 s and extension at 72°C for 30 s with the final extension of 20 min at 72°C. Based on their expected amplicon sizes and dyes, PCR products were pooled together along with internal size standard (LIZ-500) and capillary electrophoresis was performed using ABI 3700xl Genetic Analyzer (Applied Biosystems, USA). The results of the run were then analyzed with GeneScan 3.7 software (Applied Biosystems, USA) and fragment sizes were automatically scored in base pairs based on the relative migration of the internal size standard using Genotyper 3.7 software (Applied Biosystems, USA). The analysis was performed at the Centre of Excellence in Genomics at the International Crops Research Institute for the Semi-Arid Tropics, India.

Data Analysis

The SSR data were analyzed for the levels of polymorphism with respect to primer and sample by counting the number of polymorphic bands and generating summary statistics of band frequencies. Shannon entropy was calculated following Russell et al. (1993) to estimate the diversity content per locus, as this estimate does not require strict genetic assumptions such as marker inheritance and sample ploidy. The entropy-based diversity content provides a measure of the effective number of alleles per marker locus (Reyes-Valdes and Williams 2005). These analyses were performed by using a SAS program written in SAS IML (SAS Institute Inc. 2004).

To assess the genetic structure of the potato germplasm, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) that was based on the dissimilarity matrix of pairwise accessions was also performed using Arlequin version 3.1 (Excoffier et al. 2005). This analysis permitted the partition of the total SSR variation into within- and among- group variation components, and provided measures of inter-group genetic distance as the proportion of the total SSR variation residing between any two groups (Phi statistic; Excoffier et al. 1992). Four models of genetic structuring were examined as (1) germplasm class (modern, farmer and local), (2) tuber skin color (white vs red), (3) breeding period (before vs after 1980), and (4) germplasm of various origins (Kenya, Europe, CIP and unknown). Significance of resulting variance components and inter-group genetic distances was tested with 10,100 random permutations.

The genetic associations of the potato accessions were assessed using two approaches. A principal component analysis of 48 accessions was performed using NTSYS-PC 2.01 (Rohlf 1997) based on the similarity matrix of 122 SSR alleles, and plots of the first three resulting principal components were made to assess the accession associations

Table 1 List of 48 potato accessions studied, along with some characteristics and related SSR variation

Code	Name	Parents	Class[a]	Color[b]	Period[c]	Origin[d]	AD[e]	Cluster[f]
60101	Roslin Tana	Unknown	M	W	B	E	0.273	III
60102	Roslin Bvumbwe	Unknown	M	W	B	E	0.274	I
60103	Tigoni	378493.15 x bk precoz	M	W	A	I	0.299	III
60104	Arka	MPI 19268 X Gineke	M	R	B	E	0.252	I
60105	Roslin Eburu (B53)	SDL.882 (5) x SDL. 1104 c (2)	M	W	B	E	0.301	I
60106	Kenya Chaguo	Unknown	M	W	A	K	0.264	III
60107	Komesha	Unknown	F	R	B	U	0.274	II
60108	Kihoro	Unknown	F	W	A	U	0.249	III
60109	Dutch Robijn (white skinned)	Rode Star x Preferent	L	W	A	U	0.243	I
60110	Kenya Karibu (purple flowers)	676064 x 800946	M	R	A	K	0.302	II
60111	Asante	378493.15 x bk precoz	M	R	A	I	0.302	III
60112	Mugaruru	Unknown	F	R	A	U	0.250	I
60113	Kenya Baraka	SDL.3680 e (18) x SDL. 3070 d (4)	M	W	A	E	0.267	I
60114	Tana Kimande	Unknown	F	W	A	U	0.326	II
60115	Nyayo	Unknown	F	W	A	U	0.257	I
60116	Zangi	Unknown	F	R	A	U	0.295	II
60117	Meru	Unknown	F	R	A	U	0.259	III
60118	Dutch Robijn (red skinned)	Rode Star x Preferent	M	R	B	E	0.240	I
60119	Maritta	S 66/102 x Mittelfruhe	M	W	B	E	0.255	III
60120	Furaha	Atlantic x 7XY.1	M	W	A	I	0.289	I
60121	Ndera Mwana	Unknown	F	R	A	U	0.267	II
60122	Kenya Dhamana	Atzimba x A-1 (Katahdin x 316.3)	M	W	A	I	0.259	III
60123	Kenya Karibu (white flowers)	676064 x 800946	L	R	A	K	0.292	II
60124	Ngure	Unknown	F	R	B	U	0.279	I
60125	Anett	S. *demissum* x S. *acaule* d	M	W	B	E	0.310	II
60126	Tigoni (oval)	Unknown	F	W	A	U	0.241	III
60127	Romano	Draga x Desiree	M	R	B	E	0.230	I
60128	Kerrs' Pink	Fortyfold x Smith's Early	M	R	B	E	0.275	I
60129	Kenya Sifa	Unknown	M	R	A	I	0.289	II
60130	Desiree	Urgenta X Despesche	M	R	B	E	0.229	I
60131	Kenya Mavuno	381378.18 x 720084	M	W	A	K	0.250	III
60132	Pimpernel	Bravo x Alpha	M	R	B	E	0.262	I
60133	393524.9	BWH87.409 x 386042.8	L	W	A	I	0.277	III
60134	393385.39	387231.7 x 387170.9	M	W	A	I	0.276	III
60135	393371.58	387170.16 x 389746.2	M	W	A	I	0.290	II
60136	Mukori	Unknown	F	R	A	I	0.266	II
60137	395438.1	BWH87.344R X TXY.11	L	W	A	I	0.305	II
60138	396286.9	393614.3 x 67008	L	R	A	I	0.284	II
60139	396286.6	TXY.3 x I-1039	L	R	A	I	0.296	II
60140	382171.4	380086.3 x Bulk Mex	L	W	A	I	0.294	III
60141	394904.17	72011.1 x C90.205	L	W	A	I	0.287	II
60142	575049	(Alpha x Hol 32) x [USDA-133.3 x (Leona x Penn 3PO-23)]	L	W	A	I	0.278	II
60143	394903.3	720118.1 x BWH8.183	L	W	A	I	0.284	II
60144	395196.4	(C83.621 x Katahdin) x Bulk I-RKN	L	W	A	I	0.295	II
60145	394905.8	Cruza-148 x C90.205	L	W	A	I	0.305	II
60146	Alika	Unknown	F	R	A	U	0.229	I

Table 1 (continued)

Code	Name	Parents	Class[a]	Color[b]	Period[c]	Origin[d]	AD[e]	Cluster[f]
60147	Thima Thuti-1	Unknown	F	W	A	U	0.283	II
60148	Thima Thuti-2	Unknown	F	W	A	U	0.289	III

[a] Accessions were classified with *M* as modern variety; *F* farmer's variety; *L* local genotype

[b] Tuber skin color with *W* white; *R* red

[c] Breeding periods with *B* before 1980 and *A* after 1980

[d] Origin of an accession was labeled with *E* from Europe; *K* Kenya; *I* International Potato Centre; U unknown

[e] *AD* average SSR dissimilarity

[f] *Cluster* grouping based on SSR similarity

and to identify genetically distinct accessions. A neighbor-joining analysis of 48 accessions was also made using PAUP* (Swofford 1998) and a circulating tree was displayed using MEGA 3.01 (Kumar et al. 2004) to confirm the genetic association of individual accessions and to identify any genetic clustering without restriction to known characteristics.

To assess the genetic distinctiveness of the potato accessions, the similarities of each accession with the remaining accessions assayed were calculated using the simple matching coefficient (Sokal and Michener 1958) as: $S_{ij} = (a+d)/(a+b+c+d)$, where S_{ij} is the SSR similarity between the accession i (i=1 to n) and the other accession j [j=1 to (n-1)], a is the number of alleles (from

Table 2 Details of 22 SSR markers applied in this study

Linkage group	SSR primer	Marker type/ source[a]	PCR label	Annealing temperature	Multiplex group[b]	Size range (bp)[c]	Allelic count/ primer	Entropy based diversity content
I	STG0016	*E*/f	VIC	55	2	137–174	7	2.833
I	STM5127	*G*/f	PET	55	4	248–291	7	2.458
II	STM5114	*G*/f	PET	60	2	297–322	4	2.417
II	STM1064	*G*/c	NED	55	3	201–213	5	1.792
III	STG0010	*E*/f	6-FAM	60	2	175–192	5	1.833
III	STM1053	*G*/c	6-FAM	53	6	170–196	3	0.833
IV	STI0001	*E*/e	6-FAM	60	1	194–215	6	2.708
IV	STI0012	*E*/e	6-FAM	56	5	183–234	6	3.375
IX	STI0014	*E*/e	VIC	54	5	127–137	4	1.750
IX	STM1052	*G*/c	6-FAM	50	3	214–263	6	1.833
V	STI0032	*E*/e	VIC	61	3	127–148	6	3.083
V	STPoAc58	*G*/d	PET	55	3	243–263	3	0.208
VI	STI0004	*E*/e	PET	60	1	83–126	6	1.750
VI	STM0019	*G*/a,b,c	VIC	48	6	99–206	8	0.625
VII	STI0033	*E*/e	NED	61	5	131–155	6	2.708
VII	STM0031	*G*/c	PET	53	5	185–211	5	2.792
VIII	STM1104	*G*/c	NED	53	4	178–198	5	2.125
X	STG0025	*E*/f	VIC	56	1	208–223	2	0.458
X	STM1106	*G*/c	NED	51	6	145–211	4	1.708
XI	STG0001	*E*/f	NED	58	1	137–163	8	3.375
XI	STM0037	*G*/c	NED	52	2	87–133	7	3.792
XII	STI0030	*E*/e	6-FAM	58	4	94–137	9	3.417

[a] *E* EST-derived SSR markers; *G* Genomic SSR markers. The reference of the SSR markers are: a, Veilleux et al. (1995); b, Kawchuk et al. (1996); c, Milbourne et al. (1998); d, Ghislain et al. (2004); e, Feingold et al. (2005); and f, Ghislain et al. (2009). [b] Six groups of primer pairs for multiplex PCR reactions, [c] *bp* base pairs

all SSR loci) shared in both *i* and *j*, *b* is the number of alleles present in *i* but not shared in *j*, *c* is the number of alleles present in *j* but not shared in *i*, and *d* is the number of alleles absent from both *i* and *j*. The SSR dissimilarity for each pair of accessions can be defined as 1- S_{ij}. The average SSR dissimilarity for the accession *i* can be obtained by averaging all of the *n*-1 SSR dissimilarities that the accession was associated with. This average dissimilarity measures the overall genetic difference between the accession (*i*) of interest and the remaining accessions assayed. A higher average dissimilarity obtained from unlinked markers means that the accession has a genetic background more distinct from the other accessions (Fu 2006). This assessment was done using a specific SAS program written in SAS IML (SAS Institute 2004).

Results and Discussion

SSR Variation

The 22 SSR primer pairs detected a total of 122 alleles scattered on all 12 homologous linkage groups of potato (Table 2). Interestingly, 11 EST-derived SSR primer pairs detected more alleles (65) than the 11 genomic SSR primer pairs (57). The most informative primer pair was the genomic STM0037 on linkage group XI with a Shannon entropy of 3.79 and seven alleles detected, followed by the EST-derived STI0030 on linkage group XII with a Shannon entropy of 3.42 and nine alleles detected. The less informative primer pair was the genomic STPoAc58 with a Shannon entropy of 0.21 and three alleles detected, followed by the EST-derived STG0025 with a Shannon entropy of 0.46 and two alleles detected. Some of these primer pairs should sample SSR alleles in both transcribed and non-transcribed chromosomal regions and provide an adequate measure of genetic diversity.

The observed occurrence frequencies of the 122 alleles ranged from 0.02 to 0.98 with an average of 0.35. There were five alleles with an occurrence frequency of 0.95 or higher in the assayed accessions and 32 with frequencies greater than 0.60, while 19 alleles were detected with frequencies less than 0.021 (i.e., occurring only in one accession). Some of the rare alleles may be useful as diagnostic markers for some of the assayed potato accessions. Also, tracing these rare alleles in the larger potato collection held by CIP to enhance the germplasm introduction for future breeding is possible, if the standard sizing for all the SSR alleles as Ghislain et al. (2009) proposed was applied. However, this study employed a different genotyping platform with a different internal size standard, thus making the tracing effort less effective.

Genetic Structure of Potato Accessions

This assessment considered four models of genetic structuring. The proportion of the total SSR variation which resided among three germplasm classes was 4.36% (Table 3), but slightly more diversity was observed within local genotypes (Table 4). The highest SSR differences resided in accessions between farmer varieties and local genotypes (Table 4). This result is not surprising, as the local genotypes are materials probably originating from CIP. Also phenotypically, the farmer varieties tend to have lesser resistances to late blight and viruses in addition to having shorter dormancy.

The proportion of the total SSR variation explained by germplasm origin was 6.32% (Table 3). The materials originated from CIP had the most within group diversity and were distantly apart from those accessions of European origin (Table 4). The highest proportion (0.115) of total SSR variation was found to reside between the accessions of CIP and European origin, reflecting the different breeding gene pools. Interestingly, the accessions originating from CIP are also generally taller, more vegetative and have longer stolons than those of European origin (data not shown).

There was 4.79% of the total SSR variation present between the accessions obtained before and after 1980 (Table 3), but the accessions after 1980 had more within-group variation (Table 4). Interestingly, there was a non-significant (P=0.1959) difference in SSR variation between the accessions with white and red tuber skins (Table 3), indicating that tuber skin color alone is not a good indicator of potato genetic diversity.

The genetic associations of 48 potato accessions obtained (Fig. 1) revealed several patterns of variation. First, the accessions with different origins displayed a large variation and were widely spread over the plot (Fig. 1a). The accessions from CIP displayed more divergence than those from Europe. Second, the accessions released after 1980 had a wider spread over the plot than those released before 1980 (Fig. 1b). Third, the modern varieties were more divergent than the other classes of the potato accessions, while the farmer varieties were largely located within the centre of the plot (Fig. 1c). The two principal components accounted for 17.0% and 12.6% of the total SSR variation, respectively.

These results indicate that the representative set of the Kenyan *Solanum* germplasm displayed considerable SSR variation, which is at least more than those observed in the Canadian improved potato gene pool (Fu et al. 2009). This reflects well the diverse germplasm origins and the low selection pressure applied on the improved gene pool. Thus, the continuous exploration of the existing variation for the potato breeding would most likely be fruitful.

Table 3 Results for analysis of molecular variance for 48 potato accessions with four models of genetic structuring

Sources of variation	*Df*	Sum of squares	Variance component	Percentage of variation	*P*-value [a]
Class [b]					
Among population	2	55.426	0.742	4.36	<0.0001
Within population	45	732.553	16.279	95.64	
Total	47	787.979	17.021		
Origin [c]					
Among population	3	84.463	1.078	6.32	<0.0001
Within population	44	703.516	15.989	93.68	
Total	47	787.979	17.068		
Breeding period [d]					
Between population	1	32.115	0.8273	4.79	<0.0001
Within population	46	755.864	16.439	95.21	
Total	47	787.978	17.259		
Tuber color [e]					
Between population	1	19.601	0.124	0.74	0.1959
Within population	46	769.379	16.704	99.26	
Total	47	787.979	16.828		

[a] The probability that the among-type variance component was larger than zero, as computed with random permutations, [b] Germplasm class includes modern, farmer variety, and local genotype, [c] Germplasm origin includes Kenya, Europe, International Potato Centre, and Unknown, [d] Breeding period includes pre-1980 and post-1980, [e] Tuber color refers to white and red skinned tubers

Genetic Association of Potato Accessions

Clustering of 48 potato accessions by the neighbor-joining analysis revealed several variation patterns (Fig. 2). First, about three major clusters were detected, but they were not well separated due to relatively low SSR variation. Second, Cluster I consisted mainly of 15 accessions with European origin, Cluster II of 19 accessions mainly from CIP, and Cluster III of 14 accessions mixed from Europe and CIP (also see Table 1). Third, four varieties developed in Kenya appear to be more associated with the accessions originated from CIP than Europe. Fourth, three germplasm classes (modern, farmer, local) and two breeding periods (before and after 1980) were not particularly associated with any three clusters; and all of their accessions were widely spread over the three clusters.

Table 4 The proportion of total SSR variation within and among class, origin, and breeding period of Kenyan potato accessions

Group	Sample size	Within group *F*st	Pairwise group *F*st [a]		
Class			Farmer	Local	
Modern variety	22	0.0426	0.0114 ns	0.0641***	
Farmer variety	14	0.0462		0.0663**	
Local genotype	12	0.0423			
Origin			Europe	CIP	Unknown
Kenya	4	0.0790	0.1560*	0.0105 ns	0.0471 ns
Europe	12	0.0744		0.1153***	0.0311 ns
CIP [b]	18	0.0520			0.0363**
Unknown	14	0.0635			
Breeding period			After 1980		
After 1980	35	0.0453	0.0479**		
Before 1980	13	0.0551			

[a] *F*st is the proportional SSR variation observed between two groups to measure the genetic differentiation between subpopulations. The significance levels of the random permutation test with ns, *, **, *** are $P>0.05$, <0.05, 0.01, 0.001, respectively, [b] CIP = International Potato Centre

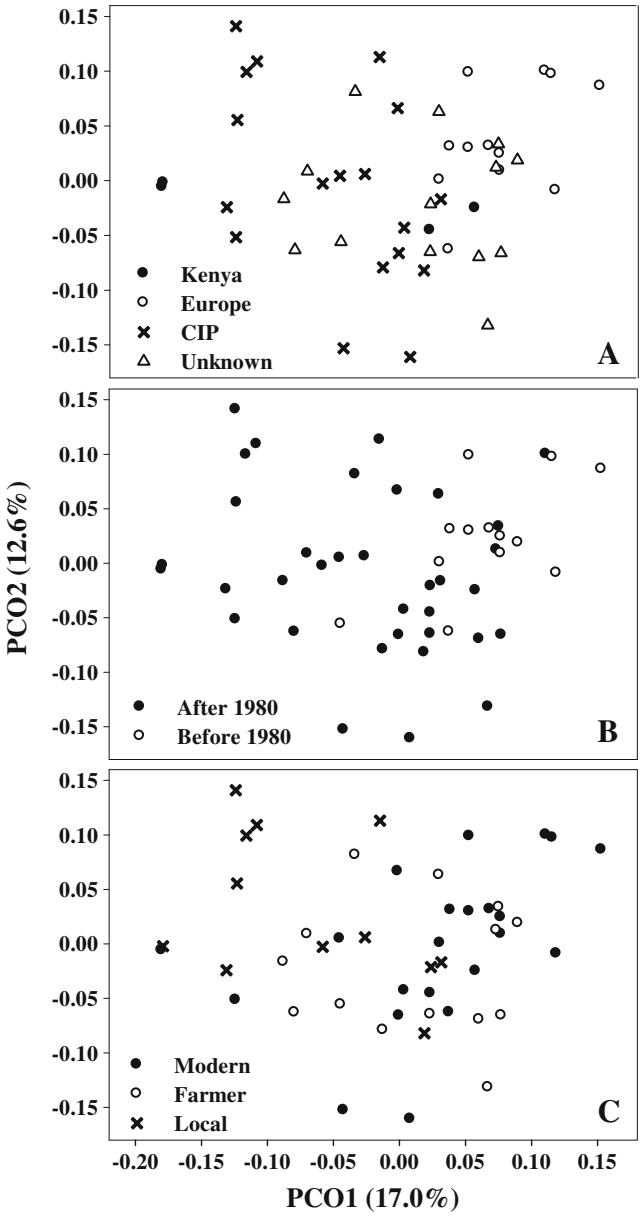

Fig. 1 Plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 122 SSR alleles for 48 potato accessions. These two components accounted for 17.0% and 12.6% of the total SSR variance, respectively. **a**: Accessions are labeled for germplasm origin (from Kenya, Europe, International Potato Centre, and unknown). **b**: Accessions are identified with their breeding periods (after or before 1980). **c**: Individual accessions are separately labeled for three classes (modern variety, farmer variety, and local genotype). Note that **a**, **b** and **c** are the same plot with different labeling for clear illustration

Further assessments on the clustering with respect to known parentage (Table 1) revealed five pairs of the accessions with known pedigree. They are (1) Tigoni vs Asante, (2) Dutch Robijn (white skinned) vs Dutch Robijn (red skinned), (3) Romano vs Desiree, (4) Kenya Karibu (purple flowers) vs Kenya Karibu (white flowers); and (5) 394904.17 vs 394905.8, as numerically labeled in Fig. 2. Such a large congruency of clustering with known parentage is encouraging, as one could identify similar parentage of some accessions with unknown pedigree records, if based on the observed relatedness. For example, the farmer variety Alika (60146) may share the same parentage with the modern cultivars Romano and Desiree, as genetically it was nearly identical to these two cultivars (see Fig. 2). Also, the estimated genetic relationships could offer some useful guide for parental selections in potato breeding, as they are more informative than parental selection (Hosaka et al. 1994; Demeke et al. 1996; Kim et al. 1998) and traditional pedigree analysis (sentence appears not so clear) (Mendoza and Haynes 1974; Loiselle et al. 1989a, b). However, caution is needed because many inconsistencies between grouping and known pedigree have also been reported in potato research due to a narrow genetic base (Loiselle et al. 1989b; Kim et al. 1998; Love 1999).

Genetic Distinctiveness of Potato Accessions

The genetic distinctiveness of a potato accession was measured by the average dissimilarity (AD) of the accession against the remaining accessions assayed. The higher the AD, the greater is the distinctiveness of the genetic background. The AD of the accessions ranged from 0.229 for Desiree (modern variety) and Alika (farmer variety) to 0.326 for Tana Kimande (farmer variety) with a mean of 0.275 (Table 1). The seven most distinctive accessions with AD greater than 3 were Tana Kimande (0.326), Anett (0.310), 395438.1 (0.305), 394905.8 (0.305), Kenya Karibu (purple flowers) (0.302), Asante (0.302), and Roslin Eburu (B53) (0.301). These distinct accessions are well mixed with various germplasm classes, breeding period and origin. The seven less distinctive accessions with AD smaller than 0.25 were Alika (0.229), Desiree (0.229), Romano (0.230), Dutch Robijn (red skinned) (0.240), Tigoni (Oval) (0.241), Dutch Robijn (white skinned) (0.243), and Kihoro (0.249). These accessions represent all three germplasm classes and the majority of them originated from Europe.

On average, the local genotypes displayed the highest mean (0.287) of AD and thus were more genetically distinct than the modern and farmer varieties (0.272 and 0.269, respectively). Similarly, the accessions from CIP had the highest mean (0.286) of AD, followed by the Kenyan accessions with a mean (0.277) of AD and the accessions from Europe with a mean (0.264) of AD. These measures of genetic distinctiveness are consistent with the patterns of SSR variation reported above.

The ADs shown in Table 1 are limited to only the 48 accessions assayed. The AD values would change if more potato accessions were assessed. This method can recognize the distinctiveness, but not necessarily the relatedness, of accessions (Fu 2006). For example, two closely related

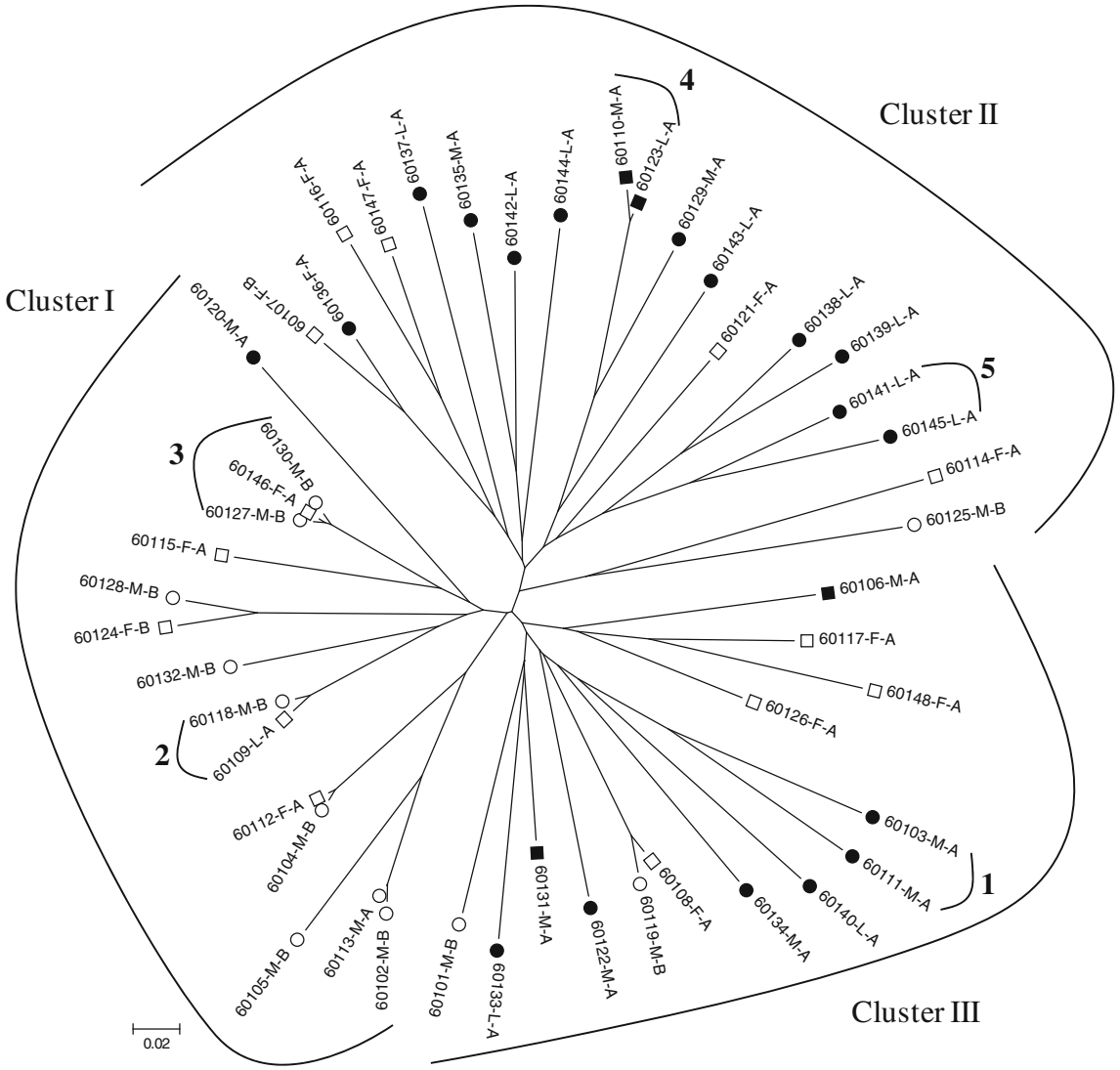

Fig. 2 Clustering of 48 potato accessions obtained from the neighbor-joining analysis of 122 SSR alleles. Accession label includes the code following with two letters, the first for germplasm class (M-modern variety, F-farmer variety, and L-local genotype) and the second for the breeding period (A for after 1980 and B for before 1980) (see Table 1). These accessions are marked for their origins with four symbols: filled circle for International Potato Centre, open circle for Europe, filled diamonds for Kenya, and open diamond for unknown. Five pairs of the accessions with known parentage are numerically labeled. Three clusters are also illustrated

cultivars that were quite distinct from the remaining cultivars could have similar higher levels of AD than the others and both cultivars would have been identified as genetically distinct. It is important to recognize these limitations when the relative measure of genetic distinctiveness reported here is used as a guide for selecting specific germplasm with distinct genetic background in potato breeding (Demeke et al. 1996; Braun and Wenzel 2005; Fu et al. 2009).

Implications for Potato Germplasm Management and Breeding

This SSR analysis, the first molecular characterization of Kenyan potato germplasm, demonstrates that the current set of the Kenyan potato germplasm harbored considerable genetic variation. This finding is encouraging for the potato breeding program in Kenya, as direct selection within the existing germplasm is feasible. The identified relationships and estimated genetic distinctiveness should provide some guide for parental selection of diverse plants for potato breeding. However, further efforts are needed to diversify the Kenyan potato gene pool to ensure a sustainable breeding program in the future. In particular, targeted acquisitions should be made of potato germplasm from those programmes in countries with tropical climates similar to Kenya. Priority should be given to those categories of germplasm with special traits such as virus resistance, tolerance of high temperatures, and drought

tolerance, as virus infections and droughts are becoming more frequent and severe while temperatures are rising due to the effects of climate change (Hijmans 2003; Pliska 2008). Also, extra efforts are still needed to conserve the potato germplasm with the rare alleles detected in this study, as the rare alleles are more vulnerable to accidental loss (Bamberg and del Rio 2003).

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