Australian Journal of Crop Science AJCS
AJCS 8(4):502-508 (2014) ISSN:1835-2707

Study of genetic relationship among Kenyan cultivated potato clones using SSR markers

Jane Muthoni*[1,2], Hussein Shimelis[1] and Rob Melis[1]

[1]African Centre for Crop Improvement, University of KwaZulu-Natal, College of Agriculture, Engineering and Science, School of Agricultural, Earth and Environmental Sciences, Private Bag X01, Scottsxille 3209, Pietermaritzburg,South Africa

[2] Kenya Agricultural Research Institute (KARI). National Potato Research Centre, Tigoni, Kenya

***Corresponding author: jayney480@yahoo.com**

Abstract

The ability to quickly and accurately identify among potato (*Solanum tuberosum* L.) clones is important to breeders, seed and commercial growers, and in marketing and utilization of cultivars. The DNA-based genotyping using simple sequence repeats (SSRs) or microsatellites has been shown to discriminate between tetraploid potato clones.The objective of this study was to determine the genetic relationships among Kenyan potato clones so as to complement other bacterial wilt-resistance data in identifying parents for a breeding programme. Twenty potato clones were genotyped with twenty four SSR primer pairs selected for high polymorphism. The twenty four SSR primers identified 160 alleles among the 20 potato clones. The average number of alleles per locus ranged from 2 to 14 with an average of 6.67. Seventeen SSR markers (71%) were highly informative and had PIC values above 0.65; the PIC ranged from 0.208 to 0.839.Three divergent clusters were identified; clone Meru was on a cluster on its own. The SSR markers did not cluster the potato clones into different bacterial wilt resistance groups. This is probably because bacterial wilt resistance is very unstable due to strong host-pathogen-environment interaction; hosts resistant to the disease in one year/environment or location may succumb to the disease in the other year/environment or location. In addition, the pedigrees of some clones are unknown; some clones are farmer selections while for others, proper records may not have been kept during breeding. Despite the discrepancies, the SSR markers generated useful information and assisted in selection of parents to include in the breeding programme.

Keywords: Bacterial wilt; polymorphic information content, potato clones; SSR markers.
Abbreviations: PIC_polymorphic information content; SSR_simple sequence repeats.

Introduction

Information on the genetic interrelationships and diversity of crop plants allows in systematic organization of the variability in the germplasm, in creating core collections in genebanks, and assisting in selection of parents in a breeding programme hence paving the way to genetic gains (Powell et al., 1991; Sun et al., 2003). The characterization of genetic diversity is also important for cultivar identification, cultivar protection (e.g. potato tuber seed) as well as to ensure the trademark and intellectual property rights (Coombs et al., 2004). In a crop like potato, information on genetic diversity is used in co-ancestry/pedigree studies to avoid closely related parents and hence inbreeding depression (Tarn et al., 1992). In determining genetic diversity, genetic markers representing genetic differences between genotypes or species are used. There are three major types of genetic markers: (1) morphological (also 'classical', 'phenotypic' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA sequence (Winter and Kahl, 1995; Jones et al., 1997). Molecular markers are the most widely used mainly because they are much more numerous than morphological markers, and they do not disturb the physiology of the organism. They reveal neutral sites of variation at the DNA sequence level. 'Neutral' means that, unlike morphological markers, these variations do not show themselves in the phenotype, and each might be nothing more than a single nucleotide difference in a gene or a piece of repetitive DNA(Jones et al., 1997). Because polymorphisms are DNA sequence variations, these markers are applicable at any plant stage and tissue and are independent of growing conditions (Hahn and Grifo, 1996). They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996).The most widely used molecular markers are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSR) or microsatellites (Collard et al., 2005) and recently Single Nucleotide Polymorphisms (SNPs) (Hamilton et al., 2011). The SSR or microsatellites (sometimes referred to as a variable number of tandem repeats or VNTRs) are short segments of DNA that have a repeated nucleotide sequences.These motifs exhibit extensive site-specific length polymorphism due to differing

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

Entry	Clone	Source	Year of release
1	TIGONI	CIP	1998
2	KIHORO	Farmers' variety	-
3	MERU	Farmers' variety	-
4	NYAYO	Farmers' variety	-
5	INGABIRE	CIP	1998
6	KENYA FURAHA	CIP	1998
7	TIGONI LONG	Farmers' variety	-
8	BISHOP GITONGA	Farmers' variety	-
9	KENYA MAVUNO	CIP	2002
10	KENYA KARIBU	CIP	2002
11	KENYA FAULU	CIP	2002
12	CANGI	Farmers' variety	
13	C1 (391919.3)	CIP	Not yet released
14	C2 (394904.9)	CIP	Not yet released
15	C3 (394905.8)	CIP	Not yet released
16	C4 (392278.19)	CIP	Not yet released
17	C5 (394895.7)	CIP	Not yet released
18	C6 (394903.5)	CIP	Not yet released
19	C7 (395438.1)	CIP	Not yet released
20	C8 (391930.1)	CIP	Not yet released

CIP=Centro Internacional de la Papa

numbers of repeat units. Length polymorphisms at a particular SSR locus can be assayed on the basis of the differing electrophoretic mobilities of polymerase chain reaction (PCR) products amplified by primers flanking the motif (Rafalski et al., 1996). The nucleotide repeat motifs can be dinucleotide, trinucleotide or tetranucleotide repeats, and they tend to occur in non-coding DNA. Simple sequence repeats (SSR) markers detect highly repetitive regions in the genome that can be derived from untranslated regions and introns (Ghislain et al., 2006. In solanaceous species the microsatellite frequency is greater in the intron untranslated regions 5' (upstream of the gene) and 3' (downstream from the gene) (Smulders et al., 1997). Moreover, although SSRs represent hypervariable areas of the genome, they are sufficiently conserved to be inherited for several generations in a Mendelian fashion (Morgnate and Olivieri, 1993). In this respect, the long-term stability of allele profiles in potato has been demonstrated (Love et al., 1992). Unlike other DNA-based markers RFLP, RAPD, SNPs and AFLP, simple sequence repeats occur frequently in plants.Microsatellites are distributed throughout the genome of eukaryotes and prokaryotes. The frequency of SSRs varies between mammals and plants, being five times more frequent in the former (Lagercrantz et al., 1993). Within plants, the frequency is approximately one every 21.2 kb in dicots and every64.6 kb in monocots (Wang et al., 1994). In potato, it was estimated that one SSR could be found every 52 kb when screening for five different motifs (Ashkenazi et al., 2001). Microsatellites are ubiquitous, highly polymorphic and can be used to detect the heterozygosity at a locus due to their co-dominant behaviour. They also permit the analysis of multiple loci per individual (multiallelism) and can function with low-quality DNA (Morgnate and Olivieri, 1993; Wang et al., 1994). Microsatellites provide high genetic information, are highly reproducible, and simple to use. Additionally, the SSRs have the capacity to reflect ploidy status and the high heterozygosity of the tetraploid potatoes. Genetic fingerprinting using SSRs has been well established to effectively discriminate between tetraploid potato clones (Kawchuk et al., 1996; Provan et al., 1996; Mc Gregor et al., 2000b; Ashkenazi et al., 2001). Simple sequence repeats have been used to great advantage in potato for studies of diversity, genetic structure, and classification (Spooner et al., 2007); tracing germplasm migrations (Rios et al., 2007); fingerprinting (Provan et al., 1996; 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 genebanks (Del Rio et al., 2006). Previous studies have resulted in selection of a new potato genetic identity (PGI) kit based on 24 SSR markers with two markers for each of the 12 linkage groups of potato and separated by at least 10 cM. The kit provides high locus-specific polymorphic information content and high quality of amplicons as determined by clarity and reproducibility (Ghislain et al., 2009). It thus seems that SSR markers are a powerful molecular approach for establishing genetic relationship, assessing genetic diversity and germplasm characterization in tetraploid potato. Breeders commonly complement phenotypic information with a genotypic assessment of diversity and content using molecular markers to capture allelic diversity in a smaller core set of parents. They can also use genetic distance based on molecular markers to complement co-ancestry/pedigree analysis (Tarn et al., 1992; Gopal and Oyama, 2005) to avoid closely related parents and hence inbreeding depression and to ensure genetic variation for continued progress. Against this background, the current study was undertaken to determine the genetic relationships among potato clones so as to complement other bacterial wilt-resistance data in identifying parents for a breeding programme.

Results

Genetic polymorphisms

The twenty four SSR primers identified 160 alleles among the 20 potato clones. The number of alleles scored across SSR loci ranged from 2 to 14, with an average of 6.67 alleles (Table 2).The PIC estimated for all loci ranged from 0.839 to 0.208 with an average of 0.649. Expected heterozygosity (He) values, as a measure of allelic diversity at a locus, varied from 0.856 to 0.236 with an average of 0.69 (Table 2). Correlations were positive and strong between PIC and He (r= 0.986), PIC and number of alleles (r=0.772) and, He and number of alleles (r=0.715). Only seven SSR loci had PIC values less than 0.65 i.e. (STM1016=0.3750, STM0019a

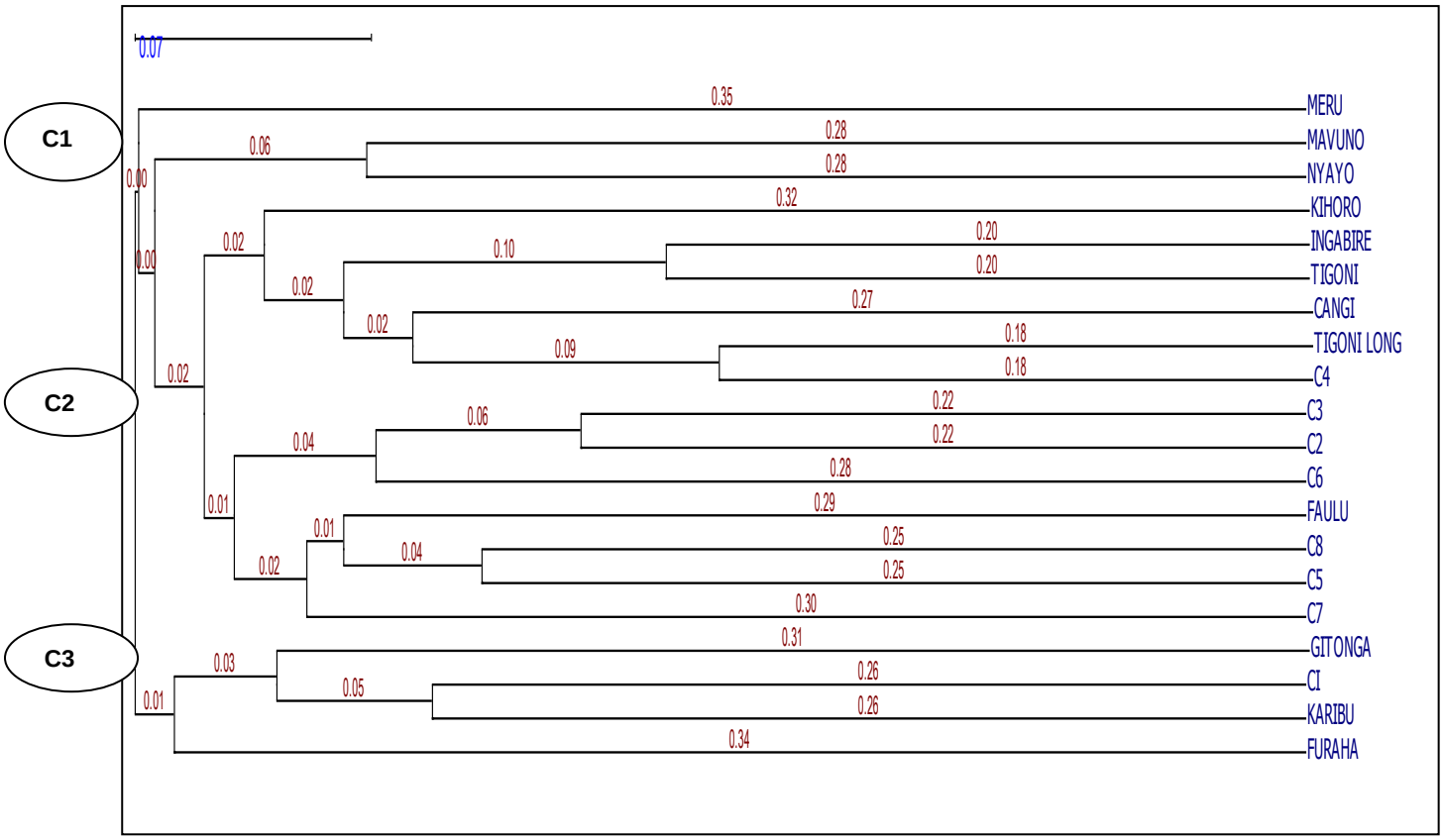

Fig 1. Dendrogram showing genetic relationship among 20 potato clones obtained using 24 SSR markers generated by UPGMA. The three clusters identified are C1, C2 and C3. .
FURAHA=KENYA FURAHA; KARIBU=KENYA KARIBU; GITONGA=BISHOP GITONGA; FAULU=KENYA FAULU; MAVUNO=KENYA MAVUNO

=0.5859, STPoAc58=0.2997, StI031=0.3750, STM1031= 0.2078, STM2022 =0.4482 and STM5121=0.3737). The remaining 17 SSR makers had potential to detect differences among the twenty potato clones.

Cluster analysis of potato clones

The dendrogram constructed using the UPGMA clustering algorithm based on SSR data matrices grouped the potato clones into three major clusters (Fig. 1). The first cluster consisted of Meru alone while the third cluster consisted of Bishop Gitonga, C1, Kenya Furaha and Kenya Karibu. The shortest genetic distance was found between Tigoni Long and C4. With the exception of Meru, Bishop Gitonga, Cangi, Nyayo, Tigoni Long and Kihoro, the rest originated from CIP where they could have shared some parents and hence high level of similarity. In addition, Tigoni Long is suspected to have escaped from CIP germplasm during national performance trials (NPT) in Kenya (Kabira, Pers.Comm). Among the 24 clones, Meru was the least genetically related to the other clones, followed by Kenya Furaha while Kihoro was third (Fig. 1). Meru is suspected to be a farmers' selection from Kerr's Pink. Kerr's Pink is an old Scottish variety released in Kenya in 1927(ASARECA, 2004). This may explain the least genetic relationship between Meru and other potato clones. The results also show that the 24 microsatellite markers distinguished all 20 potato clones. The genetic distance between clones ranged from 0.36 to 0.85 (Table 3).The short genetic distance between C4 and Tigoni Long (0.36) confirms the suspicion that Tigoni Long might have escaped from CIP germplasm. The short genetic distance between Tigoni and Ingabire (0.39) could be due the fact that both of them are selections from a single cross.

Discussion

The current study was undertaken to determine the genetic relationships among potato clones so as to complement other bacterial wilt-resistance data in identifying parents for a breeding programme. We chose to use SSRs for potato genetic identification because of their high genetic information content, high reproducibility, and simplicity of use. They are appropriate, cost-effective and simple tools for laboratories in developing countries with financial constraints. The high PIC values in most of the SSR markers observed in this study could be due to the fact that most of the potato clones used in this study were from CIP and could be closely related. Some markers used in the present study had different PIC values in a previous study i.e. STM1016 had 0.84; STM1031 had 0.499; STM2022 had 0.621; STM5121 had 0.733 while STPoAc58 had 0.754 (Ghislain et al., 2009). In yet other studies, STM0019a had 0.8808; STM1031 had 0.6584; STM1016 had 0.7757; STM2022 had 0.7531 while STPoAc58 had 0.7033 (Ghislain et al., 2004); StI031 had 0.92 (Feingold et al., 2005) and StI046 had 0.97 (Rocha, 2010). This could be due to the fact that microsatellites are often useful for only closely related germplasm sources; amplification of moderately divergent cross species can lead to significant distortion in genetic similarity estimates (Peakall et al., 1998). In addition, differences in laboratory procedures may have also led to the

Table 2. Description of repeat types, primer sequence, allelic information and PIC values of the 24 SSR loci used to genotype 20 potato clones.

NO	Marker name (at SCRI)	Repeat	Primer sequences(5'-3') Forward-Reverse	No of alleles	Allele Size (bp)	PIC	He	PGI Kit
1	STM1052	(AT)14GT(AT)4(GT)6	CAATTTCGTTTTTTCATGTGACAC ATGGCGTAATTTGATTTAATACGTAA	7	224-248	0.7603	0.7846	Yes
2	STM2013	(TCTA)6	TTCGGAATTACCCTCTGCC AAAAAAAGAACGCGCACG	7	160-185	0.7594	0.7901	No
3	STM1104	(TCT)5	TGATTCTCTTGCCTACTGTAATCG CAAAGTGGTGTGAAGCTGTGA	10	182-200	0.7997	0.8233	Yes
4	STM1016	(TCT)9	TTCTGATTTCATGCATGTTTCC ATGCTTGCCATGTGATGTGT	2	163-175	0.3750	0.5000	No
5	STM1049	(ATA)6	CTACCAGTTTGTTGATTGTGGTG AGGGACTTTAATTTGTTGGACG	10	136-212	0.7838	0.8115	No
6	STM0019a	(AT)7(GT)10(AT)4 (GT)5(GC)4(GT)4	AATAGGTGTACTGACTCTCAATG TTGAAGTAAAAGTCCTAGTATGTG	8	195-256	0.5859	0.6089	Yes
7	STM1106	(ATT)13	TCCAGCTGATTGGTTAGGTTG ATGCGAATCTACTCGTCATGG	5	165-184	0.7127	0.7562	Yes
8	STM0037	(TC)5(AC)6AA(AC)7(AT)4	AATTTAACTTAGAAGATTAGTCTC ATTTGGTTGGGTATGATA	10	85-108	0.6834	0.728	Yes
9	STM0030	Compound(GT/GC)(GT)8	AGAGATCGATGTAAAACACGT GTGGCATTTTGATGGATT	9	152-186	0.8244	0.8432	No
10	STI0012	(ATT)n	GAAGCGACTTCCAAAATCAGA AAAGGGAGGAATAGAAACCAAAA	6	182-208	0.7167	0.7538	Yes
11	STI0023	(CAG)n	GCGAATGACAGGACAAGAGG TGCCACTGCTACCATAACCA	9	80-220	0.7949	0.8194	No
12	STI0030	(ATT)n	TTGACCCTCCAACTATAGATTCTTC TGACAACTTTAAAGCATATGTCAGC	6	73-122	0.6807	0.7284	Yes
13	STI0036	(AC)n(TC)imp	GGACTGGCTGACCATGAACT TTACAGGAAATGCAAACTTCG	9	131-163	0.8389	0.8555	No
14	STI0032	(GGA)n	TGGGAAGAATCCTGAAATGG TGCTCTACCAATTAACGGCA	6	124-150	0.6945	0.7323	Yes
15	STM5127	(TCT)n	TTCAAGAATAGGCAAAACCA CTTTTTCTGACTGAGTTGCCTC	7	254-295	0.8140	0.8357	Yes
16	STGBSS	(TCT)n	AATCGGTGATAAATGTGAATGC ATGCTTGCCATGTGATGTGT	4	161-177	0.7031	0.7500	No
17	STWAX-2	(ACTC)n	CCCATAATACTGTCGATGAGCA GAATGTAGGGAAACATGCATGA	6	232-259	0.7083	0.7519	No
18	StI046	(GAT)n	CAGAGGATGCTGATGGACCT GGAGCAGTTGAGGGCTTCTT	10	196-229	0.8362	0.8533	No
19	STPoAc58	(TA)13	TTGATGAAAGGAATGCAGCTTGTG ACGTTAAAGAAGTGAGAGTACGAC	5	246-254	0.2997	0.31	Yes
20	STM0031	(AC)5...(AC)3(GCAC)(AC)2(GCAC)2	CATACGCACGCACGTACAC TTCAACCTATCATTTTGTGAGTCG	14	110-210	0.7964	0.8207	Yes
21	StI031	(TCA)n	AGGCGCACTTTAACTTCCAC CGGAACAAATTGCTCTGATG	2	141-167	0.3750	0.5000	No
22	STM1031	(AT)13	TGTGTTTGTTTTTCTGTAT AATTCTATCCTCATCTCTA	2	276-290	0.2078	0.2355	No
23	STM2022	(CAA)3...(CAA)3	GCGTCAGCGATTTCAGTACTA TTCAGTAACTCCTGTTGCG	4	190-210	0.4482	0.5463	No
24	STM5121	(TGT)n	CACCGGAATAAGCGGATCT TCTTCCCTTCCATTTGTCA	2	300-310	0.3737	0.4974	Yes

SCRI= Scottish Crop Research Institute
PIC=Polymorphic information content
He= heterozygosity

Table 3. Jaccard's similarity matrix for 20 potato clones analyzed using 24 SSR markers

	MERU	GIT.	FUR.	CI	MAV.	INGA.	TIG.	C3	CANGI	C8	KIH.	TIG. LONG	C7	C6	C4	C2	C5	NYAYO	FAU.	KAR.
MERU																				
BISHOP GITONGA	0.67																			
KENYA FURAHA	0.79	0.63																		
CI	0.74	0.58	0.67																	
KENYA MAVUNO	0.79	0.70	0.73	0.70																
INGABIRE	0.71	**0.83**	0.73	0.81	0.81															
TIGONI	0.65	0.67	0.73	0.63	0.65	**0.39**														
C3	0.70	0.67	0.68	0.60	0.62	0.76	0.65													
CANGI	0.65	0.75	0.65	0.65	0.70	0.56	0.63	0.61												
C8	0.74	0.75	0.67	0.68	0.58	0.72	0.65	0.67	0.63											
KIHORO	0.74	0.67	0.65	0.61	0.68	0.61	0.71	0.67	0.59	0.63										
TIGONI LONG	0.65	0.70	0.70	0.68	0.67	0.63	0.61	0.53	0.56	0.60	0.65									
C7	0.70	0.79	0.78	0.65	0.60	0.74	0.63	0.63	0.72	0.56	0.72	0.67								
C6	0.76	0.74	0.68	0.55	0.70	0.71	0.75	0.58	0.72	0.58	0.68	0.65	0.63							
C4	0.74	0.71	0.60	0.65	0.70	0.53	0.56	0.50	0.53	0.63	0.61	**0.36**	0.74	0.58						
C2	0.71	0.75	0.73	0.77	0.82	0.73	0.73	0.44	0.74	0.68	0.75	0.67	0.75	0.55	0.53					
C5	0.70	0.79	0.77	**0.85**	0.70	0.67	0.67	0.72	0.69	0.50	0.76	0.63	0.63	0.74	0.71	0.61				
NYAYO	0.74	0.79	0.78	0.82	0.57	0.81	0.70	0.70	0.63	0.65	0.75	0.72	0.74	0.75	0.74	0.70	0.78			
KENYA FAULU	0.65	0.78	0.75	0.71	0.67	0.65	0.68	0.63	0.65	0.61	0.74	0.56	0.63	0.58	0.65	0.60	0.56	0.58		
KENYA KARIBU	0.68	0.67	0.76	0.53	0.75	0.74	0.56	0.70	0.67	0.76	0.69	0.68	0.61	0.72	0.75	0.81	0.78	0.67	0.58	

GIT=GITONGA; FUR= KENYA FURAHA; MAV= KENYA MAVUNO; INGA=INGABIRE; TIG. = TIGONI; KIH=KIHORO; FAU=KENYA FAULU; KAR= KENYA KARIBU

discrepancies in PIC values. The SSR markers did not cluster the potato clones into different bacterial wilt resistance groups. This is probably because resistance is very unstable due to strong host-pathogen-environment interaction; hosts resistant to the disease in one year/environment or location may succumb to the disease in the other year/environment or location (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992b; Tung et al., 2006). In addition, the pedigrees of some clones are unknown; some clones are farmer selections while for others, proper records may not have been kept during breeding. Despite the discrepancies, the SSR markers generated useful information that will assist in identifying parents to include in the breeding programme.

Materials and Methods

Plant materials

Twenty potato clones were used in the study (Table 1). The eight clones C1 to C8 are advanced clones from International Potato Center (CIP) and are reported to have high levels of resistance to bacterial wilt (Merideth, Pers. Comm). The other clones are susceptible to bacterial wilt in varying degrees but are popular in Kenya because they are high yielding, early maturing or have other preferred market qualities.

DNA sampling

Fresh young leaves were picked from one month old plants in the field for DNA extraction. The DNA collection was done using Whatman FTA cards. The sampling protocol was done according to the modified protocols of FTA paper technology (Mbogori et al., 2006). The FTA_ classic card (Whatman Inc., Clifton, NJ) is a Whatman paper that has been impregnated with a patented chemical formulation that lyses cells, then captures and immobilizes nucleic acids in the paper matrix. In addition, they contain compounds for denaturing, chelating and trapping free radicals which prevent damage of the nucleic acids (http://www.whatman.com). One FTA classic card measures 750 x 130 mm and each was labeled prior to the day of sampling. For sampling, ten plants were sampled from each clone, 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 soaked with the sap. Ethanol (70 %) was used to clean the pliers between samples to prevent cross contamination. The FTA card was then hung on a drying line using a paper clip for air drying under room temperature for 2–5 hours. After drying, the FTA cards were packed in an envelope and sent to the laboratory for analysis.

SSR analysis

In the laboratory, (INCOTEC South Africa (Pty) Ltd.), samples on FTA cards from the twenty potato clones (10samples per clone) were analysed. All the samples from each clone were bulked. A single punch of each card per submission was taken and homogenized in the Finnzymes dilution buffer (Kit). Then 2 uL of each of the bulked sample was used in the polymerase chain reaction (PCR).Twenty four SSR markers were used in this study. These were selected from previous studies based on their high polymorphic information content (PIC) (Ghislain et al., 2004; Feingold et al., 2005; Ghislain et al., 2009; Rocha, 2010). Twelve 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). Analysis was performed using GeneMapper 4.1. Euclidian distances were calculated between bulked samples, using the program GGT 2.0 (Van Berloo, 2007). Because potato is an autotetraploid, each individual could contain between one and four different alleles at any one locus. The SSR marker alleles were scored for presence or absence of the band for all the 20 potato clones and treated as dominant marker. Therefore, the bands generated by SSR markers were not considered allelic but evaluated as dominant markers, so the data were considered binary. Thus, to evaluate the results of SSR markers, each amplified fragment was considered as one locus. The genetic similarity matrix of the 20 potato clones was calculated using the Jaccard's coefficient (Anderberg, 1973).

Statistical analysis

The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA). The polymorphic information content (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 i^{th} allele in the accessions (Nei, 1973; Rafalski et al., 1996).

Conclusions

The SSR markers did not cluster the potato clones into different bacterial wilt resistance groups. This is probably because bacterial wilt resistance is very unstable due to strong host-pathogen-environment interaction; hosts resistant to the disease in one year/environment or location may succumb to the disease in the other year/environment or location (French and Lindo, 1982; Tung et al., 1990; Tung, 1992; Tung et al., 1992b; Tung et al., 2006). In addition, the pedigrees of some clones are unknown; some clones are farmer selections while for others, proper records may not have been kept during breeding. Despite the discrepancies, the SSR markers generated useful information that will assist in identifying parents to include in the breeding programme.

Acknowledgements

Due thanks go to the Alliance for a Green Revolution in Africa (AGRA) for funding this research and Kenya Agricultural Research Institute (KARI) management for granting study leave to the first author. Thanks to members of staff at INCOTEC South Africa (Pty) Ltd for DNA extraction and data analysis.

References

Anderberg MR (1973) Cluster analysis for applications. Academic Press, New York.

ASARECA (2004) Regional variety list for Kenya, Uganda and Tanzania. Association for Strengthening Agricultural Research in Eastern and Central Africa.

Ashkenazi V, Chani E, Levy D, Hillel J, Eeilleux RE (2001) Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. Genome 62*:* 44-50.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142*:* 169-196.

Coombs JJ, Frank LM, Souches DS (2004) An applied fingerprinting system for cultivated potato using simple sequence repeats. Ame J Pot Res. 81*:* 243-250.

Del Rio A, Bamberg J, Huama´n Z (2006) Genetic equivalence of putative duplicate germplasm collections held at CIP and US potato genebanks. Ame J Pot Res. 83*:* 279-285.

Feingold S, Lloyd J, Norero N, Bonierbale M, Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). Theor Appl Gen. 111*:* 456-466.

Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM (2006) Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. Theor Appl Gen. 113*:* 1515-1527.

Ghislain M, Nu´n˜ez J, Herrera MR, Pignataro J, Guzman F, Bonierbale M, Spooner DM (2009) Robust and highly informative microsatellite-based genetic identity kit for potato. Mol Breed. 23: 377-388.

Ghislain M, Spooner DM, Rodríguez F, Villamón F, Núñez J, Vásquez C, Waugh R, Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor Appl Gen. 108*:* 881-890.

Gopal J, Oyama K (2005) Genetic base of Indian potato selections as revealved by pedigree analysis. Euphytica 142*:* 23-31.

Hahn WJ, Grifo FT (1996) Molecular markers in plant conservation genetics. p. 114-136. *In* B.W.S. Sobral (ed.) The impact of plant molecular genetics. Birkh/iuser, Boston.

Hamilton JP, Hansey CN, Whitty BR, Stoffel K, Massa AN, Deynze AV, Jong WSD, Douches DS, Buell CR (2011) Single nucleotide polymorphism discovery in elite north american potato germplasm. 12:302. Available at http://www.biomedcentral.com/1471-2164/12/302 (Accessed 25 March 2013) BMC Genomics 12:302.

Jones N, Helen O, Howard T (1997) Markers and mapping: We are all geneticists now. New Phytol. 137*:* 165-177.

Kawchuk LM, Lynch DR, Thomas J, Penner B, Sillito D, Kulcsar F (1996) Characterization of *Solanum tuberosum* simple sequence repeats and application to potato cultivar identification. Ame J Pot Res. 73*:* 325-335.

Lagercrantz U, Ellegren H, Andersson L (1993) The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Res. 21*:* 1111-1115.

Love SL, Thompson AL, Baker TP, Corsini DL (1992) Comparison of Russet Burbank clones from various geographical regions of the United States and Canada. Ame J Pot Res. 69*:* 299-307.

Mbogori MN, Kimani M, Kuria A, Lagat M, Danson JW (2006) Optimization of FTA technology for large scale plant DNA isolation for use in marker assisted selection. Afr J Biotech. 5:693-696.

Mc Gregor CE, Lambert CA, Greyling M, Louw JH, Warnich L (2000b) A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica. 113*:* 135-144.

Moisan-Thiery M, Marhadour S, Kerlan MC, Dessenne N, Perramant M, Gokelaere T(2005) Potato cultivar identification using simple sequence repeats markers (SSR). Pot Res. 48*:* 191-200.

Morgnate M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J. 3: 175-182.

Nei M (1973) Analyses of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 70*:* 3321-3323.

Paterson AH (1996) Making genetic maps.p. 23-39. *In* A.H. Paterson (ed.) Genome Mapping in Plants. R. G. Landes Company, San Diego, California. Academic Press, Austin, Texas.

Powell W, Phillips MS, McNicol JW, Waugh R (1991) The use of DNA markers to estimate the extent and nature of genetic variability in *Solanum tuberosum* cultivars. Ann App Biol. 118*:* 423-432.

Provan J, Powell W, Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum* L.). Theor Appl Gen. 92*:* 1078-1084.

Rafalski DJA, Vogel JM, Morgante M, Powell W, Andre S, Tingey SV (1996) Generating and using DNA markers in plant. p. 75-134. *In* B. Birren and E. Lai (ed.) Nonmammalian genomic analysis: A practical guide. Chapman and Hall, New York.

Rios D, Ghislain M, Rodriguez F, Spooner DM (2007) What is the origin of the European potato? Evidence from Canary Island landraces. Crop Sci. 47*:* 1271-1280.

Rocha EA (2010) Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. Crop Breed Appl Biotech. 10*:* 204-210.

Smulders MJM, Bredeeijer G, Rus-Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other Lycopersicon species. Theor Appl Gen. 97:264-272.

Spooner DM, Nu´n˜ez J, Trujillo G, Herrera RM, Guzma´n F, Ghislain M (2007) Extensive simple sequence repeat genotyping of potato landraces supports a major re-evaluation of their gene pool structure and classification. Proc Natl Acad Sci USA. 104*:* 19398-19403.

Sun G, Wang-Pruski G, Mayich M, Jong H (2003) RADP and pedigree-based genetic diversity estimates in cultivated diploid hybrids. Theor Appl Gen. 107*:* 110-115.

Tarn TR, Tai GCC, Jong HD, Murphy AM, Seabrook JEA (1992) Breeding potatoes for long-day, temperate climates. Plant Breed Rev. 9*:* 219-332.

Van Berloo R (2007) GGT graphical genotypes. Laboratory of plant breeding Wageningen University [Online]. Available at http://www.dpw.wau.nl/pv/pub/ggt/ (Accessed 10 April 2013). Wageningen Agricultural University. The Netherlands.

Wang Z, Weber JL, Zhang G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Gen. 88*:* 1-6.

Wang Z, Weber JL, Zhang G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Gen. 88*:* 1-6.

Winter P, Kahl G (1995) Molecular marker technologies for plant improvement. World J Microbio Biotech. 11*:* 438-448.