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Note

A set of tetra-nucleotide core motif SSR markers for efficient identification of potato (*Solanum tuberosum*) cultivars

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Simple sequence repeat (SSR) is a popular tool for individual fingerprinting. The long-core motif (e.g. tetra-, penta-, and hexa-nucleotide) simple sequence repeats (SSRs) are preferred because they make it easier to separate and distinguish neighbor alleles. In the present study, a new set of 8 tetra-nucleotide SSRs in potato (*Solanum tuberosum*) is reported. By using these 8 markers, 72 out of 76 cultivars obtained from Japan and the United States were clearly discriminated, while two pairs, both of which arose from natural variation, showed identical profiles. The combined probability of identity between two random cultivars for the set of 8 SSR markers was estimated to be 1.10×10^{-8}, confirming the usefulness of the proposed SSR markers for fingerprinting analyses of potato.

Key Words: potato, *Solanum tuberosum*, cultivar identification, SSR markers.

Introduction

Potato (*Solanum tuberosum*) is the fourth-largest food crop in the world, following maize, wheat, and rice. More than 4,500 potato varieties are cultivated in over 100 countries (Pieterse and Judd 2014). As the number of known varieties increases, it becomes difficult to identify them by morphological markers. Thus, reliable methods of correctly identifying cultivars are strongly needed to assess the genetic diversity of the potato germplasm.

SSR markers, or microsatellites, consist of tandemly repeated DNA sequences with a core unit of 1–6 base pairs (bp). They have many positive features useful for the genetic profiling of individuals, including abundance in plant genomes, multi-allelic co-dominant patterns, ease of use, and high variability in the number of core-motif repeats. In the long-core motif (e.g. tetra-, penta-, and hexa-nucleotide) SSRs, neighbor alleles are more easily separated from each other, while di-nucleotide SSRs are subject to a lower level of separation of neighbor alleles and a higher level of stuttering, which make the interpretation of electropherograms and the allele call less reliable (Cipriani *et al.* 2008). Long nucleotide repeats are widely adopted for genetic profiling in humans and animals (Butler *et al.* 2004, Butler 2006, Hammond *et al.* 1994, Hellmann *et al.* 2006, Ruitberg *et al.* 2001). Meanwhile, regarding plants, the use of long nucleotide repeats has been limited to the variety identification of a few crops: grape (Cipriani *et al.* 2008, 2010), *Eucalyptus* (Faria *et al.* 2011), olive (De la Rosa *et al.* 2013), peach (Dettori *et al.* 2015), and tea (Wang *et al.* 2016).

In potato, SSRs have been used to study the genetic relationships and distances between wild and cultivated potato (Ghislain *et al.* 2004, 2009, Milbourne *et al.* 1998). However, as with other crops, di- and tri-nucleotide SSRs have mainly been used, and few long-core motif SSRs have been reported (Ghislain *et al.* 2004, 2009, Milbourne *et al.* 1998).

In the present paper, we propose a new set of long-core motif SSR markers for potato with the aim of minimizing genotyping errors.

Materials and Methods

Plant materials and DNA extraction

Ten potato cultivars of in vitro cultures were obtained from the University of Idaho as representative cultivars in the United States. Potato tubers of Japanese cultivars were obtained from the Hokkaido Research Organization (HRO) Kitami Agricultural Experiment Station (9 cultivars), the Nagasaki Agricultural and Forestry Technical Development Center (8 cultivars), and the NARO Hokkaido Agricultural Research Center (49 cultivars) (**Table 3**). For each cultivar tested, DNA was extracted using the GM quicker 2 kit (Nippon Gene, Toyama, Japan) according to the supplier's protocol.

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PCR and DNA fragment analysis

Fifty-six SSR markers with a tetra-nucleotide motif from Spud DB (Hirsch *et al.* 2014) were initially selected. Using 4 Japanese and 4 US major cultivars, a preliminary test of PCR amplification was performed. After the screening, 8 markers were selected for efficient discrimination of cultivars.

Octaplex PCR reactions were carried in a 5 µL reaction mixture with 2.5 ng genomic DNA, 0.1 U of KOD -Multi & Epi- (Toyobo, Osaka, Japan) and appropriate concentrations of the primer pairs shown in **Table 1**. The forward primers were labeled with any of 6-FAM, HEX, NED, and PET fluorescent dyes. The PCR reactions were carried out with the following thermal profile: one cycle at 94°C for 2 min followed by 30 cycles at 98°C for 10 sec, 63°C for 30 sec, and 68°C for 30 sec. Electrophoresis was performed in a Genetic Analyzer 310 (Thermo Fisher Scientific, Waltham, MA, USA). The PCR products were analyzed using GeneMapper v3.7 software (Thermo Fisher Scientific). For each locus, peaks were assigned letters in alphabetical order from the smallest to the largest (**Table 2**). The number of peaks and the number of profiles per marker were evaluated based on amplification of the 76 test cultivars. Discrimination power (DP) was calculated as $DP = 1 - \sum P_i^2$ where P_i is the frequency of the ith profile.

Results and Discussion

A total of 1,729 tetra-nucleotide SSRs were annotated by Spud DB (Hirsch *et al.* 2014). Among them, 56 SSRs were selected based on a high number of repeats, and were tested according to the following criteria: (1) two or more peaks detected in a preliminary screening with 8 cultivars, (2) no null peak, (3) at most one marker in each chromosome to ensure independence of individual markers. As a result, 8 SSRs were further selected and an 8-plex PCR condition was designed (**Table 1**).

Based on the analysis of the 76 test cultivars, the number of peaks ranged from 6 to 12 (average 7.4), and the number of profiles ranged from 16 to 36 (average 24.4). For each locus, the discrimination power (PD) ranged from 0.725 to 0.942, averaging 0.886 (**Table 2**). The probability of finding two random individuals with identical profiles at all 8 loci was an estimated 1.10×10^{-8}, which provided enough discriminant power to identify the tested cultivars.

Among all peaks, the differences of peak sizes between B and C of marker 8242, C and D of 12002, and A and B of 46514 were within two bases, suggesting that these small differences of peak sizes may have been caused by insertion or deletion of nucleotide except for difference of SSR motif replication. Because one base difference of peaks is sometimes difficult to discriminate in peak callings, we propose to use reference cultivars in a practical discrimination experiment or to consider results of other markers profile when these peaks are used in cultivar discrimination.

The profiles of the 76 cultivars generated from the set of 8 SSR markers are shown in **Table 3**. By using 8 markers, 72 cultivars were distinguished from each other, except for two combinations of cultivars: the combination of "Red Moon" and "Destroyer", and that of "Inca no mezame" and "Hokkai 98 (Inca Rouge)". Both of the latter cultivars are known as skin color mutants derived from the former ones,

Table 1. Eight tetra-nucleotide SSR primers selected for identification of potato cultivars

Marker ID*	Chr.	Motif	Forward (5′ to 3′)	Reverse (5′ to 3′)	Conc. (µM)	peak range (bp)
4026/4027	1	(CTAT)n(CTAG)n	NED-AACTTGCGGGAATAAGTGACG	ACTATACACACGTGCCCTGAAACTAG	0.09	265–346
8242	2	(CTTT)n	FAM-CGTCTTGGATGTCTTAGTTGTGG	GCAAAACCAGAAAGGCTAACAAAC	0.08	191–218
12002	3	(ACAT)n	NED-CCATGAACCTGAAGTTTTTCTGC	TGGATATCTTGTGCCTACAAGCTAG	0.10	209–235
16410	4	(ATAC)n	FAM-GTATGTTTGAGTAAAATCCTCCACCA’	TTCTCTGCCCCCTTTTAATTTG	0.16	258–354
31924	8	(ATAC)n	VIC-CGAAGACACCAAATCGCTCAG	GAAACGCCATTAACATTTTACATCG	0.07	136–250
35584	9	(GAAA)n	VIC-AGTAAGTCAAACTCAACTCCAAGGTG	GTTCTAGATTATCTCACTCATGCCTTTC	0.08	84–111
43016	11	(ATCC)n	PET-CAAGCTGCATGAAAGCCATC	TTTGCCTAAAAGTTTGTAGTGTGAGG	0.07	184–227
46514	12	(TATC)n	PET-TGCTTTTTGTTTCCTTTTGTGTG	GGAATGAAACTAAGCCTTGCTCTG	0.12	130–172

*Marker IDs are the same as in Spud DB (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml).

Table 2. Characteristics of 8 tetra-nucleotide SSR primers

Marker ID	No. of peaks	No. of profiles	Discrimination power	Averaged peak size (bp)											
				A	B	C	D	E	F	G	H	I	J	K	L
4026/4027	7	27	0.912	265.0	307.4	312.9	319.7	339.3	343.0	345.5					
8242	7	31	0.942	190.6	193.5	194.5	198.4	206.3	214.2	218.2					
12002	7	28	0.920	208.9	212.9	216.9	217.8	224.5	230.7	234.6					
16410	12	36	0.927	257.6	266.9	271.0	279.0	281.0	310.3	325.2	335.5	339.5	346.3	349.9	353.6
31924	6	22	0.918	135.6	213.5	218.2	222.2	230.3	249.9						
35584	6	17	0.874	84.0	91.9	95.9	99.8	103.7	111.2						
43016	7	16	0.725	184.3	188.0	192.0	199.5	203.5	215.0	226.9					
46514	7	18	0.869	129.9	131.2	152.5	156.9	161.1	165.1	172.0					

For each locus, peaks were assigned letters in alphabetical order from the smallest to the largest.

Table 3. Profiling of 76 potato cultivars using 8 tetra-nucleotide SSR primers

Cultivar	Source	4026/4027	8242	12002	16410	31924	35584	43016	46514
Ainoaka	Nagasaki	AB	CF	BCD	BE	BD	CF	D	ABE
Aiyutaka	Nagasaki	ABF	ABF	BD	BE	BD	C	G	AF
Alturas	Idaho	C	BC	CE	DEK	BDE	B	DFG	AE
Astarte	NARO	BD	BCF	D	DE	ABD	BE	D	AE
Atlantic	NARO	BDG	ACD	CDEF	BK	BD	BCE	DFG	A
Beniakari	NARO	BG	BF	DFG	BEK	DE	CEF	D	AC
Benimaru	NARO	BDG	ABD	CDFG	EHIK	AB	BE	CE	AE
Cal white	Idaho	DG	ABC	BCF	CDK	BE	BF	D	E
Chelsea (Jenny)	NARO	B	BCG	ACD	DEFK	BCD	ABE	D	AE
Cherie	NARO	BD	ABCD	CD	DEG	AD	B	DF	E
Clearwater	Idaho	A	B	BCF	DK	BE	B	D	EG
Cynthia	NARO	AB	BF	CD	DEHK	BD	B	CD	ABE
Dansyakuimo (Irish Cobber)	NARO	B	AB	CF	BCIK	AB	BC	D	E
Dejima	Nagasaki	ABF	ABF	BCD	BE	B	BC	Null	AFG
Destroyer	NARO	B	BDF	CD	AEJK	CF	AB	BCDF	ACE
Early Starch	NARO	B	CF	CD	BE	ABD	BE	D	ACG
Eniwa	NARO	G	ABF	CDF	DEKL	BDE	BC	D	ACE
Hanashibetsu	Hokkaido	BCD	AF	DFG	BE	BCD	BE	AD	AE
Haruka	NARO	BC	BF	BCDG	BEG	BD	BCEF	DEG	AEF
Hikaru	NARO	BCFG	BDEF	DE	BEK	ABCE	BCF	D	ACEF
Hokkai 50	NARO	B	BF	CDF	BCIK	AB	BC	D	AE
Hokkai 98 (Inca Rouge)	NARO	B	CG	F	EK	F	B	D	E
Hokkaikogane	NARO	BG	AB	CDF	EH	ABE	BC	D	AC
Hugenmaru	Nagasaki	ABF	AF	BCD	BE	AB	B	G	AE
Inca no hitomi	NARO	D	FG	FG	K	F	B	D	E
Inca no mezame	NARO	B	CG	F	EK	F	B	D	E
Inca Purple	NARO	BCG	BCF	CDF	EK	AB	B	AC	ACE
Inca Red	NARO	B	BF	DF	EK	AC	BC	D	ABE
Kitaakari	NARO	BF	ABF	CDF	BCEK	BE	BC	D	AEG
Kitahime	NARO	BC	F	CG	EFGK	BCE	BEF	DE	E
Kitamurasaki	NARO	BCG	B	CD	BEK	ADE	EF	D	ACE
Kitamusashi	NARO	FG	BDF	BD	DE	BD	BE	E	ACEF
Koganemaru	NARO	BF	AB	CD	BEK	BDE	ABE	DG	ACE
Konahubuki	Hokkaido	BG	ABD	CD	CE	BD	B	D	AG
Konayuki	Hokkaido	BG	AB	CDF	EHIK	AB	E	CD	AE
Konayutaka	Hokkaido	BDF	ABE	DFG	BEK	BE	BC	D	AE
Matilda	NARO	BD	CDG	DG	BEI	CD	ABE	D	EG
May Queen	NARO	BDG	CDF	CFG	BEGI	BE	BE	EF	ABE
Nishiyutaka	Nagasaki	B	BCF	CD	BEK	B	BE	Null	AEG
Norin 1	NARO	B	AB	CDF	BEHK	AB	BCE	D	AE
Norking Russet	NARO	BF	BC	BCD	DE	BCE	BF	Null	AEG
Northern Ruby	NARO	CG	BCF	CD	CEK	ABD	BF	DE	AE
Okhotsk Chip	Hokkaido	BG	CDF	BCF	EK	BE	BEF	DF	AF
Oojiro	NARO	B	AD	DF	BHIK	A	BCE	D	E
Piruka	NARO	BF	ABC	BCD	BEK	BD	CE	CDG	ACE
Prevalent	NARO	CD	CDG	BD	DE	BCD	AB	CE	E
Ranger Russet	Idaho	B	CF	BDG	DEK	CD	BC	Null	AE
Ranran Chip	NARO	BDF	BCF	BCD	BEK	BD	BCF	DG	ABE
Red Andes	NARO	B	ABG	F	BDF	BDF	BE	D	ADE
Red Moon	NARO	B	BDF	CD	AEJK	CF	AB	BCDF	ACE
Rira Chip	Hokkaido	BCG	ACF	BCD	BEK	BE	BC	DG	AE
Russet Bannock	Idaho	ABC	BCF	CF	DK	BDE	BC	D	EG
Russet Burbank	Idaho	B	BDG	BCFG	DGK	BC	BEF	DE	AE
Russet Norkotah	Idaho	BD	BCDG	CDFG	BDK	BCD	BC	D	AEF
Saikai 31 (Dragon Red)	Nagasaki	ACE	AF	BCD	BEL	BD	BCF	D	ABE
Sakurahubuki	NARO	EG	AB	CD	CEKL	BD	BC	D	AEG
Sanjumaru	Nagasaki	AC	BDF	BC	BE	B	D	DG	AEF
Sanyenimo (Vermont Gold Coin)	NARO	BG	ABDG	BCF	BCGK	B	BE	Null	AE
Sayaakane	Hokkaido	BD	CF	CDF	E	BCD	BCE	D	AE
Sayaka	NARO	BC	BF	CG	BFG	BD	BEF	DE	AE
Setoyutaka	Nagasaki	DF	ABF	BDF	BEHK	ABD	BCE	CD	AEF
Shadow Queen	NARO	CG	BCF	CD	BEK	ABCD	BEF	DE	AC
Shepody	Idaho	ABG	CDFG	BCDG	BDG	BE	BE	Null	ABEG
Shigetsu	NARO	BF	ABF	CD	BEK	ABD	BC	DG	ACE
Snow March	Hokkaido	BDG	ABCF	CDE	BEK	B	BCE	DG	AE
Snowden	NARO	BD	BCF	CDF	BEK	B	BC	D	ABEG
Star Ruby	NARO	BDG	BCF	BCDF	BDK	BCE	BF	D	ACE
Tawaramurasaki	NARO	BF	ACF	BDF	BE	D	ABE	C	AC
Tokachikogane	NARO	BF	BF	BCD	BEK	ABD	BCE	DG	ACE
Toya	NARO	BF	AC	BDF	BEK	BDE	BCE	G	A
Toyoshiro	NARO	BG	ABD	CDF	EL	BDE	BC	D	AE
Umatilla Russet	Idaho	BG	CG	BC	DK	BDE	B	D	CE
Waseshiro	NARO	F	CDF	CDG	EK	ABE	BE	C	AE
Western Russet	Idaho	BD	CF	BDG	DEK	BD	B	D	ABCE
Yukirasya	NARO	BF	BCF	C	EK	D	B	D	ACE
Yukitsubura	Hokkaido	BC	C	CDF	BDG	DE	BC	E	AE

Symbols of the peaks are described in **Table 2**. Null indicates a cultivar in which no peaks are obtained with the corresponding primer pair.

suggesting that these two cultivar pairs respectively have the same genomic organization other than the corresponding gene for skin color.

Cultivar identification of potato has been reported previously, and the markers described by Ghislain *et al.* (2004, 2009) have been used widely. Since these markers are mainly di- and tri-nucleotide SSRs, the lower separation of neighboring alleles and the relatively high level of stutter bands are inevitable. In fact, Reid *et al.* (2011) reported that one allele of STM3023 (di-nucleotide SSRs) is located at the stutter position for the other allele, resulting in a complication of the allele call. Additionally, simplex PCR and the various annealing temperatures of the primers are time-consuming and labor-intensive.

The set of tetra-nucleotide SSRs described here has no or extremely little stuttering, resulting in good reproducibility and reliability of allele calling. The 8-plex PCR conditions designed in this study allow simple and rapid analysis of cultivars. These markers will be helpful for the rapid identification of potato cultivars, and consequently for protecting plant breeders' rights.

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