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 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.
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.

Octplex PCR reactions were carried out 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 $i^{th}$ 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 for each locus, the 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 $i^{th}$ profile.

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

| Marker ID* | Chr. | Motif | Forward (5’→3’) | Reverse (5’→3’) | Conc. peak range (bp) |
|-----------|------|-------|-----------------|-----------------|-----------------------|
| 4026/4027 | 1    | C(TAT)n(TAG)n | NED-AACCTTGCGAGGATAAGTGCAGC  | ACTATACACAGCTGCCCCTGAAACTACAG | 0.09 265–346 |
| 8242      | 2    | C(TTT)n | FMG-CATCGTATGGCTTAGTGTTGG  | GCAAACCGAGAAAGCTAACAAC  | 0.08 191–218 |
| 12002     | 3    | A(CAT)n | NED-CCATGAACTGAGTTTTTCTGGC  | TTGAATCTTGTGCTACTAAAGCTAG  | 0.10 209–235 |
| 16410     | 4    | A(TAC)n | FM-GATGTTTTGAGTAGAATTCTCACCAG  | TTTCCTGCCCCCTTTTTATTTG  | 0.16 258–354 |
| 31924     | 8    | A(TAC)n | VIC-CGAGAACACACAAATGCTCAG  | GAAACGCAATTACATTTTACATCG  | 0.07 136–250 |
| 35584     | 9    | G(AAA)n | VIC-ACTAGTGCACAACTCAGTCGAGGTTG  | GTTCATGATATTCTCCTACGTGCTTTG  | 0.08 84–111 |
| 43016     | 11   | A(TCC)n | PET-CAAGCTCTAGTAAAGCCTAC  | TTTGCCTAAAAAGTTGTAGTTGAGG  | 0.07 184–227 |
| 46514     | 12   | T(ATC)n | PET-TGGTCTTTGTCTCTTTTGTG  | GGAATGGAACTACGGCTTCTG  | 0.12 150–172 |

*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) |
|------------|--------------|----------------|----------------------|-------------------------|
| 4026/4027  | 7            | 27             | 0.912                | 265.0 307.4 312.9 319.7 339.3 343.0 345.5 |
| 8242       | 3            | 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                | 257.6 266.9 271.0 279.0 281.0 310.3 325.2 335.9 339.5 346.3 349.9 353.6 |
| 31924      | 6            | 22             | 0.918                | 135.6 213.5 218.2 222.2 223.0 249.9 |
| 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 |
| Alyutaka              | Nagasaki | ABF | ABF | BD | BE | BD | C | G | AFE |
| Alturas               | Idaho   | C | BC | CE | DEK | BDE | B | DFG | AE |
| Astarte               | NARO BD | BDCF | D | DE | AB | BD | BE | D | AE |
| Atlantic              | NARO BDG | ACD | CDEF | BDK | BD | BCE | DFG | A | AC |
| Beniakari             | NARO BG | BF | PDFG | BEK | DE | CEF | D | AC |
| Benimaru              | NARO BDG | AB | CDFG | EHIK | AB | BE | CE | AE |
| Cal white             | Idaho   | DG | ABC | BCF | CDK | BE | BF | D | E |
| Chelsea (Jenny)       | NARO B | BDCF | ACD | DEFK | BCD | ABE | D | AE |
| Cherie                | NARO BD | ABCD | CD | DEG | AD | BF | DF | E |
| Clearwater            | Idaho A | B | BCF | DK | BE | B | D | E |
| Cynthia               | NARO AB | BF | CD | DEHK | BD | B | CD | ABE |
| Dansyakuimono (Irish Cobber) | NARO B | AB | CF | CBK | AB | BC | D | E |
| Hanashibetsu          | Hokkaido | BCD | AF | PDFG | BE | BCE | B | AD | AE |
| Haruka                | NARO BC | BF | BCDG | BEG | BD | BCEF | DEG | AEF |
| Hikaru                | NARO BCFG | BFDEF | DE | BEK | ABCE | BCF | D | ACF |
| Hokkaido 50           | NARO B | BF | CDF | BEK | AB | BC | D | AE |
| Hokkai 98 (Inca Rouge) | NARO B | CG | F | EK | F | B | D | E |
| Hokaikogane           | NARO BG | AB | CDF | EHE | ABE | BC | D | AE |
| Hugenmara             | Nagasaki | ABF | AF | BCD | BE | AB | B | G | AE |
| Inca no hitomi        | NARO D | F | FG | FG | K | E | B | D | E |
| Inca no meizame       | NARO B | CG | F | EK | F | B | D | E |
| Inca Purple           | NARO BCFG | BCF | CDF | EK | AB | B | AC | ACE |
| Inca Red              | NARO B | BF | DF | EK | AC | BD | C | D | ABE |
| Kitakari              | NARO ABF | ABF | CDF | BCEK | BE | D | C | AFG |
| Kitahime              | NARO BC | F | CG | EFGK | BCE | BEF | DE | E |
| Kitamurasaki          | NARO BCFG | BC | BD | BEK | ADE | EF | D | AE |
| Kitamurasani          | NARO F | GFG | BD | BE | DE | BD | BE | E | ACE |
| Koganemaru            | NARO B | BF | AB | CD | BEK | BDE | ABE | DG | ACE |
| Konabukichi           | Hokkaido | BG | AB | CD | CE | BD | B | D | A |
| Konayuki              | Hokkaido | AB | CDF | EHIK | AB | E | CD | AE |
| Konayutaka            | Hokkaido | BDF | AB | DEF | BEK | BE | BC | D | AE |
| Matilda               | NARO BD | CDG | DG | BEI | CD | ABE | D | EG |
| May Queen             | NARO BDG | CDF | CGF | BEG | BE | EF | ABE |
| Nishiyutaka           | Nagasaki | B | BCF | CD | BEK | B | BE | Null | AEG |
| Norin I               | NARO B | AB | CDF | BHIK | AB | BCF | D | AE |
| Norking Russet        | NARO BF | BC | BCD | DE | BCE | BF | Null | AEG |
| Northern Ruby         | NARO CG | BCF | CD | CEK | ABDF | BF | DE | AE |
| Okhotchk Chip         | Hokkaido | BG | CDF | BCF | EK | BD | BDEF | DF | AF |
| Oojoiro               | NARO B | AD | DF | BHIK | A | BCE | D | E |
| Piruka                | NARO BF | ABC | BCD | BEK | BD | CE | CGD | ACE |
| Prevalent             | NARO CD | CDF | BD | D | BCE | AB | CE | E |
| Ranger Russet         | Idaho B | CF | BDG | DEK | CD | BC | Null | AE |
| Runnar Chip           | NARO BDF | BCF | BCD | BEK | CBDF | DB | DFG | ABE |
| Red Andes             | NARO B | ABG | F | BDF | BDF | BE | D | ADF |
| Red Moon              | NARO B | BDF | CD | AEK | CF | AB | BCDF | ACE |
| Rina Chip             | Hokkaido | BCFG | ACF | BCD | BEK | BE | BC | DG | AE |
| Russet Bannock        | Idaho AEC | BCF | CF | DK | BDE | BC | D | EG |
| Russet Burbank        | Idaho BG | 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 |
| Sakurahubiki          | NARO EG | AB | CD | CEK | BD | BC | D | AEG |
| Sanjumaru             | Nagasaki | AC | BDF | BC | BE | B | D | DFG | EA |
| Sanyenimo (Vermont Gold Coin) | NARO BG | ABGD | BCF | BCGK | B | BE | Null | AE |
| Sayakane              | Hokkaido | BD | CF | CDF | E | BCD | BCE | D | AE |
| Sayaka                | NARO BC | BF | CG | BFG | BD | BDEF | DE | AE |
| Setoyutaka            | Nagasaki | DE | ABF | BDF | BEH | AB | BCF | CC | DAE |
| Shadow Queen          | NARO CG | BCF | CD | BEK | ABCD | BCF | DE | DFG | AE |
| Shepody               | Idaho ABG | CDFG | BCDG | BDG | BE | Null | ABEG |
| Shigetsu              | NARO BF | ABF | CD | BEK | ABD | BC | DG | ACE |
| Snow March            | Hokkaido | BDG | ABCF | CDE | BKE | B | BCE | DEG | AE |
| Snowden               | NARO BDG | ABCF | CDF | BEK | B | BCD | D | ABE |
| Star Ruby             | NARO BDG | BCF | BD | CD | BCE | DFG | A | ACE |
| Tawaramurasaki        | NARO BAC | AC | BDF | BEK | BDF | B | G | A |
| Tokachikogane         | NARO BF | BCF | BD | BEK | ABD | BCE | DG | ACE |
| Toyu                  | NARO BF | AC | BDF | BEK | BDF | BE | CG | D |
| Toyoshirou            | NARO BGD | AB | CDF | EL | BDE | BC | D | AE |
| Umatilla Russet       | Idaho BG | CF | BC | DK | BDE | B | D | CE |
| Waseshio              | NARO F | CDF | CG | AEK | BE | B | C | AE |
| Western Russet        | Idaho BD | CF | BDG | DEK | BD | B | D | AEF |
| Yukitasara            | NARO BF | BCF | C | EK | D | B | D | ACE |
| Yukitsubara           | 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.
suggested 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.

Acknowledgments

The authors would like to thank the University of Idaho, HRO Kitami Agricultural Experiment Station, Nagasaki Agricultural and Forestry Technical Development Center, and NARO Hokkaido Agricultural Research Center for providing us with potato materials.

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