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Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.)

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Abstract Microsatellites, or simple sequence repeats (SSRs) are very useful molecular markers for a number of plant species. They are commonly used in cultivar identification, plant variety protection, as anchor markers in genetic mapping, and in marker-assisted breeding. Early development of SSRs was hampered by the high cost of library screening and clone sequencing. Currently, large public SSR datasets exist for many crop species, but the number of publicly available, mapped SSRs for potato is relatively low (~100). We have utilized a database mining approach to identify SSR-containing sequences in The Institute For Genomic Research Potato Gene Index database (http://www.tigr.org), focusing on sequences with size polymorphisms present in this dataset. Ninety-four primer pairs flanking SSR sequences were synthesized and used to amplify potato DNA. This study rendered 61 useful SSRs that were located in pre-existing genetic maps, fingerprinted in a set of 30 cultivars from South America, North America, and Europe or a combination thereof. The high proportion of success (65%) of expressed sequence tag-derived SSRs obtained in this work validates the use of transcribed sequences as a source of markers. These markers will be useful for genetic mapping, taxonomic studies, marker-assisted selection, and cultivar identification.

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Introduction

Molecular markers detect DNA sequence variation among genotypes. In potato, they have been used for construction of genetic linkage maps (Bonierbale et al. 1988, 1994; Gebhardt et al. 1991), trait tagging (Gebhardt et al. 1993; Bryan et al. 2002), fingerprinting analysis (Görg et al. 1992; Milbourne et al. 1997; Mc Gregor et al. 2000a; Norero et al. 2004), phylogeny studies (Debener et al. 1990; Raker and Spooner 2002; Izpizua 2004; Raimondi et al. 2005), and characterization of accessions from germplasm banks (Gebhardt et al. 2004; Ghislain et al. 2004).

Since 1980, when restriction fragment length polymorphisms (RFLPs) were first used by Botstein et al. (1980) to construct the first molecular map of the human genome, several types of DNA-based markers were developed (reviewed by Vekemans and Jacquemart 1997). These markers were used to make the first linkage maps for potato (Bonierbale et al. 1988; Gebhardt et al. 1989).

Among DNA markers, microsatellites [synonymous with simple sequence repeats (SSRs), Tautz 1989] have been chosen over RFLPs or other PCR-based methods such as random amplified polymorphic DNA (Williams et al. 1990) or amplified fragment length polymorphism [(AFLP) Vos et al. 1995] because of their simplicity and low DNA requirement. Additional advantages include their ubiquity, distribution across the genome, co-dominant behavior, multiallelism, reproducibility, and high level of polymorphism detected (Milbourne et al. 1997; Marcucci Poltri 1998; Mc Gregor et al. 2000b).

SSR markers consist of a variable number of tandem repeats of a simple motif sequence, typically a mono-, di-, tri-, or tetranucleotide repeat. Polymorphisms are detected by PCR amplification with specific flanking primers and subsequent size sieving in agarose or denaturing polyacrylamide gels.

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 every 64.6 kb in monocots (Wang et al. 1994). In potato, Ashkenazi et al. (2001) estimated that one SSR could be found every 52 kb when screening for five different motifs. This relatively low frequency of SSRs constituted one of the major drawbacks in early SSR developments, as the number of microsatellites found by sequencing libraries made the cost per marker very expensive (Rafalski and Tingey 1993). The first SSRs were developed by screening large numbers of clones from genomic libraries with repetitive probes (Akkaya et al. 1992; Röder et al. 1995; Liu et al. 1996; Bryan et al. 1997). To overcome this constraint, processes were designed to enrich libraries for microsatellite motifs prior to screening, such as by hybridizations to repetitive oligos bound to magnetic beads (Kijas et al. 1994) or membranes (Edwards et al. 1996), triplex affinity capture (Milbourne et al. 1998), or selective pre-amplification using oligos with repeat motifs (Bryan et al. 1997). However, enrichment techniques can lead to a high level of redundancy (Scott 2001).

A more practical, economical, and straightforward approach for species with reasonable DNA sequence database representation is a database search for SSR sequences. This strategy has been successfully used for tomato (Smulders et al. 1997), sorghum (Brown et al. 1996), rice (Miyao et al. 1996), and soybean (Akkaya et al. 1992), among other plant species.

Early development of potato SSRs from GenBank and European Molecular Biology Laboratory (EMBL) databases searches was done by Veilleux et al. (1995) and Provan et al. (1996), rendering around 30 working markers. Milbourne et al. (1998) utilized the EMBL database, an expressed sequence tag (EST) database, and cDNA library screening to develop 112 potato SSRs, of which 65 were mapped. Ashkenazi et al. (2001) screened three libraries (two of them enriched for ATT and GT repeats) and searched public genomic databases to obtain 30 new microsatellites. More recently, Ghislain et al. (2004) reported the location of 13 new EST-derived SSRs (STM5000 series) for which primer sequences may be found at http://www.potgenebank.org/.

However, more markers are needed for the construction of saturated and consensus genetic maps, marker-assisted breeding, and germplasm bank management. Because ESTs are from expressed genes, they present the possibility of direct gene tagging through candidate gene mapping and contribute to "functional maps." Moreover, selective pressure may reduce genetic variability in the primer region and enhance transferability of the markers across related species (Scott et al. 2000).

Milbourne et al. (1998) reported that 451 potato sequences were publicly available (of which 75 contained SSRs and 30 were suitable for primer design). Subsequent large-scale sequencing programs for potato, tomato, and other related species have released sequences for a large number of ESTs. At the time of writing this paper, 157,197 potato EST sequences were available from http://www.tigr.org, organized into 19,225 tentative consensus sequences (TCs) and 13,226 singletons (release 9.0, May 2004). The objective of this work is to generate new SSRs derived from gene index databases. We report here the development of 61 new SSRs using The Institute for Genomic Research (TIGR) Potato Gene Index database and the mapping of 57 of these.

Materials and methods

Plant material and DNA isolation

Thirty cultivars from Europe, North America, and South America were used for fingerprinting analysis (Table 1), as a sample of the variability in commercial potato germplasm. The selection also included genotypes of *Solanum tuberosum* ssp. *andigena* (4×), ssp. *tuberosum* × ssp. *andigena* (4×) and *S. phureja* (2×). Genomic DNA was isolated from in vitro plants supplied by the Propapa Germplasm Bank (INTA-Balcarce), according to Haymes (1996).

Forty-two genotypes from each of two segregating diploid populations (BCB and BCT, Bonierbale et al. 1994) were selected to represent the most informative subset based on selective mapping using MapPop,

Table 1 Potato cultivars used for fingerprinting and their origin

Cultivar	Origin
Solanum tuberosum ssp. *tuberosum*	
Achat	Germany
Achirana INTA	Argentina
Alpha	The Netherlands
Americana INTA	Argentina
Araucana INTA	Argentina
Atlantic	USA
Bintje	The Netherlands
Bonaerense la Ballenera MAA	Argentina
Chieftain	USA
Cruza 148	Mexico
Danva	Denmark
Desiree	The Netherlands
Frital INTA	Argentina
Granola	Germany
Huinkul MAG	Argentina
Kelune INTA	Argentina
Kennebec	USA
Norland	USA
Pampeana INTA	Argentina
Pentland Crown	UK
Ranger Russet	USA
Russet Burbank	USA
Serrana INTA	Argentina
Shepody	Canada
Spunta	The Netherlands
Yagana INIA	Chile
S. tuberosum ssp. *andigena*	
Collareja 621	CIP
Jaspe	Bolivia
Ssp. *tuberosum* × ssp. *andigena*	
Canchan-INIA	Peru
S. phureja	
Yema de huevo	Colombia

Table 2 Simple sequence repeat (SSR) mapping and fingerprinting results, including map location, StI codes, flanking primer sequences, repeat motif, working annealing temperatures, expected and observed amplicon size, number of alleles, number of patterns, number of expected bands per genotype, and diversity index (*DI*) after analyzing 30 diverse potato cultivars and four diploid mapping populations

SSR Name	Repeat motif	forward primer	reverse primer	Annealing temperature (°C)[b]	expected size (bp)	size range (bp)	# of Alleles	# of patterns	DI	mean expected bands	map location
StI001	(AAT)n	cagcaaaatcagaacccgat	ggatcatcaaattcaccgct	60-54	188	185-208	7	22	0,94	2,8	4
StI002	(ATT)n	acaggaatcacacctgcaca	ttcaacatccgcctgtcata	60-54	122	107-128	6	10	0,81	2,0	9
StI003	(ACC)n	accatccaccatgtcaatgc	ctcatggatggtgtcattgg	60-54	144		4	6	0,77	2,8	8
StI004	(AAG)n	gctgctaaacactcaagcagaa	caactacaagattccatccacag	60-54	103	78-112	9	17	0,90	2,6	6
StI005	(ACA)n (GCA)n	ctaatttgatgggaagcga	cggagataaaacccaagtcc	58-52	157	110-172	12	23	0,95	6,8	3 & 8
StI006	(AT)n(GT)n	ctttagtccttggcagagctt	cgggctgattcttcttcatc	62-56	250	199-250	10	19	0,92	3,0	5
StI007	(GTT)n(GAT)n	tatgttccacgccatttcag	acggaaactcatcgtgcatt	60-54	134	117-134	6	17	0,91	3,4	12
StI008	(AGC)n	catctccttcacctgctcct	cgacaaaggaggaaatccaa	58-52	152	137-155	4	6	0,79	2,3	7
StI009	(AGC)n(AAC)n	gcgaaaaccttgaagcaact	ctgctgttgctgttgatggt	60-54	237	270-297	9	17	0,90	3,0	1
StI011	(AC)n(AT)n	tggtgtgcacaaacttaagagg	gaggagatcacaattcctttga	60-54	99	59-113	7	10	0,88	2,3	6
StI012	(ATT)n	gaagcgacttccaaaatcaga	aaagggaggaatagaaaccaaaa	58-52	183	168-192	7	14	0,90	2,9	4
StI013	(ACC)n	ccacttcctccacttccaaa	ccatggttgcaccaactaga	60-54	157	255-300	4	12	0,88	2,5	3
StI014	(TGG)n(AGG)n	agaaactgagttgtgtttggga	tcaacagtctcagaaaaccctct	60-54	120	130-142	3	6	0,76	2,2	9
StI015	(AT)n(AG)n(AT)n	gcatgtcttcgaaggtacgttta	ttcttcacagcagcaaggtg	60-54	241	230-260	9	17	0,90	2,5	6
StI016	(CCT)n	gaattgcagagaggacctgg	actccctgttgtcggagatg	58-52	197	285-300	5	11	0,88	1,9	6
StI017	(CAT)n(TAG)n(AAG)n	tatggaaattccggtgatgg	gacggtgacaaagaggaagg	65-63	163	154-172	6	13	0,88	2,7	11
StI018	(GTT)n	ccactactgcttcctccacc	gcagcaacaacaagctcaac	60-54	189	157-192	7	10	0,80	2,5	11
StI019	(ATCT)imp	tccctgttgccttgaacaat	tgggaaaaggtacaaagacga	60	126	86-130	7	14	0,90	3,3	7
StI020	(AAT)n	gacgcagaactcatcttgttca	gcaaaatttgaaaaactatggatg	60	117		8	6	0,67	2,6	4
StI021	(CAT)n	tcatcaagtcgtcgtcatcaa	tcgaatgatccaaagcttcc	60-54	106	85-109	6	13	0,86	2,7	6
StI022	(ACCCG)n	tctccaattacttgatggaccc	caatgccatacacgtggcta	63	133		9	13	0,85	3,3	8
StI023	(GGC)n (GGT)n	gcgaatgacaggacaagagg	tgccactgctaccataacca	60-54	193	142-205	11	11	0,77	2,5	10
StI024	(CAA)n	cgccattctctcagatcactc	gctgcagcagttgttgttgt	60-54	168	138-171	7	14	0,85	1,9	2
StI025	(CTCC)n	ctgccgcaaaaagtgaaaac	tgaatgtaggccaaattttgaa	60	116	104-116	3	4	0,25	1,1	7
StI026	(TA)n (GT)n	caacgctactcaatggctca	acaactctagaacgagaggaaca	62-56	182	176-232	8	14	0,83		4
StI027	(GAA)n	cgcaaatcttcatccgattc	tccggcggataatacttgtt	62-56	148	109-148	6	11	0,82	1,8	8
StI028	(CAA)imp	ataccctccaatgggtcctt	cttggagatttgcaagaagaa	60	192	156-201	8	10	0,76	2,7	11
StI029	(CA)imp (TC)imp	gactggctgaccctgaactc	gacaaaattacaggaactgcaaa	60-54	155	124-172	11	13	0,87	2,8	2
StI030	(ATT)n	ttgaccctccaactatagattcttc	tgacaactttaaagcatatgtcagc	60	107	77-117	9	14	0,89	2,6	12
StI031	(TCA)n	aggcgcactttaacttccac	cggaacaaattgctctgatg	60-54	138	129-171	11	16	0,92	4,0	1
StI032	(GGA)n	tgggaagaatcctgaaatgg	tgctctaccaattaacggca	64-60	121	106-127	9	23	0,94	3,0	5
StI033	(AGG)n	tgagggttttcagaaaggga	catccttgcaacaacctcct	64	134	116-143	6	15	0,91	2,9	7
StI034	(CTT)n	caagaaaccaagagcaaatttca	tggcgaatgtgagaaacaaa	63	158		6	12	0,81	3,2	
StI035	(TTC)n	acctttgaggaattgcagga	cattgaaggagttccagtcc	60-54	102	102-117	4	5	0,69	2,6	
StI037	(AT)n	ggacaaccaagtgagcaaca	tgaggagaaaggcacacaaa	60	180	142-180	6	12	0,79	2,3	
StI039	(AAC)n (AAT)n	gattgatccaatcacgcaca	aattattcgcgcaattcgtc	60-54	236						11
StI040	(ACA)n (GCA)n	tctttccctttttatcctcactg	gggattgggtttgaagtagttg	60-54	180	155-189	4	4	0,54	1,4	7
StI041	(GAA)n	ctctgtttctctaatcggccgta	aagcgttggccaccgcca	60-54	135	126-138	4	6	0,66	2,2	11
StI042	(AT)n	tcacgaggtgcccaactg	tccattcgtcaacaaggt	60-54	133	117-135	9	21	0,94	2,8	
StI043	(AAC)imp	caatgcgaatgttgctactggt	atccaccaagacctccagaa	60-54	138	129-144	6	12	0,88	2,3	1
StI044	(AT)n	gagaaccccacccaccaa	ggtattgtgcttgaacagcca	60-54	162	130-162	8	10	0,87	2,5	8
StI045	(CTT)n	ctgtacccattacttctctgctga	gcaactttgaagggtgtttgc	60-54	91	79-91	5	6	0,45	2,0	6
StI046	(GAT)n	cagaggatgctgatggacct	ggagcagttgagggcttctt	60-54	191	167-215	16	24	0,95	5,4	11[a]
StI047	(TAA)n	actgctgtggttggcgtc	acggcatagatttggaagcatc	60-54	143	128-146	6	13	0,86	2,8	8
StI048	(GAT)n	cgagtccgtggatctcacg	gattcccgccggtaaagc	60-54	170	164-191	5	6	0,81	2,4	8
StI049	(TCTT)n	ggaagtcctcaactggctg	tcaactatatgcctactgcccaa	60-54	157	137-157	5	8	0,80	2,4	5
StI050	(ATA)n	ttcctctaagcggcaaaagg	ggaggagacttgggttttctcc	58-52	91	153-168	6	12	0,82	2,5	3
StI051	(TA)n	ggtctccattagccctctgag	acataaatggatcacaca	58-52	170	149-187	8	12	0,84	1,9	12
StI052	(CTT)n	tcatcacaacgtgacccca	gggcttgaatgatgtgaagctc	60-54	173	153-173	4	6	0,76	2,6	2
StI053	(AT)imp	tcagaccgggttcgatgg	cggcttgaatcattgccca	60-54	160	112-160	7	15	0,91	2,4	2
StI054	(CCA)n	gccactatgcaagcccatttg	gggtcgatgtttcggttgag	60-54	176	152-179	7	7	0,61	2,2	12
StI055	(AAG)n	ccgttgatgggattgcaca	tgatattaaccatggcagcagc	60-54	220	214-244	5	6	0,56	1,3	4
StI056	(TA)n	gacagagaatatgggaccacca	gcagcaccttaaatggctgac	60-54	195	179-195	2	2	0,50	1,5	2
StI057	(AAG)n	ccttgtagaacagcagtggtc	tccgccaagactgatgca	60-54	196	172-202	8	17	0,90	3,0	9
StI058	(TA)n	caagcacgttacaacaagcaa	ttgaagcatcacatacacaaaca	60-54	121	77-103	8	14	0,90	2,5	5
StI059	(AT)n	agacgggtgcacacgcac	tgcttgagtatgacagcacttga	58-52	141	126-154	8	10	0,82	2,2	6
StI060	(ATA)n	acttctgcatctggtgaagc	ggtctggattcccaggttg	60-54	173	158-173	5	8	0,81	1,9	3
StI061	(GAA)n	agcaaccaccacagcagc	ccggcgattggatcgacg	60-54	137	131-137	3	4	0,61	2,1	
StI062	(GAA)n	ggggtcaagctccatacg	actaaaaccacaacccatgagc	60-54	117	117	1	1	0,00	1,0	1
StI063	(GAT)imp	gcattctatggccaacattgg	agattcctccaatttcccagc	60-54	252						12
StI064	(AT)n(GT)n	caaattctccccattttgga	aaccgattcaaaaaccctca	60-54	171						7

Blank cell means data not available

[a]Suspected multilocus marker, additional location possible

[b]Range of annealing temperatures denotes a touchdown profile with 0.5°C decrease per cycle, until lower annealing temperature was reached

version 1.0 software (Vision et al. 2000). Genomic DNA from these clones was isolated using the DNeasy plant isolation kit (Qiagen, Valencia, Calif., USA).

Database search for SSR sequences and primer design

Initial candidate SSRs were selected from the Potato Gene Index database (version 4.0, Aug. 2001; http://www.tigr.org/tdb/tgi/plant.shtml) using the BLAST search algorithm. Subsequent candidate SSRs were selected from a downloaded version (version 7.0, April 2003) of the same database using Sputnik (http://espressosoftware.com/pages/sputnik.jsp), a C-language program that searches DNA sequence files in FASTA format for microsatellite repeats. In some cases, candidate ESTs were prioritized based on presence of motif size polymorphisms in the EST database. Flanking oligonucleotides were designed using the program Primer3 (http://www.genome.wi.mit.edu/genome_software/other/primer3.html) or FastPCR (Kalendar 2004. http://www.biocenter.helsinki.fi/bi/bare-1_html/fastpcr.htm), using the following initial parameters: target primer length: 20 nucleotides, target T_m: 58°C, target amplicon length: 75–300 bp. Primer sequences and annealing temperatures for SSRs reported in this paper are given in Table 2. Information on additional primer pairs that failed to amplify, were not polymorphic in these mapping populations, or that could not be easily scored will be provided upon request.

Polymerase chain reactions conditions and product electrophoresis

Polymerase chain reactions (PCRs) were performed in 20-µl volumes containing approximately 30 ng template DNA in 1.5 m*M* $MgCl_2$, 1X PCR buffer (50 m*M* KCl; 10 m*M* Tris-HCl, pH 8.3), 0.2 m*M* dNTP mix, 0.12 µ*M* of forward and reverse primers, and 1 U of *Taq* polymerase. Reactions were performed in 96-well plates in MJ thermocyclers (PTC-100 or PTC-200; MJ Research, Watertown, Mass., USA) under the following profile: 3-min initial denaturation step at 95°C, 35 cycles of 30 s at 95°C, 30 s at annealing temperature (Table 2), 1 min at 72°C, with a final extension step of 5 min at 72°C. The indication of a range in the annealing temperature column in Table 2 denotes the use of a touchdown profile (Don et al. 1991). In this case, the annealing temperature used in the first cycle was the higher temperature of the range, with a 0.5°C decrease per cycle until the lower temperature was reached; additional cycles at the lower annealing temperature were added to complete 35 cycles in total.

Success of amplification was checked on 1% agarose gels run in 1X TBE (89 m*M* Tris-Borate, 20 m*M* ethylenediaminetetraacetic acid) and stained with 0.5X GelStar (Cambrex Biosciences, Rockland, Me., USA). Band size on fingerprinting panel was determined by comparison with a 10-bp DNA ladder (InvitroGen Life Technologies, Carlsbad, Calif., USA). PCR products were run on 5% denaturing polyacrylamide gels and silver-stained as detailed elsewhere (Bassam et al. 1991).

Mapping and genetic analysis

Band scoring was performed manually for both mapping and fingerprinting purposes. The genetic location of SSR markers was based on the RFLP framework maps for the BCB and BCT mapping populations (Bonierbale et al. 1994) using MapManager QTX program (Manly et al. 2001). Markers were considered linked to pre-existing RFLP or SSR markers when LOD scores were equal or greater than 3.0. A consensus map derived from the four parental maps from BCB and BCT was obtained using JoinMap, version 3.0 (van Ooijen and Voorrips 2001).

A diversity index (DI) was calculated from fingerprint data as $1-\Sigma pg^2$ where *pg* is the frequency of an individual genotype, assuming each SSR reveals one locus (Milbourne et al. 1997).

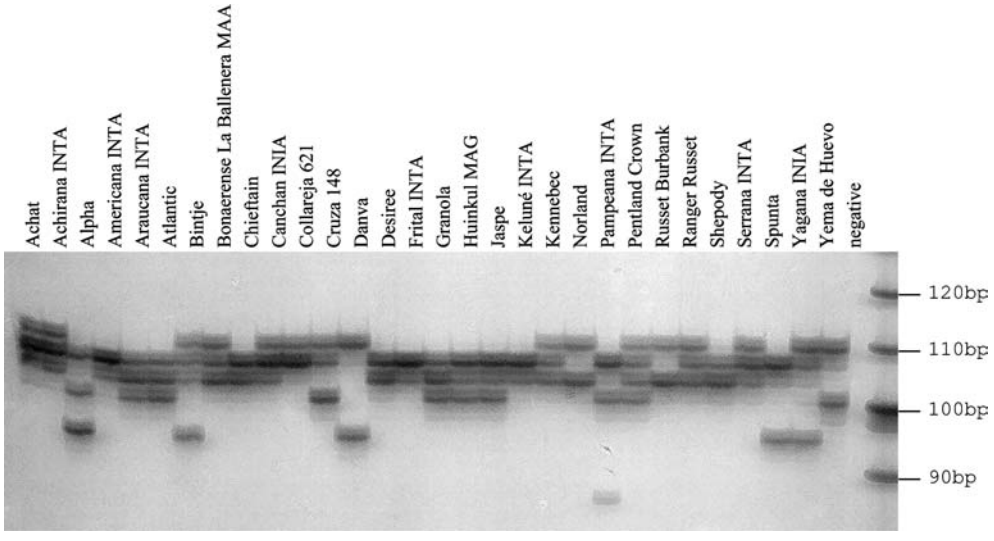

Fig. 1 Allelic variation of 30 potato genotypes revealed by StI021

Table 3 Original and current (version 9.0) identifiers of the sequences used as source of SSRs, along with their most recent tentative annotation

code	Original ID	Current (v.9.0) ID	tentative annotation
StI001	AF122053	NP280807	tuber-specific and sucrose-responsive element binding factor
StI002	AJ133765	NP182395	invertase, putative
StI003	AW096896	AW096896	*similar to* SP|O08550|TRX2_MOUSE Trithorax homolog 2 (WW domain binding protein 7) (Fragment). {Mus musculus;}, partial (9%)
StI004	BE471335	TC98002	*similar to* UP|Q944L3 (Q944L3) AT3g45260/F18N11_20, *partial (10%)*
StI005	BE340719	TC105083	*similar to* UP|Q16861 (Q16861) Super cysteine rich protein (Fragment), *partial (46%)*
StI006	BG096868	TC100002	*similar to* GB|AAD14477.1|4249380|T2K10 ESTs gb|Z37637, gb|AA042498 and gb|AA042269 come from this gene. {Arabidopsis thaliana;}, *partial (45%)*
StI007	BG594771	TC96544	*weakly similar to* Zeatin O-glucosyltransferase (EC 2.4.1.203) (Zeatin O-beta-D- glucosyltransferase)., *partial (16%)*
StI008	BG596262	TC96259	*similar to* PIR|T10265|T10265 arabinogalactan-protein AGP2 - Persian tobacco {Nicotiana alata;}, *partial (36%)*
StI009	BG888053	TC102749	*homologue to* PIR|T01932|T01932 RNA binding protein homolog - common tobacco (fragment) {Nicotiana tabacum;}, *partial (87%)*
StI011	BG888359	TC98906	UP|Q61402 (Q61402) Gcap1 protein (Fragment), *partial (21%)*
StI012	STU69633[1]	U69633[1]	>gi|2055384|gb|AAB53203.1| (U69633) cold-stress inducible protein
StI013	TC16934	TC93187	*similar to* PIR|T09555|T09555 fibrillarin - Arabidopsis thaliana {Arabidopsis thaliana;}, *partial (88%)*
StI014	TC18962	TC99264	*similar to* UP|Q9C5W8 (Q9C5W8) IAA14, *partial (71%)*
StI015	TC19128	TC105552	SP|Q42429|AGL8_SOLTU Agamous-like MADS box protein AGL8 homolog (POTM1-1). {Solanum tuberosum;}, *complete*
StI016	TC19146	TC93393	*homologue to* PIR|S51529|S51529 SPF1 protein - sweet potato {Ipomoea batatas;}, *partial (57%)*
StI017	TC20237	TC95133	*similar to* PIR|T48868|T48868 zinc finger protein [imported] - garden pea {Pisum sativum;}, *partial (45%)*
StI018	TC20973	TC98351	*weakly similar to* GB|AAK96446.1|15450537|AY052732 AT4g00850/A_TM018A10_22 {Arabidopsis thaliana;}, *partial (26%)*
StI019	TC57834	TC92630	*homologue to* UP|O24372 (O24372) Metallocarboxypeptidase inhibitor, *complete*
StI020	TC58214	TC103360	*similar to* UP|Q8L5W2 (Q8L5W2) bZIP transcription factor ATB2, *partial (60%)*
StI021	TC58421	TC94434	*similar to* GB|BAB01258.1|9279701|AB022223 heat shock transcription factor-like protein {Arabidopsis thaliana;} , *partial (49%)*
StI022	TC58754	TC94901	*similar to* UP|Q94F45 (Q94F45) AT4g18020/T6K21_200, *partial (19%)*
StI023	TC58809	TC93150	*weakly similar to* PIR|S71779|S71779 glycine-rich RNA-binding protein GRP1 - wheat {Triticum aestivum;}, *partial (78%)*
StI024	TC59697	TC104888	*similar to* PIR|F85438|F85438 nucleoporin-like protein [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}, *partial (25%)*
StI025	TC59810	TC105200	*similar to* GB|AAO63401.1|28950955|BT005337 At2g18870 {Arabidopsis thaliana;}, *partial (49%)*
StI026	TC60726	TC106976	*weakly similar to* PIR|S65081|S65081 wound-induced protein Sn-1, vacuolar membrane - pepper {Capsicum annuum;}, *partial (52%)*
StI027	TC67985	TC105598	*similar to* SP|Q12355|PST1_YEAST Protoplast secreted protein 1 precursor. {Saccharomyces cerevisiae;}, *partial (4%)*
StI028	TC68264	TC104539	*homologue to* UP|O13577 (O13577) YLR437CP, *partial (10%)*
StI029	TC68604	TC97044	*similar to* UP|Q8NH31 (Q8NH31) Seven transmembrane helix receptor, *partial (8%)*
StI030	TC69039	TC97970	*homologue to* UP|Q940Q8 (Q940Q8) AT5g61840/mac9_140, *partial (33%)*
StI031	TC69334	TC105680	SP|P00127|UCRH_YEAST Ubiquinol-cytochrome C reductase complex 17 kDa protein (Mitochondrial hinge protein) (Complex III polypeptide VI). {S. cerevisiae}, *partial (7%)*
StI032	TC72009	TC95955	*similar to* UP|Q9FJJ6 (Q9FJJ6) Genomic DNA, chromosome 5, TAC clone:K19B1 (AT5g62460/K19B1_7), *partial (65%)*
StI033	TC73984	TC111179	*similar to* UP|Q84YG9 (Q84YG9) Heat stress transcription factor HSFA9, *partial (29%)*
StI034	TC58806	TC94397	*similar to* GB|AAP31934.1|30387537|BT006590 At2g35860 {Arabidopsis thaliana;}, *partial (82%)*
StI035	TC66701	TC103721/TC1(	*similar to* GB|AAP21357.1|30102878|BT006549 At1g56300 (Arabidopsis thaliana;}, *partial (24%)*
StI037	TC70263	TC108138	*similar to* UP|CAE53273 (CAE53273) 1,3-beta-glucan glucanohydrolase , *partial (97%)*
StI039	BG593132	TC99449	*homologue to* PIR|T07393|T07393 myb-related transcription factor - tomato {Lycopersicon esculentum;}, *partial (80%)*
StI040	TC19603	TC93508	(Q7MZZ7) Similar to unknown protein YhhN of Escherichia coli, *partial (6%)*
StI041	TC58603	TC102743	*similar to* unknown protein {Arabidopsis thaliana;}, *partial (54%)*
StI042	TC59236	TC95093	*similar to* AAQ89615 (AAQ89615) At3g17205, *partial (39%)*
StI043	TC59569	TC95982	UP|Q8MQL7 (Q8MQL7) SD06302p, *partial (3%)*
StI044	TC59590	TC105347/TC1(	(Q9W2R6) CG15226 protein, *partial (5%)*
StI045	TC59679	TC105942	*similar to* PIR|T39903|T39903 serine-rich protein - fission yeast (Schizosaccharomyces pombe) {Schizosaccharomyces pombe;}, *partial (4%)*
StI046	TC59924	TC94217	*similar to* UP|Q9SJF1 (Q9SJF1) T27G7.9, *partial (69%)*
StI047	TC60280	TC106106	UP|CAF74710 (CAF74710) MYC transcription factor (Fragment), *partial (43%)*
StI048	TC60350	TC95585	*homologue to* PIR|S21495|S21495 tomato leucine zipper-containing protein - tomato {Lycopersicon esculentum;}, *partial (55%)*
StI049	TC61790	TC107030	*weakly similar to* UP|Q84W04 (Q84W04) At1g12390, *partial (88%)*
StI050	TC62248	TC106737	*similar to* (Q7X989) Putative MSP1(mitochondrial sorting of proteins) protein, *partial (7%)*
StI051	TC63163	TC99682	*no tentative annotation*
StI052	TC64563	TC101374	*weakly similar to* UP|Q8H1T9 (Q8H1T9) Phospholipase D delta isoform 1a , *partial (14%)*
StI053	TC64749	TC106350	*similar to* GB|AAD30253.1|4835787|F25C20 ESTs gb|R65381 and gb|T44635 come from this gene. {Arabidopsis thaliana;}, *partial (33%)*
StI054	TC65995	TC104083	*similar to* UP|Q9LIR4 (Q9LIR4) Dihydroxy-acid dehydratase (AT3g23940/F14O13_13), *partial (90%)*
StI055	TC66130	TC102626	SP|P29677|MPPA_SOLTU Mitochondrial processing peptidase alpha subunit, mitochondrial precursor (Alpha-MPP) (Ubiquinol-cytochrome C reductasesubunit II) {S. tuberosum;}, *complete*
StI056	TC67582	TC95818	*similar to* UP|P93341 (P93341) Phosphoinositide-specific phospholipase C, *partial (32%)*
StI057	TC68393	TC105136	*homologue to* UP|Q9XGA8 (Q9XGA8) Starch branching enzyme II precursor , *complete*
StI058	TC69083	TC103278	*homologue to* UP|Q8I738 (Q8I738) TcC31.4, *partial (9%)*
StI059	TC71385	TC108804	*no tentative annotation*
StI060	TC72082	TC100258	*homologue to* GB|AAM10292.1|20147151|AY091693 AT3g13810/MCP4_2 {Arabidopsis thaliana;}, *partial (15%)*
StI061	TC73738	TC105342	*similar to* UP|Q9LM88 (Q9LM88) F2D10.15, *partial (31%)*
StI062	TC73922	TC96968	*weakly similar to* unknown protein {Arabidopsis thaliana;}, *partial (95%)*
StI063	T_AW030425	T_AW030425	*weakly similar to* UP|O65815 (O65815) Cytochrome P450 81B1 (Isoflavone 2'-hydroxylase) , *partial (36%)*
StI064	BG890263	TC101810	*similar to* UP|Q9MLP5 (Q9MLP5) NADH dehydrogenase subunit 2, *partial (5%)*

[1]Actual GB number, not in current TIGR index
[2]T_ = derived from tomato EST

Results

Database search

A search of the TIGR Potato Gene Index, version 7.0, database gave 555 TC/singleton sequences with a Sputnik score ≥15 (corresponding to trinucleotide repeats with ≥18 nucleotides or dinucleotide repeats with ≥17 nucleotides). Relaxing the score to 14 added nearly another 250 candidate SSRs. Unique sequences containing SSRs with Sputnik scores ≥20 (minimum trinucleotide repeat length = 23, minimum dinucleotide repeat length = 22) were used for primer design.

Characterization of SSRs

Ninety-four primer pairs were tested in PCR reactions for the three parents (M200-30, B11B, and HH1-9) of the BCB and BCT mapping populations. Most primer pairs (85%) gave products of approximately the expected size range. Seven primer sets did not successfully amplify product, and seven primer pairs gave very high-molecular-weight bands or an apparently non-specific complex pattern. Of the 80 primer pairs that amplified clean products of approximately the expected size, 16 showed either no polymorphism between BCB and BCT parental genotypes or no segregation in the corresponding populations and were dropped from further consideration. Additional details on those primer sets may be accessed at http://marvin.ibest.uidaho.edu/~jlorenze or by request to the corresponding author.

During the course of this study, three pairs of SSRs were shown to be redundant. Although only one of each redundant pair is presented in this paper, the StI codes of the dropped SSRs were reserved, because a partial list of StI primers had been previously presented (Feingold et al. 2004). Identifiers for both, original database ID, and current version ID of the successful SSR clones along with their tentative annotation are presented in Table 3.

Sixty-one useful SSRs were then mapped, fingerprinted or both (Table 2). Forty-three of the described SSRs were trinucleotides, while the rest were dinucleotides (14), tetranucleotides (3), or a pentanucleotide (1). The most common trinucleotide motifs in this set were GAA and TAA (or their equivalents), whereas for the dinucleotides the most common motif was AT. Of all the SSRs assayed, 72% contained perfect repeats, 18% were compound motifs, and 10% had imperfect repeats. None of the 61 SSR-containing sequences shared significant homology with already published SSR sequences.

Fingerprinting analysis

The chosen SSRs were used to fingerprint a panel of 30 potato genotypes (Table 1), most of which were tetraploid cultivars. The fingerprinting panel gave a mean of 6.8 bands per SSR (Table 2), with a range of 1–16 bands per SSR. When considered on a per genotype basis, the mean number of bands per SSR varied from 1 to 6.8, with a mean value of 2.6 bands per genotype. Only three SSRs showed more than the expected four bands for some of the fingerprinted tetraploid genotypes (StI005, StI031, and StI046), suggesting the amplification of more than one locus.

Figure 1 shows an example of the fingerprinting results with StI021. Additional fingerprinting results may be accessed at http://marvin.ibest.uidaho.edu/~jlorenze.

When used for genotype identification, it is useful to determine the number of unique patterns for candidate markers. The number of unique patterns across all fingerprinted genotypes averaged 11.6, ranging from 1 to 24. One measure of informativeness is a DI (Milbourne et al. 1998), which, in this study, varied from 0 (only one pattern for all 30 genotypes) to 0.95. StI005 and StI046 showed the highest values and the highest number of alleles (23 and 24, respectively). The mean DI for the SSRs in this study was 0.81.

The majority of SSRs (84%) showed a size range similar to the expected size, based on the original cDNA sequence used for primer design. In addition to the four candidates that were not included in this study because of very high molecular weight, five of the included SSRs (StI009, StI013, StI014, StI016, and StI050) showed higher-than expected size ranges, suggesting the presence of introns within the amplicons. However, the increased size did not mask the existing allelic variation, and these SSRs were also informative. The band size of four of the markers was not determined because appropriate size markers were not available at the time of analysis.

Map location of SSRs

Of the sixty-one SSRs included in the study, 56 SSRs were mapped to 57 locations distributed across all the 12 potato chromosomes (Fig. 2). The distribution of the markers varied from one for chromosome X to eight for chromosome VI (Fig. 2; Table 2). The mapped markers revealed between one and four segregating alleles (Fig. 3) in agreement with the diploid constitution of the mapping populations used.

Because potato mapping is an example of a pseudo-testcross, independent maps were generated for each parent. Thirty SSRs were located in the map for the interspecific parent of the BCT population (M200-30), and 20 were located in the map for the recurrent *S. tuberosum* parent (HH1-9). Different alleles of 14 of the above markers segregated from each parent; these allelic fragments were assigned similar locations in both parental maps. Similarly, there were 19 SSRs in the M200-30 map for the BCB population, while eight were placed in the map for the recurrent *S. berthaultii* parent (B11B). These included four allelic pairs which each mapped to similar locations for both parents of the BCB population. Two markers, StI039 and StI048, did not

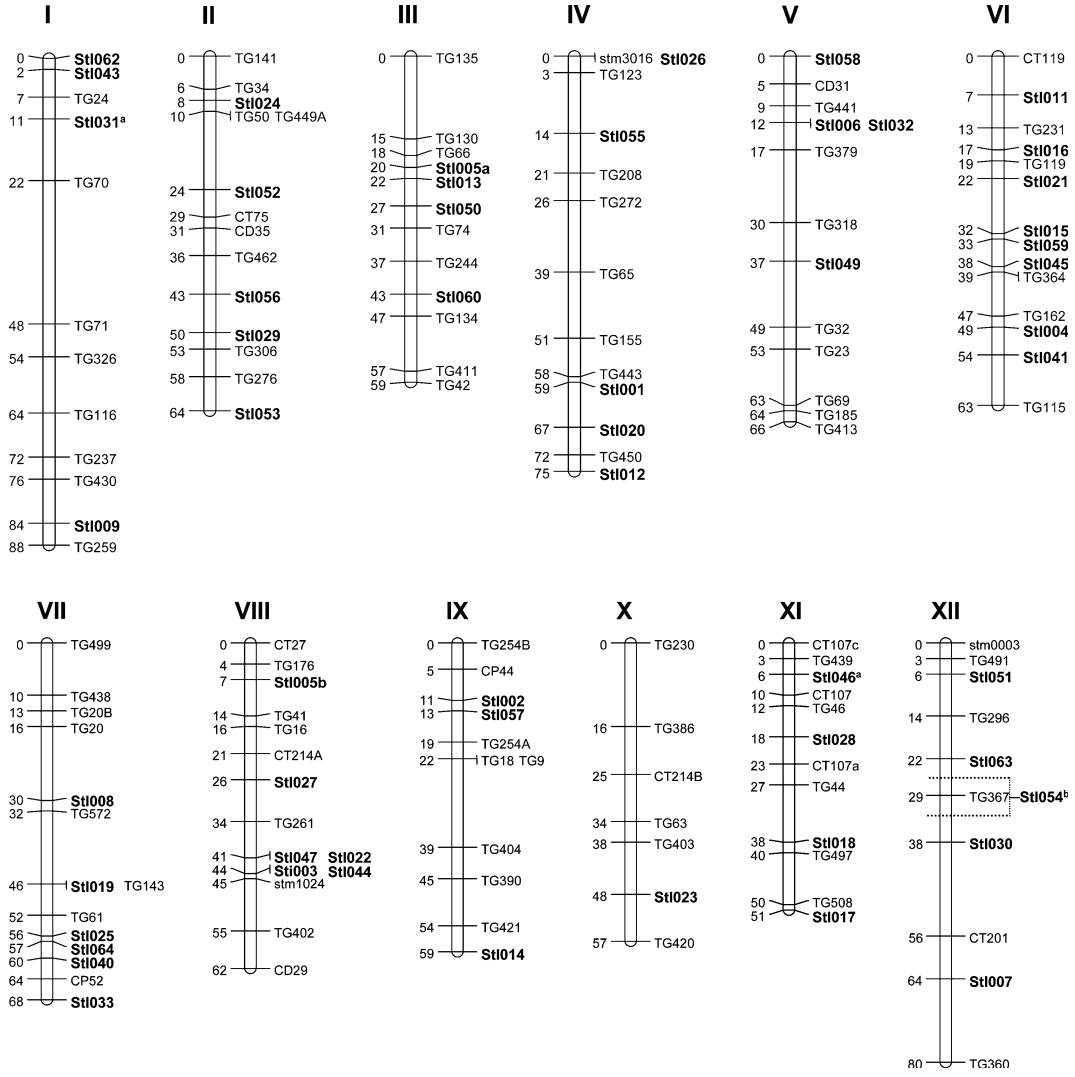

Fig. 2 Location of 55 mapped simple sequence repeats (SSRs) in a joined restriction fragment length polymorphism potato genetic map derived from BCB and BCT populations (Bonierbale et al. 1994). New SSRs are indicated by StI code and are presented in *boldface*. StI039 and StI048 were mapped in chromosomes XI and VIII, respectively, in a different population without common markers to this one. **a** Marker suspected to be multilocus; only one location found in this study. **b** StI054 was linked at 4 cM of TG367 with no additional markers to assess orientation

segregate in the BCB or BCT populations, but were mapped in a different diploid population (Saha 2003). Markers StI042 and StI061 mapped to chromosomes VII and X in the recurrent parent maps for *S. tuberosum* and *S. berthaultii*, respectively, but their exact location could not be assessed unequivocally because of low marker coverage in these maps. Although three markers (StI005, StI031, and StI046) were suspected to be multilocus based on number of bands found by fingerprinting (Table 2), only StI005 showed two map locations (chromosome III and chromosome VIII, Fig. 2).

Discussion

The search of the TIGR Potato Gene Index database (http://www.tigr.org) produced a large number of candidate sequences containing SSRs in potato. Of the 94 sequences that were selected to design primers, a high proportion (85%) gave amplification products in the range of the expected size, and only 7% did not amplify successfully. These results are in agreement with He et al. (2003), who found that 79% of the primers designed based on 139 SSR-containing sequences of Solanaceae (mostly tomato) retrieved from GenBank (mostly expressed sequences) produced the expected DNA fragments for a set of 19 tomato genotypes. Similar success rates were reported for SSRs derived from a cDNA library and an enriched genomic library (Milbourne et al. 1998). One of the concerns in using cDNA sequences for microsatellite development is the unknown final size of the PCR product that could vary depending on the number and size of intron sequences (Scott 2001). Only nine SSR-containing sequences showed larger than expected sizes due to probable introns; five of the nine were still useful (Table 2).

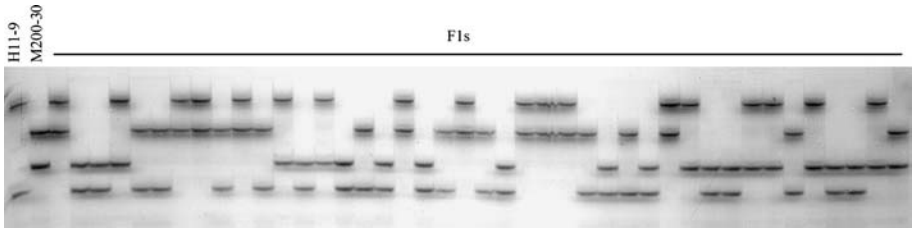

Fig. 3 Segregation of StI051 alleles in diploid population BCT. H11-9 and M200-30, parental genotypes and their derived progeny (F_1s)

Preference in this study was given to trinucleotide SSRs, based on the hypothesis that di-, tetra-, or pentanucleotide variation would produce shifts in the reading frame that would result in negative selection and a lesser degree of polymorphism. However, no differences were observed for the average DI of the two groups (tri- vs non-trinucleotides). An analysis with Framefinder and ESTscan software tools included in the TIGR Potato Gene Index database (http://www.tigr.org/tgi) predicted that most of the 17 non-trinucleotide SSR sequences were located by both tools in the untranslated region (UTR) of the ESTs, and none were unambiguously predicted by both tools to be translated.

Milbourne et al. (1998) also found that all database-derived sequences containing non-trinucleotide SSRs were found either in 3′ or 5′ UTRs, or within introns. Only 12% of the SSR-containing sequences were found in exons, all of which were trinucleotides.

The level of polymorphism of the SSRs reported in this work was high. All but one SSR amplicon showed multiple patterns among the 30 *Solanum* genotypes that were surveyed. The mean DI was 0.81, and the mean number of bands observed was 6.8 per SSR. There was no apparent association between the DI values and the motif size of the repeat, nor between imperfect/compound SSR motifs compared to perfect repeats. Milbourne et al. (1997) reported similar values (DI: 0.73, mean bands per SSR: 5.7) when screening a panel of 16 potato cultivars, although Ashkenazi et al. (2001) reported relatively little diversity in potato SSRs. However, the lower figures reported in that study might be attributed to lower genetic diversity in the chosen genotypes or simply the smaller size of the chosen subset.

Only StI062 was monomorphic across the 27 genotypes in the fingerprinting panel (except for three genotypes that did not give any product). Although there was no diversity for this marker across these mostly tetraploid genotypes, the interspecific parent M200-30 of the BCT population was heterozygous, allowing the genetic location to be assessed.

Some markers displayed a reduced DI (e.g., StI040), and were characterized by a main band and weak allelic bands, or no amplification in some genotypes. This may indicate that primers were complementary to a variable region and could be allele-specific. Re-design of such primers could give a more robust assay with a more normal DI.

Three different *Solanum* species were included in this study: *S. tuberosum* (ssp. *tuberosum*, ssp. *andigena*), *S. berthaultii*, and *S. phureja* (Table 1; Figs. 1, 2). All primers reported in Table 2 showed amplification across species. The diploid genotypes represented in the fingerprinting analysis and the mapping populations generally had fewer bands per amplification than the tetraploid genotypes (data not shown).

One of the reported advantages of cDNA based SSRs is their transferability across the species, in the belief that DNA sequence complementary to primers would be more conserved in transcribed regions versus random or nongenic regions (Scott et al. 2000). In this respect, Ashkenazi et al. (2001) reported that only half of tomato genomic library-derived SSRs gave amplification products with potato DNA as template. Rossetto (2001) analyzed transferability of SSRs in different species (ranging from *Paspalum* sp. to *Pinus* sp.) and showed that polymorphism rates are high when priming sites are conserved, even between evolutionary distant species, but decrease beyond genus taxa. The SSRs developed from *Solanum tuberosum* ssp. *tuberosum* L. in this study will likely be very useful in other related species.

Most mapped markers were monolocus in this combined map. Only StI005 mapped to two different loci, on chromosomes III and VIII. This marker also had more than the four expected bands for many of the cultivars of the fingerprinting panel. StI031 and StI046 also presented a mean number of expected bands (alleles) of four or higher (Table 2), but mapped to single genetic locations (Fig. 2). StI031 showed only two alleles per genotype in the segregating population, but StI046, presented up to seven discrete bands in some diploid genotypes. Many of these bands cosegregated. The available data do not allow us to differentiate whether these linked amplified alleles corresponded to different closely linked loci or to intragenic tandem repeats of priming sites (Milbourne et al. 1998). All other SSR markers reported in this study are believed to be unilocus, and the location of ~25% of them was confirmed by mapping in two different maps. In a previous report, a substantial proportion of potato SSRs (20%) were multilocus (Milbourne et al. 1998), and the number of multilocus SSRs in that set has been subsequently expanded (Ghislain et al. 2004).

Approximately 100 potato SSRs have been previously mapped (Kawchuk et al. 1996; Provan et al. 1996; Milbourne et al. 1998; Ghislain et al. 2004). Thus, the 57

additional mapped SSRs presented here represent a >50% increase in the number of mapped potato SSRs. In our hands, the quality of these new SSRs is also very high, comparable to the best of the previously reported SSRs (Milbourne et al. 1998).

With the exception of chromosome X, where only one marker was mapped, SSRs were distributed relatively homogeneously across chromosomes (Fig. 2). Small clusters of SSRs were found on top of chromosome I, and in proximal regions of chromosomes III, VI, VII, and VIII. Despite being closely linked, these markers should not be considered redundant, because greater map density increases the chances of finding usable polymorphisms for a region in a given population. The present study complements the Milbourne et al. (1998) study on potato SSRs, at least doubling the number of markers for 5 of the 12 potato chromosomes. Interestingly, the SSR density on chromosome X was very low in both studies.

Many recent mapping efforts utilize the AFLP technique (Vos et al. 1995), which generates a high number of anonymous markers per assay. Microsatellites are useful "anchor" markers in such studies to relate anonymous AFLP linkage groups to known chromosomes. Multilocus markers are not ideal for such studies because of their ambiguity. The construction of consensus maps is based on the existence of unequivocal common markers. Co-migrating AFLPs can be used for this task (Rouppe van der Voort et al. 1998) but require very accurate fragment sizing and depend on segregation of previously mapped fragments in the target population. The high proportion of single-locus SSRs presented here will facilitate their use as anchor markers for identifying linkage groups and comparative mapping across related species.

Microsatellites are especially useful for mapping in tetraploid potato, because they are co-dominant and allow the identification of homologous linkage groups of a given chromosome (Meyer et al. 1998). Different SSR alleles from a given locus can be combined to make an artificial duplex locus, useful in duplex–simplex mapping (Meyer et al. 1998).

Tagging of agronomically important traits is one of the main applications of markers, both for understanding the genetic base of a trait and for marker-assisted selection in breeding programs. In this respect, the cDNA-based markers developed and characterized in this study will not only provide reproducible co-dominant, easily scored markers, but also may include candidate genes that have the potential of being causally linked to the trait of interest. In this respect, these SSRs can be directly included in "functional maps," based on the putative function of the clones they have been generated from (Table 3) and may be helpful to understand a causal-effect relationship in co-located, previously mapped QTLs.

Development of SSRs has become much more convenient with the advent of EST databases and bioinformatics tools. A high proportion of SSRs tested amplified products of the expected size range that were polymorphic among cultivated potato genotypes and segregating diploid populations. One caveat regarding design should be mentioned. Size variants of several SSRs were not placed in the same contigs in the TIGR database (e.g., StI035 and StI044). This highlights the need to compare chosen target sequences against existing and intended target sequences to minimize redundancy.

Since the advent of these studies, two new resources have become available for assisting in the development of potato/solanaceous SSR markers. The Solanaceae Genomics Network [(SGN) http://www.sgn.cornell.edu/cgi-bin/legacy/microsats_top.pl] currently lists 609 SSR sequences with suggested primer combinations based on tomato and potato sequences, with map locations for about 15% of those SSRs. However, some map locations were determined by hybridization, and degree of polymorphism in potato remains to be determined. An additional resource (http://hornbill.cspp.latrobe.edu.au/ssrresults/potato/) lists potato SSR-containing ESTs and suggested primer combinations. However, these ESTs were not first organized into contigs as for the TIGR database utilized in this study or the SGN resource, and would require additional processing to eliminate redundancy before commencing validation with that resource. On the other hand, a recent search of the version 9.0 Potato Gene Index database, which was based upon an additional 63,000 ESTs compared to version 7.0 and has 34% more "unique" sequences (TCs + singletons) than version 7.0, gave 896 sequences with a Sputnik score ≥15, and 1,223 sequences with a score ≥14 (61% and 52% more than version 7.0, respectively). Many of these sequences can be used as a source for future SSRs.

In conclusion, this study reports on the development and mapping of new potato SSRs. Most of these are of very high quality and will be valuable for characterization of genetic diversity, fingerprinting tetraploid potato cultivars, mapping, and synteny studies within the Solanaceae.

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