An Applied Fingerprinting System for Cultivated Potato Using Simple Sequence Repeats

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ABSTRACT

The ability to quickly and accurately identify potato (*Solanum tuberosum* L.) clones is important to potato-breeding programs, seed and commercial potato growers, and marketing and utilization of potato cultivars. Since 1990, the Michigan State University Potato Breeding and Genetics Program has used an isozyme-based fingerprinting system to identify potato cultivars. Isozyme analysis is an economical and effective means of discriminating potato clones; however, isozyme analysis requires fresh, healthy tuber or leaf tissue. DNA-based fingerprinting using simple sequence repeats (SSRs or microsatellites) has been shown to discriminate between potato clones. The objective of this study was to identify the most useful SSR primer pairs that accurately and efficiently distinguish clones for an applied fingerprinting system of cultivated potato. SSR primer pairs with high polymorphism were selected from previous tetraploid potato studies. DNA isolated from 17 potato clones representing round-white, russet, and red market classes were visualized on both polyacrylamide (PAGE) and agarose gel systems. Polymorphism was observed in all 18 primer combinations on PAGE and 14 using agarose gel electrophoresis. All 17 cultivars were discriminated on PAGE with various combinations of two primer pairs: STIIKA using STACCAS3, STINHWI, or STM0031; and STACCAS3 using STGBSS1, POTM1-2, STM1104, or STM0031. The combination of STM0019, STM0031, STGBSS1, and POTM1-2 was able to differentiate all 17 clones using agarose gel electrophoresis. PAGE was determined to be the preferred system for variety identification, but agarose gel electrophoresis can be used to differentiate lines when specific varietal comparisons are needed. In addition, five different DNA source tissue types were evaluated (fresh foliar, freeze-dried foliar, fresh tuber, freeze-dried tuber epidermis, and freeze-dried tuber tissue). Amplification products were similar for all five tissue sources used for DNA isolation. This ability to isolate DNA from freeze-dried tissue will allow cultivar identification when fresh tissue is not available. The SSR primer pairs presented here can be used as a practical fingerprinting system for cultivated potato identification.

RESUMEN

La posibilidad de identificación rápida y exacta de clones de papa (*Solanum tuberosum* L.), es importante para los programas de mejoramiento, para los productores de semilla y papa de consumo, lo mismo que para la comercialización y utilización de los cultivares de papa. Desde 1990, el Programa de Mejoramiento y Genética de la Universidad del Estado de Michigan ha utilizado un sistema de tipificación genética para identificar cultivares de papa basado en isoenzimas. El análisis de isoenzimas es un método económico y efectivo para diferenciar clones de papa. Sin embargo, este análisis requiere de tejido fresco y sano de hoja o de tubérculo. Con la tipificación genética basada en el uso de ADN y utilizando repeticiones de secuencias simples (SSRs o microsatélites) se ha demostrado la existencia de diferencias entre clones de papa. El objetivo del pre-

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ABBREVIATIONS: polymerase chain reaction (PCR), simple sequence repeats (SSR), polyacrylimide gel electrophoresis (PAGE)

sente estudio fue identificar los pares de iniciadores SSR más útiles para distinguir clones de papa cultivada en forma precisa y eficiente por medio de un sistema de tipificación genética aplicada. A partir de estudios previos de papa tetraploide se seleccionaron pares de iniciadores SSR con polimorfismo amplio. El ADN aislado de 17 clones de papa representantes de las clases comerciales redondo blanco, rosado y rojo se hizo evidente tanto en el sistema de poliacrilamida (PAGE) como en el de gel de agarosa. Con el sistema PAGE se observó polimorfismo en todas las combinaciones de los 18 iniciadores, y en 14 cuando se utilizó electroforesis en gel de agarosa. Los 17 cultivares fueron diferenciados en PAGE con varias combinaciones de dos pares de iniciadores: STIKA utilizando STACCAS3, STINHWI o STM0031 y STACCAS utilizando STGBSS1, POTM1-2, STM1104, o STM0031. La combinación de STM0019, STM0031, STGBSS1 y POTM1-2 fue capaz de diferenciar los 17 clones utilizando electroforesis en gel de agarosa. Se estableció que PAGE es el sistema preferido para la identificación de variedades, pero se puede utilizar la electroforesis en gel de agarosa para diferenciar lineas, cuando se necesitan comparaciones varietales específicas. Además se evaluaron cinco fuentes de tejido (foliar fresco, foliar seco congelado, tubérculo fresco, epidermis de tubérculo seco congelado, tubérculo seco congelado). Los productos de amplificación fueron similares para las cinco fuentes de tejido para el aislamiento de ADN. Esta facultad para aislar ADN de tejido seco congelado permitirá la identificación de cultivares, cuando no se disponga de tejido fresco. Los pares del iniciador SSR presentados aquí se pueden utilizar como un sistema práctico de tipificación genética para la identificación de la papa cultivada.

INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) is the fourth most important food crop worldwide, and the top vegetable crop in the U.S. Currently, there are more than 3,200 different potato varieties that are cultivated in over 100 countries worldwide (Hamester and Hils 2003). Morphological characteristics such as plant architecture, flower color, disease reaction, and sprout and tuber type have traditionally been used to distinguish potato cultivars. These traits can be subject to environmental influence and cannot be quickly and efficiently scored. The ability to discriminate and identify unique potato clones is important to potato-breeding programs, certified potato seed programs, commercial potato growers, and increasingly necessary with intellectual property rights and other disputes of ownership and trademark issues.

Since 1990, the Michigan State University Potato Breeding and Genetics Program has used isozyme analysis to identify potato clones (Douches and Ludlam 1991). Within the past five years alone, over 1,800 samples have been analyzed (Douches unpublished data). Isozyme analysis is an economical and effective means of discriminating potato clones; however, it requires fresh, healthy tuber or leaf tissue. The limitations requiring the use of fresh tissue for isozyme fingerprinting are removed by using a DNA-based system. DNA can be isolated from various fresh and freeze-dried tissue sources and requires little source material. Freeze-dried tissue samples and isolated DNA samples can also be archived for extended storage. Freedom from fresh tissue also facilitates transportation of material across country borders that can be restricted due to phytosanitary regulations.

Simple sequence repeats (SSRs or microsatellites) are tandemly repeated short oligonucleotide sequences that are flanked by conserved DNA sequences. DNA-based fingerprinting using SSRs has been well established to effectively discriminate between tetraploid potato clones (Kawchuk et al. 1996; Provan et al. 1996; Schneider and Douches 1997; Ghislain et al. 2000; McGregor et al. 2000; Ashkenazi et al. 2001). SSRs can provide a reliable, efficient, and applied DNA-based fingerprinting system for potato. The objectives of this study were (1) to evaluate previously described SSR primer pairs to distinguish a panel of 17 North American potato clones representing three market-classes using both PAGE and agarose gel electrophoresis and (2) to evaluate the effect of DNA tissue source on SSR amplification products.

MATERIALS AND METHODS

Potato Clones

A panel of 17 potato clones of commercial importance, representing three different market classes, was used in this study (Table 1). The clones were grouped according to their market class (round white, russet, and red-skin).

TABLE 1—*Potato cultivars used for SSR variety identification, listed by market class.*

Potato Cultivar	Pedigree	Year of Introduction	Market-class
Atlantic	Lenape x Wauseon	1976	Round White
NorValley	Norchip x ND860-2	1997	Round White
Norwis	RD289-18 x Monona	1990	Round White
Onaway	USDA96-56 x Katahdin	1956	Round White
Pike	Allegany x Atlantic	1995	Round White
Proprietary 1	NA†	NA	Round White
Proprietary 2	NA	NA	Round White
Proprietary 3	NA	NA	Round White
Snowden	Lenape x Wischip	NA	Round White
Superior	USDA96-56 x M59.44	1961	Round White
MSE192-8RUS	A8163-8 x Russet Norkotah	NA	Russet
Goldrush	ND450-3Rus x Lemhi Russet	1992	Russet
Ranger Russet	Butte x A6595-3	1990	Russet
Russet Burbank	Sport of Burbank	1914	Russet
Russet Norkotah	ND9526-4Rus x ND9687-5	1987	Russet
Norland	Red Kote x ND626	1957	Red-skin
Red Pontiac	Triumph x Katahdin	1945	Red-skin

†NA: Not Applicable.

Tissue Preparation and DNA Isolation

Tuber and leaf tissue were collected from field-grown plants, under standard management practices during the 2002 season at Michigan State University Montcalm Research Farm (Entrican, MI) or the Michigan State University Soils Farm (East Lansing, MI). DNA was isolated from five different tissue sources: fresh foliar, fresh tuber, freeze-dried foliar, freeze-dried tuber epidermis, and freeze-dried tuber. All tissue samples were collected from a single plant for each variety. Foliar tissue was collected from plants at flowering and used for fresh and freeze-dried tissue. DNA was isolated immediately from fresh tissue. Tubers were harvested for fresh and freeze-dried tuber tissue epidermis. For freeze-drying, tissue was placed in 50-mL polypropylene tubes (Corning, NY) and frozen at –80 C for a minimum of 8 h before lyophilizing (Virtis Genesis 12EL, Biopharma Process Systems Ltd., Winchester, UK). Freeze-dried tissue was shaken to a fine powder using a modified paint mixer (Wolfe Machinery Co., Johnston, IA) in which glass beads (4 mm) were added prior to shaking. DNA was isolated from all tissues using the Qiagen DNeasy Plant Mini Kit as per manufacturer's instructions (Qiagen, Valencia, CA) and eluted with 50 µL of TE buffer, which yielded approximately 25 ng of DNA.

SSR Methodology

SSRs were selected from previous studies that identified SSRs that showed high levels of polymorphism in tetraploid potato (Kawchuk et al. 1996; Provan et al. 1996; Milbourne et al. 1998; Ghislain et al. 2000; McGregor et al. 2000; Ashkenazi et al. 2001). Table 2 lists the 18 primer pairs used in this study. A 10- and/or 50-bp ladder (Invitrogen, Carlsbad, CA) was used to estimate amplified fragment sizes. The primer combinations were clustered to run at annealing temperatures of 50 C, 55 C or 60 C (Table 2). All chemicals and products used were purchased from Sigma (St. Louis, MO) unless noted otherwise.

For PAGE, PCR components for 20 µL reactions consisted of PCR reaction buffer, 50mM $MgCl_2$, 0.25 units TaqDNA polymerase, 0.5 mM dNTP mixture (Gibco BRL, Carlsbad, CA), and 2-ng template DNA. Reaction conditions were as follows: 94 C for 1 min, followed by 45 cycles of 30 sec at 94 C, 45 sec at the appropriate annealing temperature (Table 2), extension for 45 sec at 72 C, and a final extension for 5 min at 72 C, and maintained at 4 C using a PTC-100 thermocycler (MJ Research, Inc., Waltham, MA). Preceding electrophoresis on polyacrylamide, 20 µL of formamide loading buffer (98% formamide, 10 mM EDTA (pH 8.0), 1 mg mL^{-1} bromphenol blue, 1 mg mL^{-1} xylene cyanol) was added to each reaction and denatured for 10 min at 94 C. Reaction products were electrophoresed on vertical denaturing 5% polyacrylamide gels (34.5 x 50 cm) for 2.5 h at 90 Watts in a Sequi-Gen® GT Sequencing Cell (Bio-Rad, Richmond, VA) and stained with Silver Sequence™ (Promega, Madison, WI) following manufacturer's instructions. Silver-stained gels were visualized on a fluorescent light box and manually scored.

TABLE 2—*Primer information of microsatellites used for tetraploid potato variety identification.*

Primer/locus	Sequence (5'-3')	Repeat Motif	Annealing Temp (°C)	Fragment Size (bp) PAGE	No. of Fragments PAGE	No. of Fragments Agarose
POTM 1-2[a]	AATAATACTGTGATGCCACAATGG GTGGCATGTCTTCGAAGGTAC	$(AT)_{20}$	60	200-246	5	4
ST13ST[ae]	GTGATTGGCAATCAGATTGAAA GTGTGTGGACTGTGGAGTGG	$(AT)_{11}$	60	200-212	3	2
STACCAS3[a]	AATTCATGTTTGCGGTACGTC ATGCAGAAAGATGTCAAAATTGA	$(AAG)_7$	60	252-2000	12	7
STGBSS1[c]	ATTCGGTGATAAATGTGAATGC ATGCTTGCCATGTGATGTGT	$(TCT)_9$	55	144-148	5	4
STGBSS2[a]	TTATGAATCGTGTTATGG GAAAAAGGGGAATCTACC	$(TCT)_9$	50	192-220	4	4
STIIKA[e]	TTCGTTGCTTACCTACTA CCCAAGATTACCACATTC	$(T)_{12}$ $(A)_9$ ATTCTTGTT $(TA)_2$ CA $(TA)_7$	50	400-490	9	6
STINHWI[e]	GGAGTCAAAGTTTGCTCACATC CACCCTCAACCCCCATATC	$(CT)_3$ TT$(CT)_8$ $(AT)_9$	60	356-420	4	3
STM0019[bc]	AATAGGTGTACTGACTCTCAATG TTGAAGTAAAAGTCCTAFRATGTG	$(AT)_7$ $(GT)_{10}$ $(AT)_4$ $(GT)_5$ $(GC)_4$ $(GT)_4$	50	170-210	4	4
STM0031[bc]	CATACGCACGCACGTACAC TTCAACCTATCATTTTGTGAGTCG	$(AC)_5$... $(AC)_3$ (GCAC) $(AC)_2$ $(GCAC)_2$	55	80-230	8	6
STM0037[bc]	AATTTAACTTAGAAGATTAGTCTC ATTTGGTTGGGTATGATA	$(TC)_5$ $(AC)_6$ AA $(AC)_7$ $(AT)_4$	55	90-108	6	3
STM1104[bc]	TGATTCTCTTGCCTACTGTAATCG CAAAGTGGTGTGAAGCTGTGA	$(TCT)_5$	60	163-186	9	4
STM2030[bc]	TCTTCCCAAATCTAGAATACATGC AAAGTTAGCARGGACAGCATTTC	$(CA)_3$ $(TA)_5$	55	175-250	2	2
STM3012[bc]	CAACTCAAACCAGAAGGCAAA GAGAAATGGGCACAAAAAACA	$(CT)_4$... $(CT)_8$	60	184-210	3	3
STPRINPSG[e]	TGTACTGGGGAGCCTCAAAG AATTTTAACCTCGTGACATGGG	$(TA)_{23}$	60	157-170	3	3
STS 1[d]	TCTCTTGACACGTGTCACTGAAAC					
STS 2[d]	TCACCGATTACAGTAGGCAAGAGA		55	248-262	5	2
STS 3[d]	TTGCCATGTGATGTGTGGTCTAGAA		55	336-388	7	2
STU6SNRN[e]	GAAGTTTTATCAGAATCC ATCACCTCATCAGCAATC	$(TGG)_5$	50	218-246	12	4
STWIN12G[e]	TGTTGATTGTGGTGATAA TGTTGGACGTGACTTGTA	$(TGAAA)_2$ $(ATA)_6$	50	167-180	5	3
Mean					5.9	3.7
Range					2-12	2-7

Reference literature cited:
[a]Ashkenazi, et al. 2001; [b]Ghislain et al. 2000; [c]Milbourne et al. 1998; [d]McGregor et al. 2000; [e]Provan et al. 1996.
Primer/locis, sequence, and repeat motif acquired from published literature (see above).

For agarose gel electrophoresis, PCR components consisted of 20-µL reactions containing RedTaq PCR reaction buffer, 0.3 units REDTaq DNA polymerase, 0.5 mM dNTP mixture (Gibco BRL, Carlsbad, CA) and 2 ng template DNA. PCR conditions were as previously stated. Reaction products were electrophoresed on horizontal 3% low melting temperature MetaPhor® (BMA, Rockland, ME) agarose gels (20.5 cm^2) using 1X Tris-borate-EDTA buffer (Sambrook and Russell 2001). Electrophoresis was conducted at 4 C for 4 h at 100 volts and bands were visualized with ultraviolet light (254 nm) using Eagle Eye™ II (Stratagene®, La Jolla, CA). The SSR amplified products, both polymorphic and monomorphic fragments, were scored as 1 or 0 for band presence or absence. Each SSR reaction was replicated a minimum of three times, and a consensus was generated to reflect bands that were consistently reproducible. Data were analyzed using NTSYSpc Version 2.11L (Rohlf 2002) to generate genetic similarity coefficients (Nei and Li 1979). Dendrograms were drawn from unweighted pair-group method, arithmetic average (UPGMA) cluster analysis using NTSYSpc (Rohlf 2002).

RESULTS

The DNA amplification patterns of 18 SSR primer combinations were evaluated using both PAGE and agarose gel electrophoresis. Polymorphism was observed with 14 of the SSRs using agarose gel electrophoresis and with all 18 SSRs using PAGE. Table 2 summarizes the SSR primer sequences, repeat motifs, DNA fragment size and number for both PAGE and agarose gel electrophoresis. PAGE resolved between 2-12 DNA fragments, while agarose gel electrophoresis resolved between 2-7 fragments. Figure 1 illustrates the greater DNA band resolution of PAGE compared to agarose gel electrophoresis.

The panel of 17 cultivars was uniquely identified using PAGE and agarose gel electrophoresis. A dendrogram was generated based upon 106 bands from the 18 primer pairs (Figure 2). The closest two cultivars based upon genetic similarity were 'Atlantic' and a proprietary chip-processing cultivar (0.90), while cultivars 'NorValley' and 'Pike' were the most distinct (0.70). No single SSR primer pair discriminated the panel of 17 cultivars using PAGE or agarose gel electrophoresis. All 17 cultivars were discriminated on PAGE with various combinations of two primer pairs: STIIKA using STACCAS3, STINHWI, or STM0031; and STACCAS3 using STGBSS1, POTM1-2, STM1104, or STM0031. The combination of STM0019, STM0031, STGBSS1, and POTM1-2 was able to differentiate all 17 clones using agarose gel electrophoresis.

PCR amplification products were compared from template DNA isolated from five different tissue sources (fresh foliar, fresh tuber, freeze-dried foliar, freeze-dried tuber epidermis, and freeze-dried tuber). DNA amplification fragment patterns were identical for all five tissue sources used for DNA isolation for all 18 SSR primer pairs for each clone evaluated. Figure 3 illustrates these results.

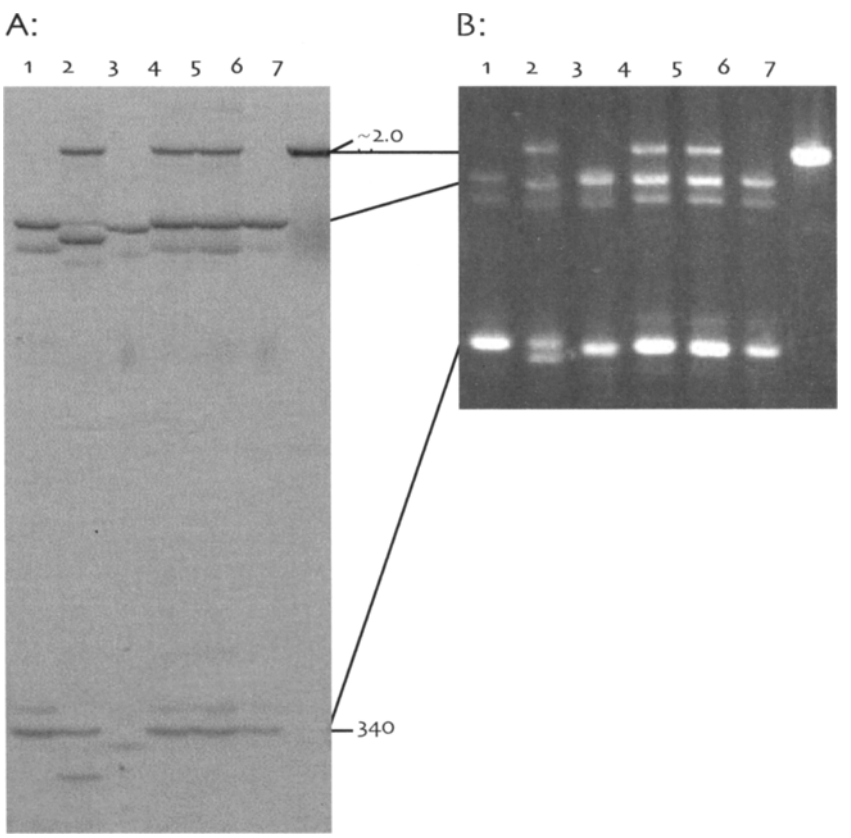

FIGURE 1.
SSR Primer STACCAS3 amplification fragments of seven potato clones visualized on (A) PAGE and (B) agarose gel electrophoresis. List of clones (left to right) include 1: Atlantic; 2: NorValley; 3: Pike; 4: Snowden; 5: Proprietary1; 6: Proprietary2; 7: Proprietary3.

DISCUSSION

Fingerprinting potato cultivars is important to potato-breeding programs, certified potato seed programs, commercial potato growers, and increasingly necessary with intellectual property rights, and other disputes of ownership and trademark issues. For marker-based cultivar identification to be useful and practical, it must be rapid, reliable, and efficient. The vegetative mode of propagation of cultivated potato allows for fixed heterozygosity at marker loci. In addition, the high levels of heterozygosity of tetraploid cultivated potato, makes genetic markers ideal for fingerprinting.

Kawchuk et al. (1996), Provan et al. (1996), Schneider and Douches (1997), McGregor et al. (2000), Ghislain et al. (2000), and Ashkenazi et al. (2001) explored SSR-based marker systems for potato. SSRs are usually species specific, costly to develop, and require prior sequence information. The initial expense of SSRs is reduced when the SSR primer pairs have been previously identified.

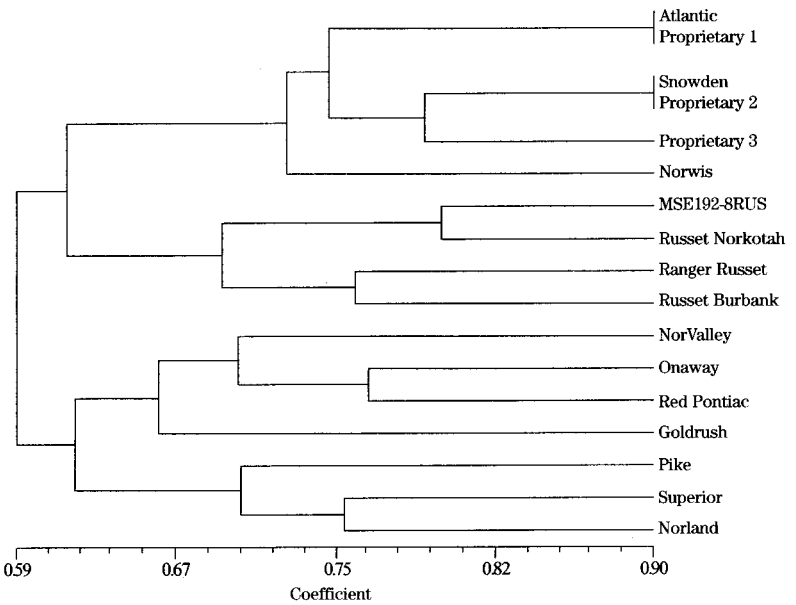

FIGURE 2.
Dendrogram of seventeen potato clones generated using 106 bands from 18 SSR primer pairs.

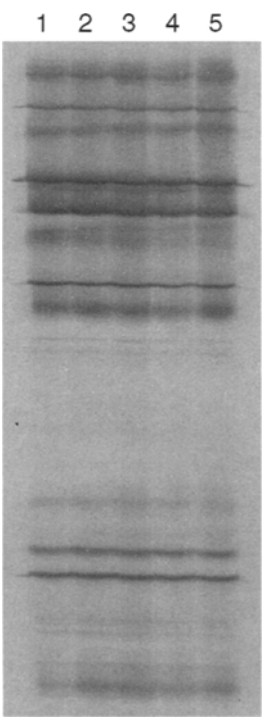

FIGURE 3.
Amplification of STACCAS3 from Norland comparing DNA isolated from five tissue type sources on polyacrylamide gel system. DNA tissue source in lanes (left to right) are 1: fresh foliar; 2: freeze-dried foliar; 3: fresh tuber; 4: freeze-dried tuber; 5: freeze-dried tuber epidermis.

Today, SSRs are attractive for fingerprinting potato cultivars and developing genetic maps because of their abundance and relatively simple technical requirements. Additionally, DNA fingerprinting samples could be archived for extended periods of time. RFLPs are limited by the need for large amounts of DNA and require several days to obtain results (Gebhardt et al. 1989); and RAPDs lack desired stringency (Demeke et al. 1993). Because of site-specific amplification of SSRs it is unlikely that amplification will be influenced by contaminating DNA from non-solanaceous sources. AFLPs have better discriminatory power, but are technically more demanding and require more time and higher costs to obtain results (McGregor et al. 2000).

This study examined highly polymorphic SSR primer pairs identified for potato on both PAGE and agarose gel electrophoresis with a panel of commercially important potato cultivars in the United States. SSR fragments were visualized on both PAGE and agarose gel electrophoresis. Agarose gel electrophoresis is technically less demanding and less expensive, and the panel of 17 cultivars was distinguished using four primer pairs. However, PAGE resolved much greater levels of polymorphism with many of the SSR primer pairs, distinguishing the 17 cultivars with various combinations of two primer pairs. The polymorphism observed on PAGE outweighs the simplicity and cost-savings of agarose gel electrophoresis.

The SSR primer pairs used in this study were rated by ease of readability (band clarity and intensity) and level of polymorphism. The most readable primer pairs were STIIKA, STGBSS1, STGBSS2, POTM1-2, and STM0019. The most polymorphic primer pairs were STACCAS3, STIIKA, STM0031, POTM1-2, and STU6SNRN. Considering ease of readability, level of polymorphism, and reproducibility, the best overall primer pairs were STIIKA, STACCAS3, POTM1-2, and STM1104. Provan et al. (1996) was able to discriminate their sample of 18 cultivars with STIIKA alone. McGregor et al. (2000) discriminated 35 of 37 cultivars using STIIKA and STS1+2. In addition, McGregor et al. (2000) noted that STIIKA gave rise to complex fragments with 11 different alleles and 30 different genotypes, while STS1+2 was their best single locus

primer pair. Ghislain et al. (2000) was able to discriminate 20 cultivars using STM0019 in combination with STM1104. Ashkenazi et al. (2001) reported an allelic range of three to six and were able to separate the 12 cultivars with a minimum of two primer pairs (e.g. STACCAS3 + ST13ST). Kawchuk et al. (1996) was able to distinguish 73 of 95 cultivars using a combination of STS1+2, STS1+3, and an SSR associated with Protein Inhibitor I.

The panel of 17 cultivars used in this study was discriminated by 18 primer pairs yielding 106 bands and a dendrogram was generated (Figure 2). Genetic similarity coefficients were calculated and all cultivars were distinct, with a closest genetic similarity coefficient of 0.90. Grouping of cultivars occurred roughly corresponding to market class. Four of the five russet clones grouped closely. MSE192-8RUS, a progeny of 'Russet Norkotah', grouped most closely to Russet Norkotah. Six of the nine round-white cultivars, which clustered together, are used for chip-processing. 'Atlantic' and 'Snowden' have a common parent (i.e., 'Lenape'). 'Pike' and 'NorValley' did not cluster with the other chip-processing cultivars possibly due to the introgression of South America germplasm in their pedigrees. Furthermore, 'Goldrush' and 'Red Pontiac' may have grouped together because of the use of red-skinned germplasm in 'Lemhi Russet' (paternal parent of Goldrush).

The isozyme-based fingerprinting system of Douches and Ludlam (1991) has been effective in distinguishing sexually derived potato cultivars. Due to the limited number of scorable isozyme loci, differences between clonal sports and line selections are not observed. With DNA-based markers, it may be feasible to discriminate line selections of a cultivar. However, Kawchuk et al. (1996) did not find differences between seven Russet Burbank clonal variants. Examination of five line selections of Russet Norkotah using 75 primer pairs (including the 18 primer pairs used in this study) and yielding over 400 scorable bands revealed no differences between these line selections (Douches, data not shown). SSRs are known to have high levels of polymorphism among sexually derived populations due to recombination-driven polymorphism. Therefore, SSRs can easily substitute for isozymes in discriminating sexually derived clones, but have the similar limitation to isozymes in not discriminating clonal line selections.

Isozyme fingerprint analysis is limited by the requirement of fresh, healthy plant tissue. Fresh tissue is not a limitation of DNA-based SSR fingerprinting. DNA may be isolated from many tissue sources, including fresh and freeze-dried samples. SSR amplification was compared across five different DNA tissue sources. Across all cultivars and all primer pairs evaluated, no differences were attributed to DNA source tissue type. Because of phytosanitary concerns, fresh tuber or leaf tissue cannot be quickly or easily transported across country borders. SSR fingerprinting could be conducted on DNA isolated outside the country or from freeze-dried tissue. A defined fingerprinting system will also provide a legal basis for SSR-based fingerprinting analysis of potato. Although SSRs are more expensive and take longer (2 days compared to 1 day), the discrimination strength and advantages of using a DNA-based fingerprinting system may prove to be more practical in the long term.

The objective of this research was to develop a rapid, efficient, and reliable DNA-based fingerprinting system. A subset of SSR primer pairs have been identified that effectively discriminated 17 important North American potato cultivars using DNA isolated from fresh and freeze-dried tissue sources, and visualized on PAGE. All fingerprinting systems have advantages and disadvantages. The advantages of SSRs that are discussed above make them a prime candidate for fingerprinting cultivated potato.

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LITERATURE CITED

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

Demeke T, LM Kawchuk, and DR Lynch. 1993. Identification of potato cultivars and clonal variants by random amplified polymorphic DNA analysis. Am Potato J 70:561-570.

Douches DS, and K Ludlam. 1991. Electrophoretic characterization of North American potato cultivars. Am Potato J 68:766-780.

Gebhardt C, C Blomendahl, U Schachtschabel, T Debener, F Salamini, and E Ritter. 1989. Identification of 2n breeding lines and 4n potato varities (*Solanum tuberosum* ssp. *tuberosum*) with RFLP-fingerprints. Theor Appl Genet 78:16-22.

Ghislain M, F Rodríguez, F Villamón, J Núñez, R Waugh, and M Bonierbale. 2000. Establishment of microsatellite assays for potato genetic identification. Research on Potato. CIP Program Report 1999-2000. pp. 167-174.

Hamester W, and U Hils (eds). 2003. World Catalogue of Potato Varieties. Que Pub., Indianapolis, IN.

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

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

Milbourne D, RC Meyer, AJ Collins, LD Ramsay, C Gebhardt, and R Waugh. 1998. Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233-245.

Nei M, and WH Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269-5273.

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

Rohlf FJ. 2002. NTSYSpc: Numerical Taxonomy System, ver. 2.11L. Exeter Publishing, Ltd., Setauket, NY.

Sambrook J, and D Russell. 2001. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schneider K, and DS Douches. 1997. Assessment of PCR-based simple sequence repeats to fingerprint North American potato cultivars. Am Potato J 74:149-164.