Am. J. Pot Res (2009) 86:38–48
DOI 10.1007/s12230-008-9059-6

Genetic Diversity of Canadian and Exotic Potato Germplasm Revealed by Simple Sequence Repeat Markers

Yong-Bi Fu · Gregory W. Peterson · Ken W. Richards · T. Richard Tarn · Jane E. Percy

Published online: 5 December 2008

Abstract Canadian potato germplasm (*Solanum tuberosum* L.) is unique in its geographic and climatic ranges of adaptation, but little is known about the genetic diversity of the improved *Solanum* gene pool established over the past century. Simple sequence repeat (SSR) markers were applied to assess the genetic diversity of 114 Canadian and 55 exotic potato accessions. Thirty-six SSR primer pairs were applied and 232 polymorphic bands were scored for each accession. The frequencies of polymorphic bands ranged from 0.01 to 0.98 and averaged 0.35. The proportion of total SSR variation occurring between Canadian and exotic germplasm was 0.6%; among the Canadian cultivars of four major breeding periods 2.7%; among heirloom varieties, modern cultivars and elite breeding lines 4%; and between tetraploid and diploid lines 3.7%. Slightly more diversity was found for exotic, than the Canadian, germplasm. The modern cultivars displayed slightly more diversity than the heirloom varieties and the early cultivars revealed slightly more variation than the recent ones. Clustering 169 accessions revealed more than ten groups, but the groups were not distantly separated. Both the genetically most distinct accessions and the possible genetically duplicated accessions were identified. These findings not only demonstrate the narrow genetic base of the Canadian potato germplasm, but also are useful for managing the existing potato collection and for selecting genetically distinct potato materials to widen the genetic background of the potato gene pool.

Y.-B. Fu (✉) · G. W. Peterson · K. W. Richards
Plant Gene Resources of Canada, Saskatoon Research Centre,
Agriculture and Agri-Food Canada,
107 Science Place,
Saskatoon, Saskatchewan, Canada S7N 0X2
e-mail: fuy@agr.gc.ca

T. R. Tarn · J. E. Percy
Potato Research Centre, Agriculture and Agri-Food Canada,
P.O. Box 20280, 850 Lincoln Road,
Fredericton, NB, Canada E3B 4Z7

Resumen El germoplasma de de papa (*Solanum tuberosum* L.) canadiense es único en su adaptación geográfica y a variedad de climas, pero se conoce poco acerca de la diversidad genética del acervo genético mejorado de *Solanum* establecido durante el siglo pasado. Para evaluar la diversidad de 114 accesiones accesiones de papa canadiense y 55 foráneas se utilizaron marcadores de secuencia simple repetida (SSR). Se aplicaron 36 pares de iniciadores SSR y se evaluaron 232 bandas polimórficas para cada accesión. Las frecuencias de bandas polimórficas tuvieron un rango de 0.01 a 0.98 con un promedio de 0.35. La proporción de la variación total de SSR que ocurrió entre el germoplasma canadiense y foráneo fue de 0.6%; entre los cultivares canadienses de los cuatro principales periodos de mejoramiento 2.7%; entre las variedades ancestrales, los cultivares modernos, las líneas élite de mejoramiento 4%; y entre las líneas tetraploides y diploides 3.7%. Se ha encontrado una diversidad ligeramente mayor para el germoplasma foráneo que para el canadiense. Los cultivares modernos mostraron una diversidad ligeramente mayor que las variedades ancestrales y los cultivares antiguos revelaron una ligera mayor variación que los recientes. La agrupación de 169 accesiones reveló más de 10 grupos, pero éstos no estaban separados distantemente. Tanto las accesiones genéticamente más distintas como las posiblemente duplicadas fueron identificadas. Estos hallazgos no sólo demostraron la estrecha base genética del germoplasma canadiense de papa, sino que también son útiles para administrar la colección existente de papa, y para

seleccionar materiales genéticamente distintos y ampliar la base genética del acervo genético de papa.

Keywords Potato · Genetic diversity · Genetic distinctiveness · SSR · Germplasm management

Introduction

The cultivated potato, *Solanum tuberosum* L., is considered the most important tuber crop in the world and among the four most valuable crops world-wide (Ross 1986). Germplasm with adaptation to different climatic and cultural conditions has been essential to the development of new potato cultivars (Tarn et al. 1992). Canadian potato cultivars are unique in their geographic and climatic ranges of adaptation (Anstey 1986; Tarn et al. 1992). Potato breeding in Canada began in 1888 with the goal to improve potato yield of early introductions (Anstey 1986; Turner and Molyneaux 2004). Since then, breeding efforts have gone through several major stages from early selection and adaptation, selection for disease resistance, obtaining disease-free stock, to selection for processing quality (Anstey 1986). Great improvements have been made in traits associated with production, utilization, disease control, *in vitro* culture, and processing quality (Tarn et al. 1992). Hundreds of potato cultivars have been developed and released, many of which have had significant impacts on Canadian agriculture (Anstey 1986; Guenthner 2001; Turner and Molyneaux 2004). There are still concerns about the narrowing of the North American *Solanum* gene pool as a result of the small number of parental lines used since potato breeding began (Love 1999; Simko et al. 2004). However, no assessment has been made on the genetic diversity of the Canadian *Solanum* germplasm.

To enhance potato breeding programs and conserve the *Solanum* germplasm released from the Canadian potato breeding programs, the Potato Gene Resources Repository was established in 1992 at the Potato Research Centre of Agriculture and Agri-Food Canada, Fredericton, New Brunswick. The Repository is a clonal collection of potato cultivars with an emphasis on heirloom varieties, Canadian-bred cultivars, and elite breeding lines from the Canadian breeding programs. Currently, the Repository contains 132 clones originating from eight countries, of which 119 are maintained *in vitro* and 13 as tubers. Recently some effort was made to recruit 37 more domestic and exotic accessions for genetic research and possible addition to widen the genetic diversity of the collection. However, little is known about the genetic diversity and genetic structure of this Canadian dominant collection.

Characterization of plant germplasm using molecular techniques has played an increasingly important role in the management and utilization of plant genetic resources (Karp 2002). It can also enhance plant breeding in selection of diverse parents to widen the breeding gene pool (Fu 2006). Efforts have been made to characterize potato germplasm using allozyme (Ortiz and Huaman 2001), restriction fragment polymorphism (RFLP) (Powell et al. 1991), random amplified polymorphic DNA (RAPD) (Hosaka et al. 1994; Demeke et al. 1996; del Rio et al. 1997), amplified fragment length polymorphism (AFLP) (Milbourne et al. 1997; Kim et al. 1998), inter simple sequence repeat (ISSR) (Bornet et al. 2002), and simple sequence repeat (SSR) markers (Ghislain et al. 2004, 2006; Braun and Wenzel 2005; Feingold et al. 2005; Ispizúa et al. 2007). These characterizations have not only provided useful information for understanding the genetic diversity and structure of various potato gene pools established in different geographic regions for an effective management of potato germplasm, but have also facilitated some potato breeding programs by identifying released cultivars and determining their genetic relationships.

The objective of this study was to assess the genetic diversity, structure, association, and distinctiveness of 169 Canadian and exotic potato accessions using SSR markers. It is our hope that this molecular assessment generates some baseline information not only for managing our potato germplasm collection, but also for identifying diverse germplasm for potato breeding.

Materials and Methods

Plant Materials

The potato germplasm used in this study consisted of 132 potato accessions currently held at the Potato Gene Resources Repository, Fredericton, New Brunswick, and 37 recently recruited clones (Table 1). The Repository maintains 68 Canadian cultivars released since the start of modern breeding in the 1930s, 56 heirloom varieties mostly of North American origin, and eight accessions introduced from Great Britain before 1900. The recruited accessions, which are not yet included in the Repository, consisted of 24 modern cultivars, mostly Canadian, and 13 long-day adapted selections of diploid and tetraploid cultivated potato species from the Andes. These Andean accessions represented a set of late blight differentials (the NRBK clones) that were derived from *S. demissum* Lindl. and some breeding lines. Ten accessions are known to be diploid and all other accessions in the study are believed to be tetraploid. To assess the diversity changes over time, the 68 Canadian cultivars released from 1919 to 2002 were classified into four groups based on date of cross (period 1 from 1919 to 1959; period 2 from 1960 to 1970; period 3 from 1971 to

Table 1 Summary of potato accessions studied and SSR variation with respect to country of origin

Country	No. of accessions[a]	Type			No. of polymorphic alleles	Mean allelic frequency (range)	Fst[b]
		Heirloom variety	Modern cultivar	Elite line			
Canada	114 (32)	26 (1)	68 (19)	20 (12)	227	0.34(0.01–0.98)	0.019
USA	25 (4)	15 (1)	6 (2)	4 (1)	170	0.44(0.04–0.96)	0.025
UK	17	10	0	7	185	0.40(0.06–0.94)	0.019
Netherlands	8 (1)	0	4 (1)	4	145	0.43(0.13–0.87)	0.026
Others[c]	5	5	0	0	127	0.46(0.17–0.81)	0.029
Total or Average	169 (37)	56 (2)	78 (22)	35 (13)	232	0.35(0.01–0.98)	0.024

[a] The number of accessions for newly recruited germplasm is given in parenthesis, [b] The proportion of the total SSR variation specific to a country, [c] Germany, two accessions; Austria, Slovenia and Sweden, one accession each

1980; period 4 from 1981 to 2002). To assess the genetic structure, 169 accessions were grouped into three germplasm types: heirloom variety, modern cultivar, and elite line.

DNA Extraction

All clones were grown in the greenhouse at the Potato Research Centre. Young leaves were harvested, placed into plastic bags with paper towels moistened with sterile RO water, placed into coolers with ice packs and shipped to Plant Gene Resources of Canada (PGRC), Saskatoon, Saskatchewan. The leaves were freeze-dried in a Labconco Freeze Dry System for 5 days to 7 days, and stored at −20°C. From each sample, dry leaves were ground to a fine powder in a 2 ml Eppendorf tube with three 3 mm glass beads in a multidirectional shaker. Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada) according to the manufacture's directions. Extracted DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical Co., St. Louis, USA), followed by the dilution to 10 ng μL^{-1} for SSR analysis.

SSR Analysis

Based on reported polymorphism and genome coverage (Milbourne et al. 1998; Ghislain et al. 2004; Feingold et al. 2005), 14 genomic and 22 EST derived SSR primer pairs were selected for this assessment. All polymerase chain reactions (PCRs) were performed in an MJ Research DYAD thermocycler (BioRad, Mississauga, ON, Canada). PCRs for the STM series of primers (Ghislain et al. 2004) were preformed in a 25 µl total volume using 25 ng genomic DNA, 1× PCR buffer (Promega, ON, Canada), 2.5 mM $MgCl_2$ (Promega, ON, Canada), 0.2 mM each dNTPs, 0.5 µM each forward and reverse primer, and 0.5 U/reaction Taq polymerase (New England Biolabs, ON, Canada). Cycling conditions were 95°C 10 s, Tm for 30 s, 72°C 40 s for 35 cycles. Those annealing temperatures indicated by a range were preformed using a touchdown PCR decreasing by 0.5°C per cycle until the lower temperature was reached. Twenty-five additional cycles were preformed at the lower temperature. PCRs for the StI series of primers (Feingold et al. 2005) were preformed in 25 µl total volume containing 50 ng genomic DNA, 1× ThermoPol buffer (New England Biolabs, ON, Canada) containing a 2 mM $MgSO_4$, 0.2 mM each dNTPs, 0.2 µM each forward and reverse primer, and 0.5 U/reaction Taq polymerase (New England Biolabs, ON, Canada). Cycling conditions were 95°C 10 s, Tm for 30 s, 72°C 40 s for 30 cycles. Those annealing temperatures indicated by a range were preformed using a touchdown PCR decreasing by 1°C per cycle until the lower temperature was reached. Thirty additional cycles were preformed at the lower temperature. The PCR products were separated on a 1.5 mm thick 6% (w v^{-1}) non-denaturing acrylamide:bis-acrylamide (19:1) gel in 1× TBE buffer with 0.5 mg L^{-1} ethidium bromide for 2 h to 2.5 h (Wang et al. 2003) and recorded on a digital gel documentation system.

Data Analysis

To generate a dataset of SSR allele counts for each accession, DNA fragments amplified by SSR primer pairs were identified based on their sizes in base pairs measured with a 10 bp DNA ladder (Invitrogen, Carlsbad, CA, USA) and compared with the fragment sizes reported in the literature (Ghislain et al. 2004; Feingold et al. 2005). Frequencies of the scored alleles were calculated with respect to primer, country origin, and germplasm type. The number of likely loci detected for each primer pair were determined by the observed allelic pattern and the possible number of alleles per single tetraploid plant. The polymorphic information content (PIC) was calculated for each primer pair, as described in Roussel et al. (2004) with adjustment to the number of likely loci, to assess the informativeness of each marker.

An analysis of molecular variance (AMOVA; Excoffier et al. 1992) that was based on the dissimilarity matrix of pairwise accessions was also performed using Arlequin version 3.1 (Excoffier et al. 2005) to assess the genetic structure of the potato germplasm. This analysis not only allowed the partition of the total SSR variation into within- and among- group variation components, but also provided measures of inter-group genetic distance as the proportion of the total SSR variation residing between any two groups (Phi statistic; Excoffier et al. 1992). Six models of genetic structuring were examined as (1) existing vs new germplasm, (2) germplasm of various country origin, (3) Canadian vs exotic germplasm, (4) Canadian germplasm of four breeding periods, (5) diploid vs tetraploid germplasm, and (6) germplasm type. Significance of resulting variance components and inter-group genetic distances was tested with 10,100 random permutations.

To assess the genetic associations of the potato accessions, two approaches were applied. First, a principal component analysis of 169 accessions was conducted using NTSYS-PC 2.01 (Rohlf 1997) based on the similarity matrix of 232 SSR alleles, and plots of the first three resulting principal components were made to assess the accession associations and to identify genetically distinct accessions. Second, a neighbor-joining analysis of 169 accessions was also made using PAUP* (Swofford 1998) and a circulating tree was displayed using MEGA 3.01 (Kumar et al. 2004) to confirm the genetic association of individual accessions and to identify any genetic clustering without restriction to country of origin or germplasm type.

To assess the genetic distinctiveness of the potato accessions, the similarities of each accession with the remaining accessions assayed were calculated using the simple matching coefficient (Sokal and Michener 1958) as: $S_{ij}=(a+d)/(a+b+c+d)$, where S_{ij} is the SSR similarity between the accession i (i=1 to n) and the other accession j [j=1 to (n−1)], a is the number of alleles (from all SSR loci) shared in both i and j, b is the number of alleles present in i but not shared in j, c is the number of alleles present in j but not shared in i, and d is the number of alleles absent from both i and j. The SSR dissimilarity for each pair of accessions can be defined as 1- S_{ij}. The average SSR dissimilarity for the accession i can be obtained by averaging all of the n−1 SSR dissimilarities that the accession was associated with. This average dissimilarity measures the overall genetic difference between the accession (i) of interest and the remaining accessions assayed. A higher average dissimilarity obtained from unlinked markers means that the accession has a genetic background more distinct from the other accessions (Fu 2006). This assessment was done using a specific SAS program written in SAS IML (SAS Institute Inc. 2004). The specific SAS program is available upon request.

Results and Discussion

SSR Variation

The 36 SSR primer pairs detected a total of 64 likely loci on all 12 homologous linkage groups of potato (Table 2; Milbourne et al. 1998; Ghislain et al. 2004; Feingold et al. 2005). Twenty-two likely loci detected by EST-derived SSR markers should represent transcribed chromosomal regions, while the other 42 likely loci detected by genomic SSR markers may largely sample non-genic chromosomal segments. A total of 232 SSR alleles were detected in this study, but they could include some null alleles, because it was difficult to separate non-amplification due to experimental errors from null alleles. The number of detected alleles per primer pair ranged from two for four primer pairs (StI062, StI064, STM1053, STM1017) to 17 for StI049 with an average of 6.4 alleles per primer pair. Values of each marker polymorphic information content (PIC) ranged from 0.01 to 0.49 with an average of 0.25. Note that these PIC values could be biased by the determination of likely loci, as this variation was not significantly (P=0.08) associated with the number of alleles detected at each primer pair. The three most informative loci were StI062 on linkage group I, StI028 on linkage group XI, and STM0030 on linkage group XII. EST-derived SSR markers appear to be more informative (average PIC of 0.28) than genomic SSR markers (average PIC of 0.19). Thus, the SSR markers revealed a relatively large amount of variation in the sampled genome.

The observed occurrence frequencies of the 232 alleles ranged from 0.01 to 0.98 with an average of 0.35 (Table 1). There were 12 alleles with an occurrence frequency of 0.95 or larger in the assayed accessions and 51 with frequencies greater than 0.60, while 55 alleles were detected with frequencies less than 0.05, and 24 alleles with frequencies less than 0.02. Some of the rare alleles may be useful as diagnostic markers for some of the assayed potato cultivars.

Genetic Structure of Potato Accessions

Six models of genetic structuring were examined in this assessment. The proportion of the total SSR variation which resided between existing and newly recruited potato accessions was 1.8% (Table 3), but slightly more diversity was observed within newly recruited accessions. The proportion of the total SSR variation explained by country origin was 2.1% (Table 3). Eight Dutch accessions displayed more within-country variation (Table 1), probably due to the introgression of *S. demissum* genetic background. The Canadian germplasm (although with 114 accessions) harbored 0.6% less SSR variation than the other 55 potato accessions from seven countries (Table 1),

Table 2 Details of 36 SSR markers applied in this study

Linkage group	Primer name / type[a]	No. of alleles	Size range (bp)	Likely loci[b]	PIC[c]
I	StI062 /Est	2	117–135	1	0.49
I	STM1049 /G	7	175–235	2	0.06
II	StI024 /Est	13	130–240	3	0.24
II	StI052 /Est	13	150–240	3	0.25
II	STM1064 /G	8	190–240	2	0.35
II	STM2022 /G	4	175–240	2	0.33
III	StI013 /Est	5	135–330	2	0.22
III	StI060 /Est	5	150–230	2	0.45
III	STM1053 /G	2	170–175	1	0.18
IV	StI020 /Est	6	100–140	2	0.39
V	StI049 /Est	17	140–215	2	0.04
V	STM1031 /G	5	260–265	1	0.13
VI	StI004 /Est	8	80–110	2	0.33
VI	StI045 /Est	5	80–110	2	0.26
VI	STM0019 /G	9	105–330	2	0.26
VII	StI008 /Est	8	140–200	2	0.20
VII	StI025 /Est	3	105–145	1	0.01
VII	StI040 /Est	3	180–240	1	0.32
VII	StI064 /Est	2	170–220	2	0.40
VII	STM0031 /G	8	110–230	1	0.01
VII	STM1052 /G	3	200–330	1	0.01
VII	STM2013 /G	6	140–250	2	0.14
VIII	StI027 /Est	12	110–230	2	0.13
VIII	StI047 /Est	3	120–180	1	0.26
VIII	STM1016 /G	11	230–350	2	0.07
VIII	STWAX-2 /G	6	220–330	2	0.12
IX	STM1017 /G	2	130–160	1	0.07
IX	STM3012 /G	5	160–330	2	0.40
X	StI023 /Est	7	155–300	2	0.28
XI	StI028 /Est	7	165–280	2	0.49
XI	StI039 /Est	4	230–320	2	0.46
XI	StI041 /Est	12	130–170	3	0.16
XII	StI007 /Est	6	120–170	2	0.15
XII	StI054 /Est	7	155–230	1	0.24
XII	STM0030 /G	4	120–220	1	0.49
Unknown	StI037 /Est	4	145–180	2	0.47
Total or Average		232		64	0.25

[a] *Est* EST-derived SSR markers described in Feingold et al. (2005); *G* genomic SSR markers described in Ghislain et al. (2004), [b] The number of likely loci were determined by the observed band pattern and the possible number of alleles per single tetraploid plant, [c] Polymorphic information content

largely due to the mixture of genetic backgrounds of diverse origin. Considering only the 68 Canadian accessions released from 1919 to 2002, it was found that 2.7% of the total SSR variation resided among accessions released over the four major breeding periods. The period 1 accessions (i.e., released before 1960) were the most diverse, followed by the accessions for period 3 (from 1971–1980), period 4 (after 1981), and period 2 (from 1960 to 1970). The proportion of the total SSR variation explained by ploidy levels was 3.7% (Table 3), but diploid accessions unexpectedly displayed more variation than tetraploid accessions. The highest proportion of the total SSR variation (4%) was found to reside among three germplasm types. The modern cultivars were the most diverse, followed by the heirloom varieties and elite lines.

These results indicate that the *Solanum* germplasm held at the Potato Gene Resources Repository is genetically narrow. Canadian germplasm displayed less SSR variation than accessions from the other countries. Existing diversity is not much improved if newly recruited germplasm is added to the collection. Interestingly, the heirloom varieties harbored slightly less diversity than the modern cultivars. These findings were well illustrated in the genetic associ-

Table 3 Results for analyses of molecular variance (AMOVA) for 169 potato accessions with six models of genetic structuring

Model / source	*df*	Sum of squares	Variance component	Percentage of variation	*P*-value[a]
Existing vs new germplasm					
Between groups	1	64.82	0.57	1.8	<0.0001
Within groups	167	5,335.38	31.95	98.2	
Germplasm of various origins					
Among countries	4	184.92	0.68	2.1	<0.0001
Within countries	164	5,215.28	31.80	97.9	
Canadian vs exotic germplasm					
Between groups	1	47.43	0.21	0.6	<0.005
Within groups	167	5,352.74	32.08	99.4	
Canadian germplasm of four breeding periods					
Among breeding periods	3	134.08	0.84	2.7	<0.0001
Within breeding periods	64	1,995.6	30.23	97.3	
Diploid vs tetraploid germplasm					
Between ploidy groups	1	114.55	1.21	3.7	<0.0001
Within ploidy groups	167	5,285.66	31.65	96.3	
Germplasm type[b]					
Between types	2	200.71	1.29	4.0	<0.0001
Within types	166	5,199.49	31.32	96.0	

[a] The probability that the among-group (or among-country, among-period, among-type) variance component was larger than zero, as computed from random permutations, [b] Germplasm type includes heirloom variety, modern cultivar, and elite line

ations of 169 potato accessions shown in Fig. 1. The two components accounted for only 8.8% and 7.9% of the total SSR variation. Also, newly recruited accessions had a better spread over the plot than existing accessions (Fig. 1a), as reflected in the lower genetic relatedness among these recruited accessions. The Canadian germplasm was well overlapped with the exotic germplasm (Fig. 1b). The modern cultivars were widely spread over the plot (Fig. 1c).

Limited genetic diversity observed in these potato accessions and little differentiation among potato groups are not surprising (Simko et al. 2006). First, only a small number of introduction events formed the basis of modern potato germplasm (Hawkes 1978; Ames and Spooner 2008). Second, the USA cultivar Katahdin is responsible for almost a quarter of the germplasm that makes up prominent North American cultivars (Love 1999). Third, the Canadian potato breeding has long been limited to a narrow genetic base with fewer introgressions of new exotic germplasm (Anstey 1986; Tarn et al. 1992; Turner and Molyneaux 2004). Recent efforts to incorporate germplasm from Andean cultivated species into some Canadian cultivars (de Jong et al. 2001; Lynch et al. 2004) yielded an improvement only in the level of genetic relatedness, but not in the magnitude of genetic diversity.

Genetic Association of Potato Accessions

Clustering of 169 potato accessions by neighbor-joining analysis revealed several variation patterns (Fig. 2). First, about ten clusters were found, but they were not well separated due to low SSR variation. Second, nine of the ten diploid accessions were clustered together in one group. Third, accessions of three germplasm types were well mixed in various clusters and no single clusters were found separately for heirloom or modern cultivars. Fourth, exotic accessions were widely spread into various clusters, reflecting their different genetic backgrounds. Fifth, five Canadian accessions (Mrs. Moehrle's Yellow Fleshed, Siberian, LRC 373-5, LRC 4373-5b and 13594-070) and one Germany accession (NRBK-05) were genetically distinct from the other accessions, as reflected in the longer branch lengths. Sixth, some highly related (or genetically duplicated) accessions could be identified. The obvious candidates were the modern cultivar pairs of Canso vs Canus and Rose Gold vs Red Gold and the elite line pair of LRC 373-5 vs LRC 4373-5b, which is expected from the known pedigrees (Lynch et al. 2004). This study confirms the results of two groupings identified by X.-Q. Li (personal communication) through fingerprinting at the Potato Research Centre. One group consists of the blue-fleshed clones Sharon's Blue, River John Blue, Nova Scotia Blue, MacIntosh Black, Congo, and British Columbia Blue, to which this study added McIntyre Blue. The other is a group of fingerlings: Banana, Kifli, Corne de Mouton, Jogeva Yellow Estonian, and Fingerling.

Further assessments on the clustering with respect to known pedigree revealed congruency of some acces-

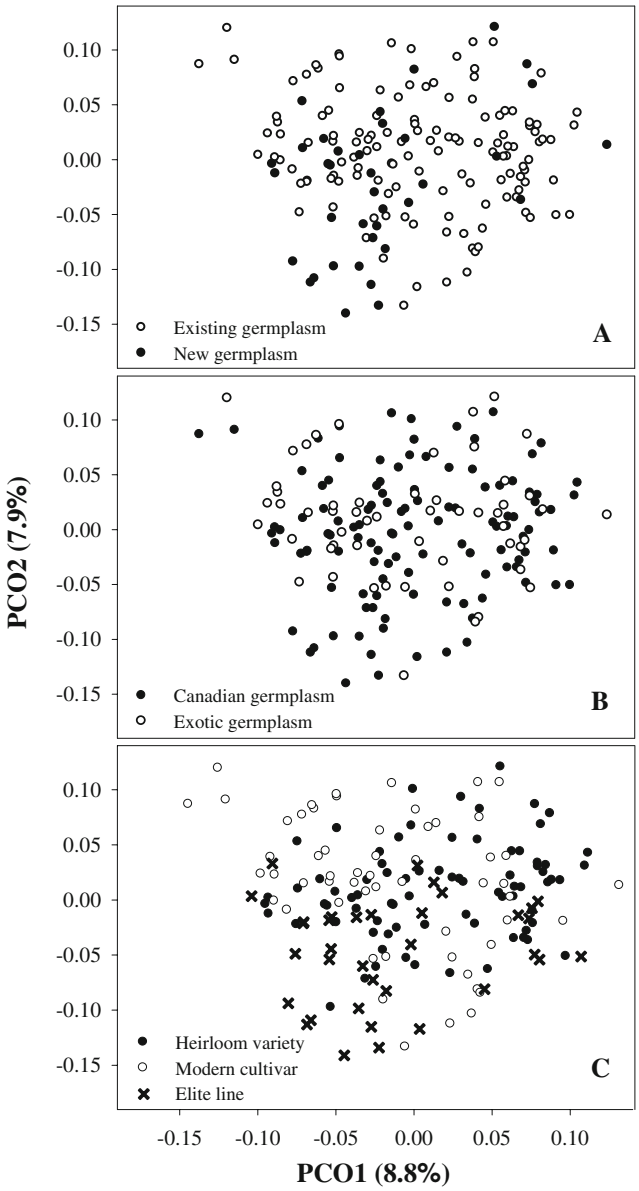

Fig. 1 Plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 232 SSR alleles for 169 potato accessions. These two components accounted for 8.8% and 7.9% of the total SSR variance, respectively. **a** Individual accessions are separately labeled for existing and newly recruited germplasm. **b** Canadian accessions are identified against those from other countries. **c** accessions are labeled for germplasm type. Note that **a, b** and **c** are the same plot with different labeling for clear illustration

sions. For example, OAC Temagami and OAC Ruby Gold shared the same parents of Rhinered and Red Gold and were grouped together. Similarly, Mirton Pearl and Anson shared the same parents of Mira and F53018 and were clustered together. There were also many inconsistencies between grouping and known pedigree. For example, Acadia Russet and Shepody shared the same female parent of Bake King, but were located in separate clusters. Similarly, Aquilon and Mouraska shared the same female parent of Hudson, but were grouped in different clusters. Inconsistencies between cultivar clustering and known pedigrees are largely expected for these potato accessions with such a narrow genetic base (Loiselle et al. 1989b; Kim et al. 1998; Love 1999). Also, the limited sampling of the potato genome revealed by only 36 SSR loci may contribute to such inconsistencies. Applications of more mapped markers across the genome would improve the resolution to the genetic relationships of these potato cultivars. However, the estimated genetic relationships should still offer a useful guide for potato breeding, as they are more informative than parental selection (Hosaka et al. 1994; Demeke et al. 1996; Kim et al. 1998) and traditional pedigree analysis (Mendoza and Haynes 1974; Loiselle et al. 1989a, b).

Genetic Distinctiveness of Potato Accessions

The genetic distinctiveness of a potato accession was measured by the average dissimilarity (AD) of the accession against the remaining accessions assayed. The higher the AD, the greater is the distinctiveness of the genetic background. The AD of the accessions ranged from 0.241 for Pink Pearl (a Canadian modern cultivar) to 0.353 for NRBK-05 (the germplasm recruited from Germany) with a mean of 0.277 (Table 4). Ranking these AD values generated the genetic distinctiveness for all the accessions assayed. Table 4 lists 31 accessions with the highest AD values and 31 accessions with the lowest AD values. The 31 genetically most distinctive accessions listed in Table 4 contains a diverse group of four diploid accessions, 16 elite lines, ten heirloom varieties, and five modern cultivars. In contrast, among the 31 genetically least distinctive accessions, there were no diploid accessions, one elite line, eight heirloom varieties, and 22 modern cultivars. This contrast demonstrates again the low level of genetic distinctiveness in modern cultivars.

The ADs shown in Table 4 are limited to only those accessions assayed. The AD values would change if more potato accessions were assessed. This method can recognize the distinctiveness, but not necessarily the relatedness, of accessions (Fu 2006). For example, two closely related cultivars that were quite distinct from the remaining cultivars could have similar higher levels of AD than the others and both cultivars would have been identified as genetically distinct. In spite of these limitations, the relative measure of genetic distinctiveness reported here could provide a guide for selecting specific germplasm with distinct genetic background for potato breeding programs (Demeke et al. 1996; Braun and Wenzel 2005).

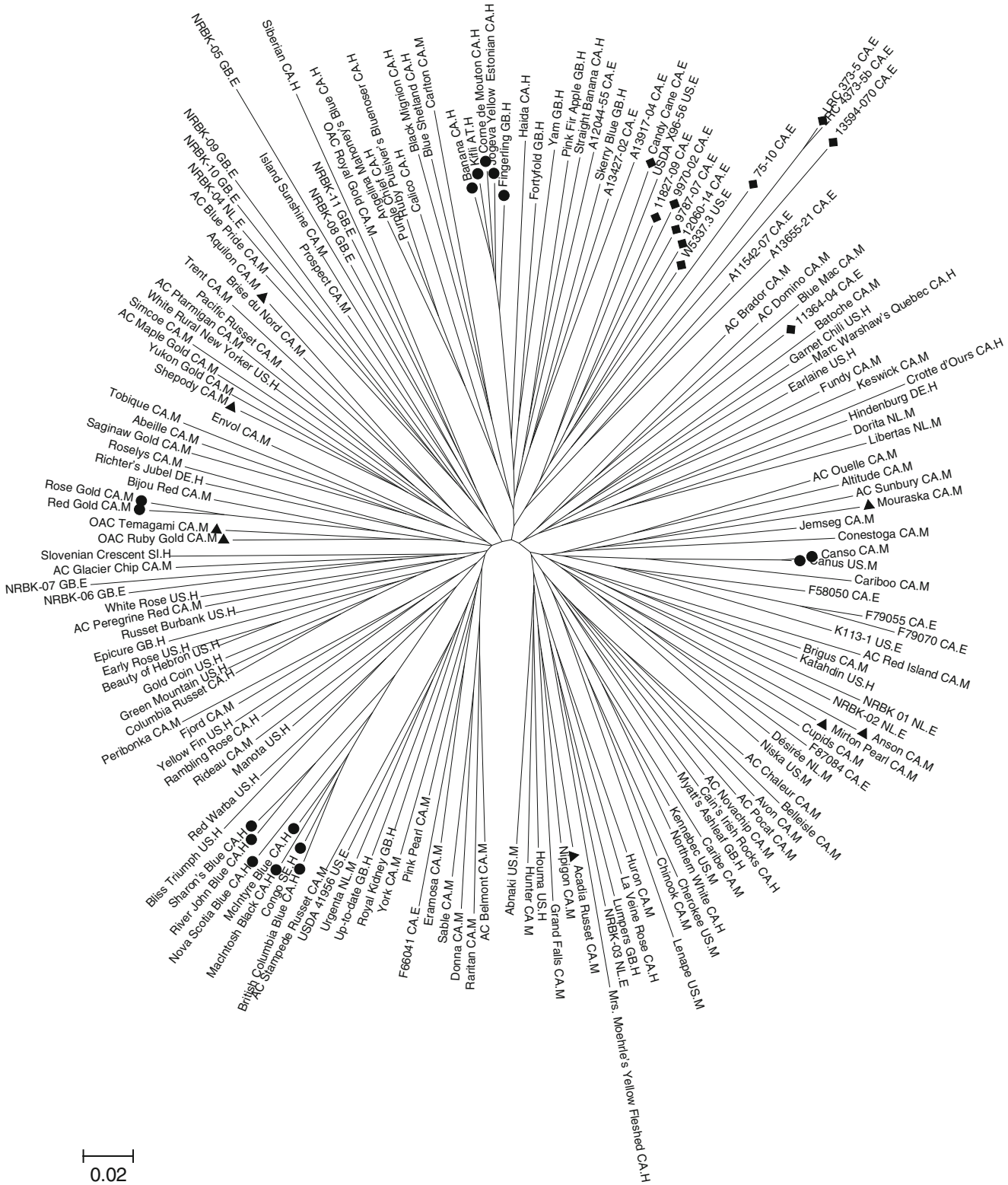

Fig. 2 Clustering of 169 potato accessions obtained from the neighbor-joining analysis of 232 SSR alleles. Accession label includes the accession name, followed by ISO two-letter code for its country origin and one letter code for its germplasm type (*H* heirloom variety; *M* modern cultivar; *E* elite line). Diploid accessions are marked with black diamonds; examples of highly related (or genetically duplicated) accessions with black circles, except the genetically distinct pair of LRC 373-5 and LRC 4373-5b; and examples of congruent or incongruent accessions with respect to known pedigree with black triangles

Table 4 Sixty-two potato accessions with the highest (left) or lowest (right) values of average dissimilarity (AD)

Accession[a]	CN[b]	AD	Accession	CN	AD
NRBK-05 GB.E	105,502	0.353	Pink Pearl CA.M	105,514	0.241
LRC 4373-5b CA.E	105,561	0.346	Envol CA.M	NG	0.242
LRC 373-5 CA.E (2×)	105,560	0.344	Manota US.H	105,491	0.246
13594-070 CA.E (2×)	NG	0.342	Canus US.M	105,457	0.247
Siberian CA.H	105,529	0.337	OAC Ruby Gold CA.M	105,544	0.250
NRBK-09 GB.E	105,506	0.324	Brise du Nord CA.M	NG	0.251
NRBK-10 GB.E	105,507	0.316	Canso CA.M	105,456	0.252
Mrs. Moehrle's Yellow Fleshed CA.H	105,495	0.315	Jemseg CA.M	105,483	0.252
Carlton CA.M	105,460	0.311	Myatt's Ashleaf GB.H	105,497	0.252
Lenape US.M	105,487	0.310	OAC Temagami CA.M	105,545	0.254
75-10 CA.E (2×)	NG	0.309	Russet Burbank US.H	105,562	0.254
Crotte d'Ours CA.H	105,465	0.307	White Rural New Yorker US.H	105,536	0.254
NRBK-04 NL.E	105,501	0.304	Pacific Russet CA.M	NG	0.254
NRBK-07 GB.E	105,504	0.301	AC Belmont CA.M	105,434	0.255
USDA X96-56 US.E	105,541	0.299	Eramosa CA.M	105,469	0.256
Candy Cane CA.E (2×)	105,455	0.297	Royal Kidney GB.H	105,523	0.256
A13917-04 CA.E	NG	0.297	Prospect CA.M	NG	0.256
Black Mignion CA.H	105,550	0.296	F58050 CA.E	105,470	0.257
Haida CA.H	105,479	0.296	Rambling Rose CA.H	105,516	0.258
A12044-55 CA.E	NG	0.295	Bijou Red CA.M	NG	0.258
A13655-21 CA.E	NG	0.295	AC Ouelle CA.M	NG	0.259
Anson CA.M	105,444	0.294	Cain's Irish Rocks CA.H	105,453	0.259
Yam GB.H	105,537	0.293	Nipigon CA.M	105,509	0.259
Blue Mac CA.M	105,449	0.292	Abnaki US.M	105,441	0.260
Blue Shetland CA.H	105,450	0.292	AC Novachip CA.M	105,439	0.260
F79070 CA.E	105,473	0.292	Acadia Russet CA.M	105,442	0.260
Bliss Triumph US.H	105,552	0.292	Brigus CA.M	105,451	0.260
Simcoe CA.M	105,530	0.291	Columbia Russet CA.H	NG	0.260
Straight Banana CA.H	105,533	0.291	Kennebec US.M	NG	0.261
NRBK-01 NL.E	105,498	0.290	Shepody CA.M	105,528	0.261
Pink Fir Apple GB.H	105,513	0.290	AC Chaleur CA.M	105,437	0.262

[a] Accession label includes accession name, followed by ISO two-letter code for country origin, germplasm type (*H* heirloom variety; *M* modern cultivar; *E* elite line), and diploid in parenthesis (if applicable), [b] *CN* the Canadian National accession number in germplasm collections held at Plant Gene Resources of Canada (PGRC); *NG* newly recruited germplasm accessions that are not part of the PGRC collection yet

Implications for Potato Germplasm Management and Breeding

This SSR analysis revealed a narrow genetic base for Canadian potato cultivars generally and specifically for the germplasm held in the Canadian Potato Gene Resources Repository. Although the mandate of the Repository focuses more on the Canadian potato germplasm, more efforts to expand the diversity range of the potato collection are warranted with targeted acquisitions of potato germplasm from countries with temperate climates similar to that of Canada. The characterization data presented here are useful for managing the existing potato germplasm in germplasm addition, accession identification, duplication verification, and germplasm structuring. Also, the analysis showed that Canadian potato germplasm harbored less SSR variation than those accessions from the other seven countries. This narrow genetic base persists in spite of the use in breeding of Andean cultivated potatoes of groups Phureja, Andigena and Tuberosum and as many as 14 wild species (Plaisted and Hoopes 1989; Spooner and Salas 2006). Canadian breeders have worked with germplasm from the Phureja and Andigena Groups (de Jong et al. 1981; Tarn and Tai 1983) and have released disease resistant germplasm (de Jong et al. 2001; Lynch et al. 2004). However, continuing efforts are still needed to diversify the Canadian potato gene pool to ensure sustainable breeding programs in the future. The findings reported here on genetic association and distinctiveness are helpful for parental selection of diverse plants for potato breeding. Moreover, the molecular characterization of potato germplasm generated not only essential information for managing the potato collection, but also provided a useful guide for selecting specific germplasm with distinct genetic background for diversifying potato breeding program.

Acknowledgments The authors would like to thank Garrett Pittenger and Seeds of Diversity Canada for assistance in acquiring heirloom varieties; Joyce and Robert Coffin (Privar Farm, North Wiltshire, PE), Pierre Turcotte (Les Buissons Research Centre, Quebec Department of Agriculture, Fisheries and Food, QC), Kirby Sawatzky (Parkland Seed Potatoes Ltd, Lacombe, AB), and Benoit Bizimungu (Lethbridge Research Centre, AAFC, Lethbridge, AB) for providing the Canadian cultivars not in the repository; colleagues in the AAFC potato breeding program at Fredericton for providing additional germplasm and cultivars; Marcie Heggie and Susan MacWilliam for their technical support; and three anonymous reviewers for helpful comments on the early version of the manuscript.

References

Ames, M., and D.M. Spooner. 2008. DNA from herbarium specimens settles a controversy about origins of the European potato. *American Journal of Botany* 95: 252–257.

Anstey, T.H. 1986. *One hundred harvests*. Research Branch, Agriculture Canada, Historical Series No. 27.

Bornet, B., F. Goraguer, G. Joly, and M. Branchard. 2002. Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *Tuberosum*) detected by inter-simple sequence repeats (ISSRs). *Genome* 45: 481–484.

Braun, A., and G. Wenzel. 2005. Molecular analysis of genetic variation in potato (*Solanum tuberosum* L.). I. German cultivars and advanced clones. *Potato Research* 47: 81–92.

de Jong, H., K.G. Proudfoot, and A.M. Murphy. 2001. The germplasm release of F87084, a fertile, adapted clone with multiple disease resistances. *American Journal of Potato Research* 78: 141–149.

de Jong, H., G.C.C. Tai, W.A. Russell, G.R. Johnston, and K.G. Proudfoot. 1981. Yield potential and genotype-environment interactions of tetraploid-diploid (4×−2×) potato hybrids. *American Journal of Potato Research* 58: 191–199.

del Rio, H., J.B. Bamberg, and Z. Huaman. 1997. Assessing changes in the genetic diversity of potato gene banks. 1. Effects of seed increase. *Theoretical and Applied Genetics* 95: 191–198.

Demeke, T., D.R. Lynch, L.M. Kawchuk, G.C. Kozub, and J.D. Armstrong. 1996. Genetic diversity of potato determined by random amplified polymorphic DNA analysis. *Plant Cell Reports* 15: 662–667.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

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

Fu, Y.B. 2006. Genetic redundancy and distinctness of flax germplasm as revealed by RAPD dissimilarity. *Plant Genetic Resources* 4: 117–124.

Ghislain, M., D.M. Spooner, F. Rodriguez, F. Villamon, J. Nunez, C. Vasquez, R. Waugh, and M. Bonierbale. 2004. Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theoretical and Applied Genetics* 108: 881–890.

Ghislain, M., D. Andrade, F. Rodríguez, R.J. Hijmans, and D.M. Spooner. 2006. Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. *Theoretical and Applied Genetics* 113: 1515–1527.

Guenthner, J. 2001. *The International Potato Industry*. Cambridge: Woodhead.

Hawkes, J.G. 1978. Genetic poverty of the potato in Europe. In *Broadening the Genetic Base of Crops*, eds. A.C. Zeven, and A. M. van Harten, 19–27. The Netherlands: Pudoc, Wageningen.

Hosaka, K., M. Mori, and K. Ogawa. 1994. Genetic relationships of Japanese potato cultivars assayed by RAPD analysis. *American Journal of Potato Research* 71: 535–546.

Ispizúa, V.N., I.R. Guma, S. Feingold, and A.M. Clausen. 2007. Genetic diversity of potato landraces from northwestern Argentina assessed with simple sequence repeats (SSRs). *Genetic Resources and Crop Evolution* 54: 1833–1848.

Karp, A. 2002. The new genetic era: will it help us in managing genetic diversity? In *Managing Plant Genetic diversity*, eds. J.M. M. Engels, V.R. Rao, A.H.D. Brown, and M.T. Jackson, 43–56. Rome: International Plant Genetic Resources Institute.

Kim, J.H., H. Joung, H.Y. Kim, and Y.P. Lim. 1998. Estimation of genetic variation and relationship in potato (*Solanum tuberosum* L.) cultivars using AFLP markers. *American Journal of Potato Research* 75: 107–112.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5: 150–163.

Loiselle, F., G.C.C. Tai, T.R. Tarn, and B.R. Christie. 1989a. The use of multivariate analyses to elimiate redunant variables when evaluating potatoes for chip quality. *Plant Breeding* 103: 153–162.

Loiselle, F., G.C.C. Tai, B.R. Christie, and T.R. Tarn. 1989b. Relationship between inbreeding coefficient and clonal selection in a potato cultivar development program. *American Journal of Potato Research* 66: 747–753.

Love, S.L. 1999. Founding clones, major contributing ancestors, and exotic progenitors of prominent North American potato cultivars. *American Journal of Potato Research* 76: 263–272.

Lynch, D.R., Q. Chen, L.M. Kawchuk, and D. Drieger. 2004. Verticillium wilt resistant germplasm-release of clone LRC18-21 and derivatives. *American Journal of Potato Research* 81: 295–297.

Mendoza, H.A., and F.L. Haynes. 1974. Genetic relationship among potato cultivars grown in the United States. *HortScience* 9: 328–330.

Milbourne, D., R. Meyer, J.E. Bradshaw, E. Baird, N. Bonar, J. Provan, W. Powell, and R. Waugh. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Molecular Breeding* 3: 127–136.

Milbourne, D., R.C. Meyer, A.J. Collins, L.D. Ramsay, C. Gehbardt, and R. Waugh. 1998. Isolation, characterization and mapping of simple sequence repeat loci in potato. *Molecular and General Genetics* 259: 233–245.

Ortiz, R., and Z. Huaman. 2001. Allozyme polymorphisms in tetraploid potato gene pools and the effect on human selection. *Theoretical and Applied Genetics* 103: 792–796.

Plaisted, R.L., and R.W. Hoopes. 1989. The past record and future prospects for the use of exotic potato germplasm. *American Journal of Potato Research* 66: 603–627.

Powell, W., M.S. Phillips, J.W. McNicol, and R. Waugh. 1991. The use of DNA markers to estimate the extent and nature of genetic variability in *Solanum tuberosum* cultivars. *Annals of Applied Biology* 118: 423–432.

Rohlf, F.J. 1997. *NTSYS-PC 2.1. Numerical taxonomy and multivariate analysis system*. Setauket: Exeter Software.

Ross, H. 1986. *Potato breeding—Problems and perspectives*. Berlin Hamburg: Verlag Paul Parey.

Roussel, V., J. Koenig, M. Bechert, and F. Balfourier. 2004. Molecular diversity in French bread wheat accessions related to temporal

trends and breeding programmes. *Theoretical and Applied Genetics* 108: 920–930.

SAS Institute Inc. 2004. *The SAS system for windows V8.02*. Cary: SAS Institute Incorporated.

Simko, I., K.G. Haynes, and R.W. Jones. 2004. Mining data from potato pedigrees: tracking the origin of susceptibility and resistance to *Verticillium dahliae* in North American cultivars through molecular marker analysis. *Theoretical and Applied Genetics* 108: 225–230.

Simko, I., K.G. Haynes, and R.W. Jones. 2006. Assessment of linkage disequilibrium in potato genome with single nucleotide polymorphism markers. *Genetics* 173: 2237–2245.

Sokal, R.R., and C.D. Michener. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* 38: 1409–1438.

Spooner, D.M., and A. Salas. 2006. Structure, biosystematics and genetic resources. In *Handbook of potato production, improvement, and postharvest management*, eds. J. Gopal, and S.M.P. Khurana, 1–39. Binghamton: Food Products.

Swofford, D.L. 1998. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sunderland: Sinauer Associates.

Tarn, T.R., and G.C.C. Tai. 1983. Tuberosum x Tuberosum and Tuberosum x Andigena potato hybrids: comparisons of families and parents, and breeding strategies for Andigena potatoes in long-day temperate environments. *Theoretical and Applied Genetics* 66: 87–91.

Tarn, T.R., G.C.C. Tai, H. de Jong, A.M. Murphy, and J.E.A. Seabrook. 1992. Breeding potato for long-day, temperate climates. *Plant Breeding Reviews* 9: 217–232.

Turner, S., and H. Molyneaux. 2004. Agricultural science, potato breeding, and the Fredericton Experimental Station, 1912–1966. *Acadiensis* 33: 1–25.

Wang, D., J. Shi, S. Carlson, P.B. Cregan, R. Ward, and B. Diers. 2003. A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. *Crop Science* 43: 1828–1832.