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Genetic Diversity in Diploid and Tetraploid Late Blight Resistant Potato Germplasm

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Abstract. **An understanding of the genetic relationship within potato germplasm is important to establish a broad genetic base for breeding purposes. The objective of this study was to assess the genetic diversity of potato (*Solanum tuberosum* subsp. *tuberosum* Hawkes) germplasm that can be used in the development of cultivars with resistance to late blight caused by *Phytophthora infestans* (Mont.) de Bary. Thirty-three diploid and 27 tetraploid late blight resistant potato clones were evaluated for their genetic diversity based on 11 isozyme loci and nine microsatellites. A total of 35 allozymes and 42 polymorphic microsatellite fragments was scored for presence or absence. The germplasm was clustered based on the matrix of genetic similarities and the unweighted pair group means analysis of the isozyme and microsatellite data, which were used to construct a dendrogram using NTSYS-pc version 1.7. Twenty-three allozymes and DNA fragments were unique to the wild species. The diploid *Solanum* species *S. berthaultii* Hawkes and *S. microdontum* Bitter formed two distinct phenetic groups. Within *S. microdontum*, three subgroups were observed. The tetraploid germplasm formed another group, with *S. sucrense* Hawkes in one subgroup and the cultivated potato and Russian hybrids in another subgroup. Based upon the genetic diversity and the level of late blight resistance, *S. microdontum* and *S. sucrense* offer the best choice for strong late blight resistance from genetically diverse sources. This potato germplasm with reported late blight resistance should be introgressed into the potato gene pool to broaden the genetic base to achieve stronger and more durable resistance.**

The cultivated potato and its wild relatives belong to the genus *Solanum* L. sect. *Petota* Dumort. There are seven cultivated and 225 wild potato species, according to the most recent taxonomic treatment of Hawkes (1990), which include diploid ($2n = 24$), tetraploid ($2n = 48$), hexaploid ($2n = 72$), and a few triploid ($2n = 36$) and pentaploid ($2n = 60$) cytotypes (Spooner and van den Berg, 1992). The cultivated potato *S. tuberosum* subsp. *tuberosum* Hawkes is an autotetraploid ($2n = 4x = 48$) that originated in South America.

Despite the wide genetic diversity that exists in the genus *Solanum*, the use of closely related germplasm in breeding programs has resulted in high genetic similarity among more than 130 potato cultivars released in North America between 1930 and 1970 (Mendoza and Haynes, 1974). The pedigrees of most cultivars can be traced back to the cultivar 'Early Rose' and one of its parents, 'Garnet Chili' (Plaisted and Hoopes, 1989). Moreover, cultivars released between 1950 and 1970 have a high genetic similarity and may have reached a yield plateau (Mendoza and Haynes, 1974).

Molecular markers have been used to confirm the relatedness among North American potato cultivars. Coefficients of similarity ranged from 0.51 to 0.89 among 28 potato cultivars, based on random amplified polymorphic DNA (RAPD) (Demeke et al., 1996), and from 0.44 to 0.81 among 18 potato cultivars from different origins, based on simple sequence repeats (SSR) or microsatellites (Provan et al., 1996). An identical chloroplast DNA (T-type) restriction pattern was found among 10 historically important potato cultivars that traced back through 'Garnet Chili' to 'Rough Purple Chili', indicating that there is only one maternal lineage (Douches et al., 1991). The predominance of the T-type cytoplasm derived from 'Rough Purple Chili' was found in the modern European cultivated gene pool. 'Rough Purple Chili' was introduced in Europe after the 1840s late blight epidemic and was extensively used as the female parent (Provan et al., 1999).

Late blight, caused by the fungal-like oomycete *Phytophthora infestans* (Mont.) de Bary, is the most devastating potato disease worldwide (Fry and Goodwin, 1997; Kamoun et al., 1999) and causes both foliar destruction and tuber decay (Ross, 1986). The development of genetic resistance to late blight in potatoes is one of the major objectives in many breeding programs (Colon et al., 1995a) and has resulted in the release of late blight resistant germplasm (Corsini et al., 1999; Goth and Haynes, 1997). Essential studies on breeding potato for late blight resistance have been done, such as identification of resistance sources (Colon and Budding, 1988; Colon et al., 1995c; Douches et al., 2001a), components of resistance (Colon et al., 1995a, 1995b, 1995c), and phenotypic stability of resistance (Haynes et al., 1998). These reported late blight resistance sources are of different origin and ploidy levels and have variable levels of resistance. Combining these sources in a breeding program will establish a broad genetic base in the cultivated potato from which the probability of selecting superior offspring is increased. Moreover, improvements in yield, adaptation, tuber quality, and disease resistance can be achieved by broadening the genetic base of potato breeding populations (Mendoza and Haynes, 1974).

The objective of this study was to assess the genetic diversity of this potato germplasm with reported late blight resistance, using a set of isozyme loci and microsatellite markers. These data can be used to characterize this germplasm that can be introgressed into cultivated gene pools to enhance late blight breeding efforts and concurrently broaden the genetic base of cultivated potatoes.

Materials and Methods

The potato late blight resistant germplasm used in this study was identified using a greenhouse fine-screening technique (Douches et al., 2001a) and represents different origins and ploidy levels (Table 1). Of the total of 60 evaluated clones, 36 were from South America (two species), 14 were tetraploid hybrids (wild x cultivated potato), and 10 were tetraploid advanced breeding clones or cultivars from North America (five), Poland (three), Sweden (one), and Russia (one). For simplification, all accessions or cultivars will only be referred to by their respective code identification (Table 1).

The genetic diversity of the potato late blight resistant germplasm was assessed using isozyme and microsatellite markers. The isozyme analysis was carried out using crude protein extraction from a newly expanded leaflet (≈120 mg), resolved in a horizontal 10% starch gel by electrophoresis with two buffer systems. Each accession or cultivar was sampled and run twice. Tissue processing, electrophoresis, staining, and nomenclature were done as described in Douches and Quiros (1988). Eleven isozyme loci of seven enzyme systems were scored according to Douches and Quiros (1988) and Douches and Ludlam (1991). Malate dehydrogenase (*Mdh-1* and *Mdh-2*), 6-phosphogluconic dehydrogenase (*6-Pgdh-3*), phosphoglucose isomerase (*Pgi-1*), and isocitric acid dehydrogenase (*Idh-1*) were resolved with a histidine-citrate pH 5.7 buffer

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Table 1. Pedigree of *Solanum* accessions or cultivars used in this study and their origin, ploidy level, and clone identification.

Accessions or cultivars	Code ID[z]	Pedigree	Origin	Ploidy level (2*n*)	Clone ID number
PI 595507	ber1	*S. berthaultii* Hawkes	South America	2*x*	3,8,12,16,20
PI 498104	ber2	*S. berthaultii*	South America	2*x*	19
PI 458358	mcd1	*S. microdontum* Bitter	South America	2*x*	8
PI 473170	mcd2	*S. microdontum*	South America	2*x*	17,33
PI 498124	mcd3	*S. microdontum*	South America	2*x*	1,5,6,7,12,17,20,21,25
PI 595509	mcd4	*S. microdontum*	South America	2*x*	12
PI 595510	mcd5	*S. microdontum*	South America	2*x*	10,14,16,19,22
PI 595511	mcd6	*S. microdontum*	South America	2*x*	2,3,5,13,14,18,22,23,25
PI 595512	scr1	*S. sucrense* Hawkes	South America	4*x*	16,17,19
VIR 595516	K97	((*S. megistacrolobum* Bitter x Gatchinski) x Umbra) x Fausta	Russia	4*x*	10,18
VIR 595517	K98	(*S. verrucosum* Schltdl. x MPI 50.140/5) x MPI 50.140/5	Russia	4*x*	1,17
VIR 595518	K99	(*S. microdontum* x Atzimba) x Earlaine	Russia	4*x*	1
VIR 595519	K100	(*S. polytrichon* Rydb. x Anoka) x Runo	Russia	4*x*	2,6
VIR 595520	K101	((*S. microdontum* x MPI 50.140/5) x Boone) x Desiree	Russia	4*x*	9
VIR 595521	K102	*S. berthaultii* x *S. tuberosum* subsp. *andigena* Hawkes	Russia	4*x*	5
VIR 595522	K103	*S. vernei* Bitter & Wittm. x MPI 50.140/5	Russia	4*x*	19
VIR 595523	K104	(*S. gourlayi* Hawkes x Hannibal) x Hannibal	Russia	4*x*	8,20,21
VIR 595524	K105	*S. berthaultii* x Taiga	Russia	4*x*	20
AWN86514-2[y]	AWN86514-2	KSA195-96 x Ranger Russet	USDA, Aberdeen, ID	4*x*	
B0718-3[y]	B0718-3	B0286-3 x B9933-27	USDA, Beltsville, MA	4*x*	
Bertita	Bertita	Ac25953 x Ac25959 Ac = (*S. tuberosum* subsp. *andigena* x (*S. demissum* Lindley x *S. tuberosum* L.))	Mexico	4*x*	
Bzura[y]	Bzura	((PG-232 x (PG-96 x Mira-1)) x ((Prosna x (Z10465 x Z951))	Poland	4*x*	
Greta[y]	Greta	Unica x ((Magnum x (Early Rose x Patersis Victoria))	Sweden	4*x*	
Libertas[y]	Libertas	((Record x (Trenctria x Energie)) x ((Bravo x Energie) x (Rode Star x Pepo))	Netherlands	4*x*	
MSG274-3[y]	MSG274-3	Tollocan x Chaleur	MSU Breeding Program	4*x*	
Stobrawa[y]	Stobrawa	Mira-1 x ((MPI55.957/54 x (MPI50.140/5 x MPI44.1016/10))	Poland	4*x*	
Tollocan	Tollocan	((58-ER-1 x (Loman x HOL-32)) x ((((Juanita x (((Loman x ((Anita x (AC25953 x USDA2131-3))))	Mexico	4*x*	
Zarevo	Zarevo	7692C/68 (*S. demissum* Lindley, *S. tuberosum* subsp. *andigena* S. *leptophyes* Bitter, *S. tuberosum*) x Berka (*S. demissum*)	Russia	4*x*	

[z]Code is for USDA Plant Introduction identification in this paper, but the clone identification number is the same as in the U.S. Potato Genebank, NRSP-6, WI.
[y]More detailed pedigree is available.

system (Stuber et al., 1988). Diaphorase (*Dia-1* and *Dia-2*), glutamate oxaloacetate transaminase (*Got-1* and *Got-2*) and phosphoglucomutase (*Pgm-1* and *Pgm-2*) were resolved with a lithium-borate pH 8.3 buffer system (Stuber et al., 1988). Nine of these isozyme loci have been previously mapped to six distinct potato linkage groups. *Mdh-2* and *Idh-1* were mapped to linkage group I; *Pgm-1* to III; *Pgm-2* to IV; *Mdh-1*, *6-Pgdh-3* and *Dia-1* to V; *Got-2* to VII; and *Got-1* to VIII (Bonierbale et al., 1988; Freyre and Douches, 1994). For statistical analysis, each allele was recorded as 1 for presence or 0 for absence.

DNA amplification, using nine pairs of microsatellite primers, was carried out in a total volume of 20 mL containing 1X REDTaq™ PCR reaction buffer, 1 unit of REDTaq™ DNA polymerase (Sigma-Aldrich Co., St. Louis), 20 ng of each dNTP, 25 ng of each microsatellite primer, and 50 ng of template DNA. The sequence of eight pairs of primers (*G28WXST*, *STPROINI*, *ST STP*, *STACCAS3*, *STWIN12G*, *POTM 1-2*, *ST13ST*, and *STLS1*) is published (Ashkenazi et al., 2001). The other primer combination, potato inhibitor *IIK* locus, was also used by Provan et al. (1996) and Milbourne et al. (1997), and the sequences are identified as STIIKA. Four microsatellites have a known position on the potato linkage map. The loci G28WXST, STACCAS3 and POTM 1-2 map to linkage groups VIII, VII, and VI, respectively (Veilleux, personal communication). The potato inhibitor IIK locus maps to the linkage group III (Meyer et al., 1998).

All amplifications were carried out on a Thermolyne Amplitron® (Barnstead™ Thermoline Corp., Dubuque, Iowa) thermal cycler. The protocol was as follows: 1) initial denaturation at 94 °C for 4 min; 2) 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 1.5 min; and 3) final extension at 72 °C for 5 min. The completed reaction products were held at 4 °C until electrophoretic separation using a 3% Metaphor™ Agarose (FMC Bioproducts, Rockland, Maine) gel with TBE (90 mM trisborate, 90 mM boric acid, and 2 mM EDTA) buffer. The gels were run at 100 V for 4 h at 10 °C, stained with ethidium bromide (1 $\mu g \cdot mL^{-1}$) for 45 min, visualized under UV light, and photographed. Each microsatellite fragment was scored as 1 for presence and 0 for absence. Fragment sizes were estimated using a 50 bp DNA ladder (Gibco BRL, Grand Island, N.Y.) in each gel.

For statistical analysis, data were scored as the presence or absence of alleles (isozymes) or fragments (microsatellites). The mean number of alleles per locus, the proportion of polymorphic loci and the mean expected heterozygosity (Nei, 1972) were estimated per accession or group of clones based on allelic frequency data. For these parameters, we did not consider allele dosage for isozymes and we evaluated DNA fragments per pair of microsatellite primers. Genetic similarity was calculated using Nei and Li's (1979) computation:

$$GS_{xy} = 2N_{xy} / (N_x - N_y)$$

where N_x and N_y are the number of bands for each genotype, and N_{xy} is the number of bands in common between the two genotypes. The unweighted pair group means analysis (UPGMA) results were used to draw the dendrogram. The distance matrix and the dendrogram were constructed with NTSYS-pc version 1.7 (Rohlf, 1992). Cophenetic correlation coefficients were used to measure the distortion between the similarity matrix and the resultant dendrogram (Rohlf and Sokal, 1981).

Results and Discussion

General genetic diversity in the late blight resistant germplasm. The total mean number of alleles per locus for isozymes was 3.18 and for microsatellites was 4.67 (Table 2). To have a better understanding of the genetic diversity, we divided the germplasm by species or group of clones. For example, there were 27 *S. microdontum* Bitter clones that we subdivided by PI numbers. We also separated cultivated PI (Russian hybrids) from cultivated potato. Based upon both marker systems, *S. sucrense* Hawkes (wild tetraploid) had one of the lowest mean numbers of alleles per locus. On the

Table 2. Number of evaluated clones per accession or group of genotypes, number of alleles/locus, proportion of polymorphic loci and expected heterozygosity in the potato late blight resistant germplasm.

Germplasm	Clones	Alleles/locus (no.)		Polymorphic loci (%)		Expected heterozygosity[z]	
code[y]	(no.)	Isozymes	Microsatellites	Isozymes	Microsatellites	Isozymes	Microsatellites
Ber1, ber2	6	2.36	2.78	60	38	0.687	0.625
Mcd1, mcd2, mcd4	4	1.91	3.00	40	36	0.688	0.580
Mcd3	9	2.18	3.67	57	57	0.704	0.532
Mcd5	5	1.64	3.22	31	43	0.678	0.593
Mcd6	9	1.91	3.78	37	60	0.579	0.554
Scr1	3	1.45	2.78	11	21	0.566	0.530
Russian hybrids	14	2.45	4.00	63	71	0.626	0.536
Cultivated potato	10	2.36	4.00	51	69	0.587	0.556
Total	60	3.18	4.67				

[z]Expected heterozygosity is the average of Hn within diploid or tetraploid groups. $Hn = 1 - \Sigma p^2$ (Powel et al., 1996).
[y]Ber = *S. berthaultii*, mcd = *S. microdontum*, and scr = *S. sucrense*; numbers refer to different accessions. See Table 1 for details.

other hand, Russian hybrids and cultivated potatoes showed the highest number of alleles per locus. *Solanum sucrense* also had the smallest proportion of polymorphic loci, indicating low genetic diversity among evaluated clones. In other marker analyses, *S. sucrense* was previously assessed to have higher levels of genetic diversity (Bamberg et al., 2000; Hosaka and Hanneman, 1991). An underestimation of *S. sucrense* diversity was expected, since only three clones of one accession were evaluated. The highest proportion of polymorphic loci was observed in *S. berthaultii* Hawkes and Russian hybrids for isozymes, and Russian hybrids and cultivated potato for microsatellites. No trend was observed to distinguish species or group of clones using mean of expected heterozygosity (Table 2). In general, there were accessions or wild species with a similar level of genetic diversity compared to cultivated potato. However, direct comparisons between diploid and tetraploid species should not be made, since tetraploids have the potential to have greater heterozygosity than diploids. In summary, genetic markers showed that a high level of genetic diversity is distributed among wild diploid, and wild tetraploid and cultivated *Solanum* species with reported late blight resistance.

Sources of unique allozyme alleles. A total of 35 allozymes was detected in the late blight resistant germplasm. The presence of allozymes that were observed in the cultivated group was similar to the Douches et al. (1991) study of 112 North American cultivars and advanced breeding clones. Evaluating the genetic diversity in 2379 accessions of *S. tuberosum* subsp. *andigena* Hawkes, Huamán et al. (2000) identified 38 allozymes; however, two allozymes had a frequency of only 0.02%.

Isozyme analysis revealed numerous allozymes in the wild germplasm that were not found in the cultivated potato group. Nine allozymes (26%), absent from the cultivated potato group, were present in other tetraploid clones and diploid *Solanum* species (Table 3). Ber1 and ber2 had six alleles not present in cultivated potatoes, and *Pgi-1*^{5} was unique to *S. berthaultii*. *Solanum microdontum* had seven allozymes that were absent in cultivated potatoes. Allozymes unique to *S. microdontum* were present in mcd6 (*Mdh-1*^{6}) and mcd5 (*Pgm-2*^{1}). *Pgi-1*^{3} was found only in one clone (mcd3-7). *Solanum sucrense* had only one allozyme (*6-Pgdh-3*^{3}) not found in the cultivated potato group. The Russian hybrids had three alleles not found in cultivated potatoes, but these alleles were found in only one clone and were also present in wild species.

Sources of unique microsatellite fragments. Forty-two of the 43 DNA fragments from nine pairs of microsatellite primers were consistently amplified and polymorphic. High levels of polymorphism were also found in other genetic studies using microsatellites in cultivated potatoes (Meyer et al., 1998; Milbourne et al., 1997; Provan et al. 1996). Six fragments were absent in the cultivated potato group (Table 4). The Russian hybrids did not possess any unique fragments, but did have two fragments that were not found in the cultivated potato group. *Solanum microdontum* possessed five fragments absent from the cultivated potatoes, and three fragments associated with three microsatellite loci (G28WXST, POTM1-2, and STLS1) were present in all evaluated clones.

Of the 23 alleles and microsatellite fragments absent in cultivated potatoes, three alleles and two DNA fragments were present in Russian hybrids. These results were, in part, expected since Russian hybrids are hybrids of wild and cultivated potato. However, only

Table 3. Unique allozymes present in unadapted late blight resistant germplasm and absent in cultivated potato.

		Percent of individuals that carry the unique allozyme							
		Diploid *Solanum* species ($2n = 2x$)					Tetraploid germplasm ($2n = 4x$)		
Isozyme system[y]	Unique allozyme	Ber[z] 1 & 2	Mcd[z] 1, 2 & 4	Mcd 3	Mcd 5	Mcd 6	Scr[z] 1	Russian hybrids	Cultivated potato[x]
MDH	*Mdh-1*^{6}	0	0	0	0	89	0	0	0
MDH	*Mdh-2*^{1}	17	0	0	20	100	0	7	0
6-PGDH	*6-Pgdh-3*^{3}	83	0	11	0	78	100	0	0
PGI	*Pgi-1*^{3}	33	0	11	0	0	0	0	0
PGI	*Pgi-1*^{5}	17	0	0	0	0	0	0	0
GOT	*Got-2*^{1}	17	0	0	0	0	0	7	0
GOT	*Got-2*^{4}	0	75	78	80	0	0	7	0
GOT	*Got-2*^{6}	33	0	0	0	67	0	0	0
PGM	*Pgm-2*^{1}	0	0	0	40	0	0	0	0
No. of alleles	9	6	1	3	3	4	1	3	0

[z]Ber = *S. berthaultii*, mcd = *S. microdontum*, and scr = *S. sucrense*; numbers refer to different accessions. See Table 1 for details.
[y]MDH = malate dehydrogenase, 6-PGDH = 6-phosphogluconic acid dehydrogenase, PGI = Phosphoglucose isomerase, GOT = glutamate oxaloacetate transminase, and PGM = phosphoglucomutase.
[x]From Table 1: AWN86514-2, B0718-3, Bertita, Bzura, Greta, Libertas, MSG274-3, Stobrawa, Tollocan, and Zarevo.

Table 4. Unique microsatellite fragments (bp) present in unadapted late blight resistant germplasm and absent in cultivated potato.

		Percent of individuals that carry the unique microsatellite fragments							
		Diploid *Solanum* species ($2n = 2x$)					Tetraploid germplasm ($2n = 4x$)		
Microsatellite locus	Unique fragments	Ber[z] 1 &2	Mcd[z] 1, 2 & 4	Mcd 3	Mcd 5	Mcd 6	Scr[z] 1	Russian hybrids	Cultivated potato[y]
G28WXST	650	0	100	100	60	56	67	57	0
STSTP	240	0	0	0	0	22	0	14	0
STWIN12G	280	0	0	0	0	56	0	0	0
POTM 1-2	145	0	75	78	100	67	0	0	0
ST13ST def 4	245	0	0	0	0	0	33	0	0
STLS1	90	17	0	89	20	100	0	0	0
No. fragments	6	1	2	3	3	5	2	2	0

[z]Ber = *S. berthaultii*, mcd = *S. microdontum*, and scr = *S. sucrense*; numbers refer to different accessions. See Table 1 for details.
[y]From Table 1: AWN86514-2, B0718-3, Bertita, Bzura, Greta, Libertas, MSG274-3, Stobrawa, Tollocan, and Zarevo.

four hybrids evaluated here (K99, K101, K102, and K105) have common wild species in their pedigrees. Therefore, the isozyme and microsatellite analyses show that Russian hybrids have incorporated some genetic diversity from wild species, and this diversity is much more accessible in the genetic background to combine with cultivated potatoes.

Genetic similarity among late blight resistant clones. The assessment of either 35 allozymes or 42 polymorphic microsatellite alleles was insufficient to completely discriminate diploid from tetraploid species. The cophenetic correlation coefficient between the similarity matrix and the dendrogram was 0.79 and 0.78, respectively, for isozymes and microsatellites. *Solanum microdontum*, *S. berthaultii,* and *S. sucrense* belong to the series *Tuberosa* (wild), and *S. tuberosum* subsp. *tuberosum* belongs to the series *Tuberosa* (cultivated) in the subsection *Petotae* (Hawkes, 1994). In both dendrograms, clones of *S. microdontum* were clustered in different groups with *S. berthaultii*, *S. sucrense,* or cultivated potatoes (data not shown).

A combined data analysis from isozyme and microsatellite markers was able to distinguish diploid from tetraploid species and also separate *S. microdontum* from *S. berthaultii*. The cophenetic correlation coefficient between the similarity matrix and the dendrogram data was 0.82. The late blight resistant germplasm formed three groups, two with each diploid wild species (*S. berthaultii* and *S. microdontum*) and one with tetraploid germplasm (*S. sucrense*, Russian hybrids, and cultivated potatoes) (Fig. 1). The genetic similarity between *S. berthaultii* and *S. microdontum* groups, excluding mcd4, was 0.58. Within *S. microdontum*, four distinct subgroups were formed according to accessions (mcd1, mcd2, and mcd3; mcd4; mcd5; and mcd6). Overall, the maximum genetic similarity (0.93) was between Mcd3-1 and Mcd3-5. The tetraploid germplasm group (group 2) formed two subgroups: one with the wild tetraploid species (*S. sucrense*) and another with Russian hybrids and cultivated potatoes, with a genetic similarity of ≈0.65. All cultivars were grouped with Russian hybrids with a genetic similarity of ≈0.77. The cultivars B0718-3, 'Tollocan', and 'AWN86514-2', reported as highly resistant to late blight, separated into three subgroups among cultivated potatoes. Moreover, both *S. berthaultii* and *S. microdontum* had more genetic diversity between and, in some cases, within accessions than cultivated potatoes.

Besides the genetic diversity quantified using isozyme and microsatellite markers, different types of late blight resistance also could be present within wild and cultivated germplasm. Several minor genes with additive effects are involved in the late blight resistance of *S. tuberosum* subsp. *andigena* (part of the pedigree of K102, 'Bertita', and 'Zarevo'), whereas major resistant genes are involved in the resistance of *S. sucrense* (Colon and Budding, 1988). If the genetic diversity present in the tetraploid germplasm might also include different genes for late blight resistance, clones from different subgroups should be hybridized as a strategy to combine potential resistance sources.

Improvements of late blight resistant cultivars with broad genetic base. Pedigree (Mendoza and Haynes, 1974; Plaisted and Hoopes, 1989) and chloroplast diversity analysis (Douches et al., 1991; Provan et al., 1999) showed that a high genetic similarity characterizes many potato cultivars released in the last century. High genetic uniformity can result in vulnerability to diseases, pests, and abiotic factors, and reduces gain from selection (Mendoza, 1989). Consequently, increasing genetic diversity in the cultivated potato gene pool is a goal in many breeding programs. Moreover, improving genetic diversity for non-T-type cytoplasm is important to reduce breeding problems associated with male sterility (Provan et al., 1999). The species studied here can be used to enhance efforts to breed late blight resistant tetraploid germplasm and to broaden the genetic base of the cultivated potato using simple crossing schemes ($4x - 4x$ and $4x - 2x$).

The late blight resistant germplasm differs in the level and source of resistance (Douches et al., 2001a; Haynes et al., 1998). *Solanum microdontum* (mcd3, mcd5, and mcd6) and *S. sucrense* had the highest level of resistance to the US8 genotype of *P. infestans*. Also, *S. berthaultii* and other *S. microdontum* accessions and the Russian hybrids had moderate to high resistance (Douches et al., 2001a). The source of resistance for the hybrids K98 and K100 is Mexican *Solanum* species, whereas all other hybrids (K97, K99 and K101–K105) have South American wild species in their pedigrees. The hybrid K102 is a cross between two South American *Solanum* species (*S. berthaultii* and *S. tuberosum* subsp. *andigena*) in which both parents could be contributing to the resistance. Among the cultivated germplasm, 'AWN86514-2' has high foliar and partial tuber resistance and has *S. acaule* Bitter, *S. demissum* Lindley, *S. phureja* Juz. & Bukasov, *S. microdontum*, *S. stoloniferum* Scheldl. & Bouche, and *S. tuberosum* subsp. *andigena* in its pedigree (Corsini et al., 1999), which can be contributing to its resistance. B0718-3 has foliar resistance to late blight derived from an Indian *S. tuberosum* introduction (PI383470B) selected in Mexico (Goth and Haynes, 1997). 'Bertita' and 'Zarevo' have *S. demissum* and *S. tuberosum* subsp. *andigena* in their pedigrees, both well-known sources of late blight resistance; these cultivars along with 'Bzura', 'Bertita', 'Greta', 'Libertas', and 'Stobrawa' exhibit foliar late blight resistance in field evaluations (Haynes et al., 1998). 'Libertas' is considered a R-gene free cultivar with both foliar (Colon et al., 1995b) and tuber resistance (Platt and Tai, 1998) and probably shares resistant genes with 'Pimpernel', 'Robijn', 'Populair', and 'Surprise' (Colon et al., 1995b). The advanced clone 'MSG274-3' is directly descended from the Mexican cultivar 'Tollocan'. 'Tollocan' and 'MSG274-3' have high foliar resistance to late blight in greenhouse and in field evaluations (Douches et al., 2001b).

The genetic diversity analysis showed that this germplasm could offer unique opportunities for late blight resistance breeding and that conventional breeding strategies may be useful to introgress and combine these different resistance sources. For a short-term strategy, combining sources of high levels of late blight resistance from different subgroups, such as 'MSG274-3' and 'Tollocan', with 'AWN86514-2' or with Russian hybrids possessing moderate resistance to late blight would be more productive than combining with *S. sucrense*, a wild $4x$ species. A long-term breeding strategy would be to introgress the resistance from *S. microdontum* and *S. berthaultii*. The fact that two clones from the same accession of *S. microdontum* had different quantitative trait loci (QTL) conferring late blight resistance (Sandbrink et al., 2000), and that there are separate clusters in the dendrogram for the different *S. microdontum* accessions, suggests that multiple selections of *S. microdontum* should be used according to the clustering. This strategy should maximize the diversity of the late blight resistant sources.

Within germplasm having moderate to high resistance to late blight it is difficult to differentiate resistant individuals and almost impossible to select recombinant offspring based upon phenotypic tests. The association between markers and QTL permits the selection of individuals with desirable QTL from different parents (Meyer et al., 1998). Therefore, mapping QTL conferring late blight resistance is required to analytically pyramid genes from diverse genetic backgrounds.

In summary, there was high genetic diversity within and among accessions and species, and between ploidy levels of the late blight resistant germplasm. This genetic diversity should be exploited using both short- and long-term strategies to broaden the genetic base of the potato gene pool and to combine different sources of resistance in a breeding program to achieve stronger and more durable resistance in the offspring.

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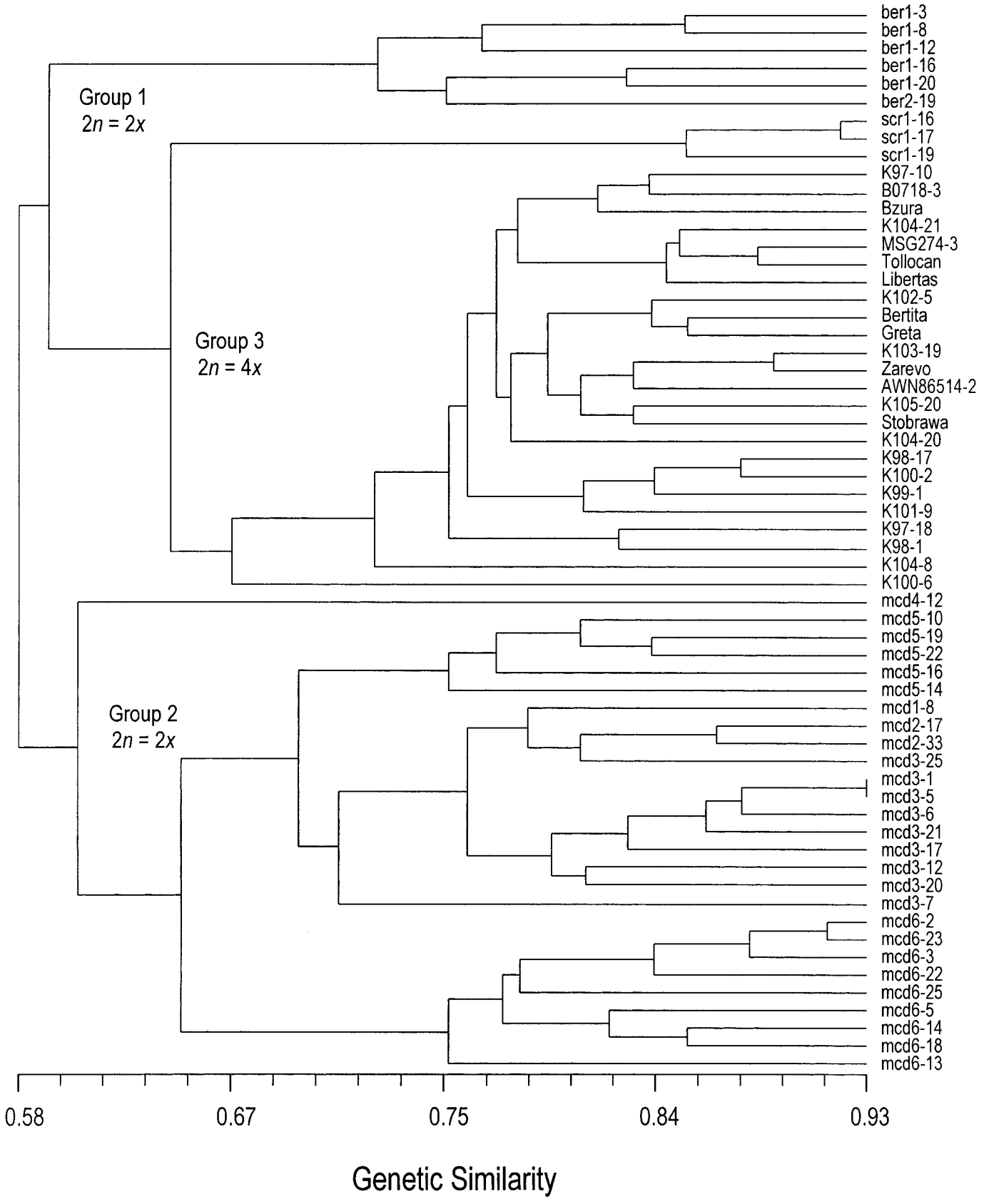

Fig. 1. Genetic similarity among diploid and tetraploid potato germplasm with reported late blight resistance based on 35 allozymes, encoding 11 isozyme loci, and 42 polymorphic DNA fragments in nine pairs of microsatellite primers. See Table 1 for codes for clones and pedigrees.

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