T-type Chloroplast DNA in *Solanum tuberosum* L. ssp. *tuberosum* Was Conferred from Some Populations of *S. tarijense* Hawkes

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ABSTRACT

The highly heterozygous and tetraploid nature of potato (*Solanum tuberosum* L. ssp. *tuberosum*) has hampered discovery of its wild ancestral species. Chloroplast DNA is a very reliable indicator to trace maternal ancestry of crops. Most of the common potato (grown worldwide) has unique, T-type chloroplast DNA derived from Chilean cultivated potato (both are *S. tuberosum* ssp. *tuberosum*). Analyzing seven different chloroplast DNA markers, I found all the T-type accessions of cultivated potatoes shared the same chloroplast DNA haplotype only with some accessions of *S. tarijense* Hawkes and its derived hybrids. Thus, I conclude that some populations of *S. tarijense* acted as the maternal ancestor of potato.

RESUMEN

La alta heterozigosis y naturaleza tetraploide de a papa (*Solanum tuberosum* L. ssp. *tuberosum*) ha dificultado el descubrimiento de su ascendiente silvestre ancestral. El DNA de cloroplastos es un indicador muy confiable para buscar el origen del ancestro materno de los cultivos. La mayoría de papas comunes (cultivadas mundialmente) tienen un singular ADN de cloroplastos tipo T derivado de la papa chilena cultivada (*S. tuberosum* ssp. *tuberosum*). Al analizar siete marcadores diferentes de ADN de cloroplastos se encontró que todas las accesiones tipo T de papas cultivadas compartían el mismo haplotipo de ADN de cloroplasto solamente con algunas accesiones de *S. tarijense* Hawkes y sus híbridos derivados. Así, se concluye que algunas poblaciones de *S. tarijense* actuaron como ancestros maternos de la papa.

INTRODUCTION

Crops were domesticated from wild species 15,000-10,000 years ago (Harlan 1992). Through thousands of years' variety differentiation, genetic constituents of a crop became complex and its history difficult to trace back. Chloroplast DNA is inherited maternally in most angiosperms and evolves relatively slowly; thus, it is a reliable indicator to trace maternal ancestry of crops (Palmer et al. 1988). The common potato (*Solanum tuberosum* L. ssp. *tuberosum*), grown worldwide, is a highly heterozygous tetraploid crop, whereas most of the potato varieties have unique, T-type chloroplast DNA (Hosaka and Hanneman 1988; Waugh et al. 1990; Powell et al. 1993; Bryan et al. 1999; Provan et al. 1999; Lössl et al. 2000) derived from Chilean cultivated potato (also *S. tuberosum* L. ssp. *tuberosum*) (Hosaka and Hanneman 1988). Although Chilean ssp. *tuberosum* seemed to be derived from Andean cultivated tetraploid potato (*S. tuberosum* ssp. *andigena* Hawkes) (Hawkes 1956), the two subspecies were distinguished from each other by nuclear DNA microsatellites (Raker and Spooner 2002) and a frequency of T-type chloroplast DNA (Hosaka and Hanneman 1988). The T-type chloroplast DNA

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According to the latest taxonomic treatment by Huamán and Spooner (2002), "common potato," "Chilean cultivated potato," "Andean cultivated tetraploid potato," and "cultivated diploid potato" in this text are all classified into *Solanum tuberosum*, and divided under the same species name as a cultivar-group (not specifically named), Chilotanum Group, Andigenum Group, and Stenotomum Group, respectively.

was rarely found in central Andes where potato was domesticated (Hawkes 1990); it was found in only five out of 113 strains of *S. tuberosum* ssp. *andigena* (Hosaka and Hanneman 1988) and one out of 54 strains of the cultivated diploid potato (*S. stenotomum* Juz. et Buk.) (Hosaka 1995). All these Andean T-type strains of cultivated potatoes were collected from the south of central Bolivia. Wild ancestral species that conferred the T-type chloroplast DNA to cultivated potatoes were not known (Hosaka 1995).

The T-type chloroplast DNA was distinguished by the presence of a 241 base pairs (bp) deletion from the other chloroplast DNA found among the Andean potatoes (Hosaka et al. 1988; Kawagoe and Kikuta 1991). In a previous paper (Hosaka 2002), an extensive survey was made by a simple polymerase chain reaction (PCR) assay using primers flanking the deleted region of chloroplast DNA for 566 accessions of 35 wild species, which covered almost all available wild species from central Bolivia to northern Argentina, and revealed that 16 out of 80 accessions of *S. berthaultii* Hawkes, *S. neorossii* Hawkes et Hjerting and *S. tarijense* Hawkes shared this deletion, thus recognizing these as T-type chloroplast DNA holders.

In this paper, a high-resolution chloroplast DNA marker system was employed for a total of 137 accessions to determine specifically a wild maternal ancestor of the common potato.

MATERIALS AND METHODS

A total of 137 accessions were used in this study (Table 1); they were *S. berthaultii* (30 accessions), *S. neorossii* (five accessions), *S. tarijense* (62 accessions), putative natural hybrids of *S. berthaultii* × *S. tarijense* (25 accessions), *S. tuberosum* ssp. *tuberosum* (six Chilean primitive accessions and one Japanese advanced cultivar) and ssp. *andigena* (three Argentine and two Chilean accessions previously identified as T-type chloroplast DNA holders by Hosaka and Hanneman [1988]), one accession of *S. stenotomum* (only accession having T-type chloroplast DNA in this species, identified by Hosaka [1995]), and one accession each of *S. phureja* Juz. et Buk. (a cultivated diploid clone having S-type chloroplast DNA) and *S. chacoense* Bitter (a wild diploid clone having W-type chloroplast DNA). Species or hybrid status for each accession of *S. berthaultii*, *S. neorossii*, *S. tarijense*, and *S. berthaultii* × *S. tarijense* hybrids was solely based on "Inventory of Tuber-bearing *Solanum* Species" (Bamberg et al. 1996).

Most accessions were obtained as seeds from the Potato Introduction Station (NRSP-6), Sturgeon Bay, Wisconsin, USA. One *S. stenotomum* accession (CIP 704089) as seeds and one Chilean *S. tuberosum* ssp. *tuberosum* (CIP 703254) as DNA samples were from the International Potato Center, Lima, Peru.

For most accessions, fresh leaves were collected from many young seedlings and bulked for DNA extraction (Hosaka 2002). PCR amplification was performed as described previously (Hosaka 2002) with primer pairs shown in Table 2. The primer pair for H1 locus was the same as the one used in the previous study (Hosaka 2002), which amplified the deleted region. H2 and H3 marker loci were first identified as restriction fragment length polymorphisms detected in *Hae*III and *Dra*I restriction digests, respectively, among T-type chloroplast DNA holders. The recognition sites causing polymorphisms were estimated from the complete sequence of tobacco chloroplast DNA (Wakasugi et al. 1998), and primer pairs flanking the recognition sites were designed. PCR products from H2 and H3 loci were ethanol-precipitated and digested with restriction endonucleases *Hae*III and *Dra*I, respectively. PCR products from H1 locus and digested PCR products from H2 locus were separated by electrophoresis in 3% NuSieve 3:1 agarose (BioWhittaker Molecular Applications, Inc., Maine) gels. PCR products from H3 locus were separated in 1.6% agarose gels. Chloroplast DNA microsatellite markers flanking mononucleotide-repeated regions have been developed by Provan et al. (1999) from *Nicotiana tabacum* chloroplast DNA (NTCP markers). Four markers were chosen, which produced consistently reliable marker bands under my PCR conditions and showed polymorphisms among the present materials. These were separated on 4% denaturing polyacrylamide gels and visualized using silver staining (Bassam et al. 1991). PCR products were sequenced using ABI PRISM 310 Genetic Analyzer by the method of BigDye Terminator Cycle Sequencing, FS (Perkin Elmer).

RESULTS

Seven chloroplast DNA markers revealed 26 banding pattern types or bands (H1, two types; H2, two types; H3, four types; NTCP6, six bands; NTCP7, four bands; NTCP14, four bands; NTCP18, four bands). A previous sequence analysis (Hosaka 2002) indicated that the H1 marker amplified a 202 bp (type 1) or 443 bp fragment (type 2) (Figure 1), the former indicating the presence of 241 bp deletion, an indicator for T-type chloroplast DNA. All the other fragments with different sizes,

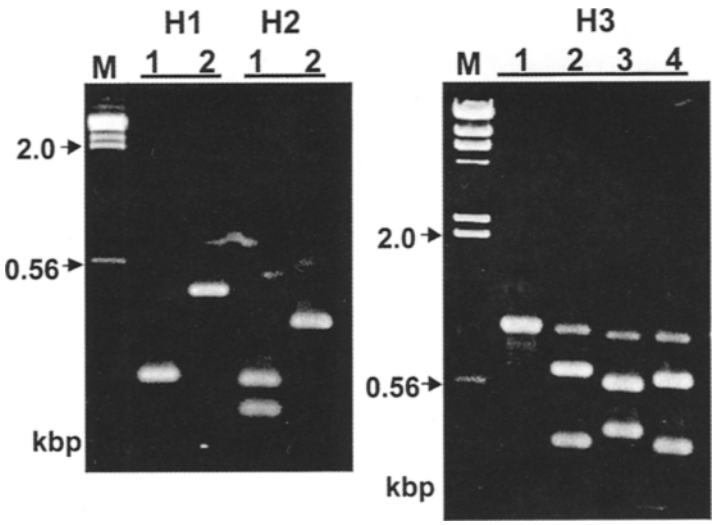

FIGURE 1.
Chloroplast DNA polymorphism types detected by H1, H2, and H3 markers. H1 marker bands were detected using undigested PCR products, while H2 and H3 marker bands were detected after digestion of PCR products with restriction endonucleases *Hae*III and *Dra*I, respectively. Lambda DNA *Hin*dIII digests were run on M.

TABLE 1—*Chloroplast DNA variation in* S. berthaultii, S. neorossii, S. tarijense, S. berthaultii *x* S. tarijense hybrids, *and some other species.*

		Marker phenotype[2]							Haplo-
Accesssion	Origin[1]	H1	H2	H3	NTCP6	NTCP7	NTCP14	NTCP18	type[3]
S. tuberosum									
ssp. *tuberosum*									
Konafubuki	Japan	1	1	1	173	173	149	188	1
CIP703254	C, ?	1	1	1	173	173	149	188	1
PI 245319	C, Maule	1	1	1	173	173	149	188	1
PI 245793	C, Los Lagos	1	1	1	173	173	149	188	1
PI 245835	C, Chiloé	1	1	1	173	173	149	188	1
PI 245839	C, Chiloé	1	1	1	173	173	149	188	1
PI 245929	C, Tarapacá	2	2	3	127	173	150	186	25
ssp. *andigena*									
PI 209421	A, ?	1	1	1	173	173	149	188	1
PI 234592	A, ?	1	1	1	173	173	149	188	1
PI 280936	A, ?	1	1	1	173	173	149	188	1
PI 245317	C, Maule	1	1	1	173	173	149	188	1
PI 245816	C, Chiloé	1	1	1	173	173	149	188	1
S. stenotomum									
CIP704089	B, Potosi	1	1	1	173	173	149	188	1
S. phureja									
1.22		2	2	3	127	173	150	186	25
S. chacoense									
chc 525-3		2	2	1	175	174	152	187	23
S. berthaultii									
PI 265857	B, Cochabamba	2	2	2	172	174	149	188	24
PI 265858	B, Cochabamba	2	2	2	172	174	149	188	24
PI 310926	B, Cochabamba	2	2	2	172	174	149	188	24
PI 473331	B, Cochabamba	2	2	1	172	174	149	189	8
PI 498095	B, Cochabamba	2	2	1	174	175	151	187	20
PI 498101	B, Cochabamba	2	2	2	172	174	149	188	24
PI 498102	B, Cochabamba	2	2	2	172	174	149	188	24
PI 498103	B, Cochabamba	2	2	2	172	174	149	188	24
PI 498104	B, Cochabamba	2	2	2	172	174	149	188	24
PI 498106	B, Cochabamba	2	2	1	173	174	149	188	11
PI 498107	B, Cochabamba	2	2	2	172	174	149	188	24
PI 498108	B, Cochabamba	2	2	2	172	174	149	188	24
PI 498109	B, Cochabamba	2	2	2	172	174	149	188	24

TABLE 1—*Continued.*

Accesssion	Origin[1]	Marker phenotype[2]							Haplo-type[3]
		H1	H2	H3	NTCP6	NTCP7	NTCP14	NTCP18	
PI 545849	B, Cochabamba	2	2	1	172	174	150	188	9
PI 545960	B, Cochabamba	2	2	2	172	174	149	188	24
PI 545961	B, Cochabamba	2	2	2	172	174	149	188	24
PI 558033	B, Cochabamba	2	2	2	172	174	149	188	24
PI 568918	B, Cochabamba	1/2	1/2	1	173/174	173/175	149/151	187/188	-
PI 568920	B, Santa Cruz	2	2	1	173	174/175	151/154	186/187	-
PI 283069	B, Chuquisaca	2	2	1	172	174	149	188	7
PI 283070	B, Chuquisaca	2	2	1	172	174	149	188	7
PI 473335	B, Chuquisaca	2	2	1	172	174	149	189	8
PI 527884	B, Chuquisaca	2	2	2	172	174	149	188	24
PI 545850	B, Chuquisaca	2	2	2	172	174	149	188	24
PI 545851	B, Chuquisaca	2	2	1	172	174	149	188	7
PI 545852	B, Chuquisaca	2	2	1	172	175	149	188	10
PI 545962	B, Chuquisaca	2	2	1	174	174	149	188	15
PI 218215	B, Potosi	2	2	2	172	174	149	188	24
PI 498075	B, Potosi	2	2	1	172	174	149	188	7
PI 527886	B, Potosi	2	2	2	172	174	149	188	24
S. neorossii									
PI 473201	A, Salta	2	2	1	175	174	151	187	22
PI 473202	A, Salta	2	2	1	175	174	151	187	22
PI 473428	A, Salta	1	2	4	173	173	149	188	5
PI 473429	A, Salta	1	2	4	173	173	149	188	5
PI 473529	A, ?	2	2	1	175	174	151	187	22
S. tarijense									
PI 265577	B, Cochabamba	2	2	1	172	174	149	188	7
PI 195206	B, Chuquisaca	2	2	3	127	173	150	186	25
PI 458394	B, Chuquisaca	2	2	1	172	174	149	189	8
PI 473332	B, Chuquisaca	2	2	1	172	174	149	188	7
PI 545920	B, Chuquisaca	2	2	2	172	174	149	188	24
PI 545921	B, Chuquisaca	2	2	2	172	174	149	188	24
PI 545922	B, Chuquisaca	1	1	1	173	173	149	188	1
PI 545923	B, Chuquisaca	2	2	2	172	174	149	188	24
PI 545924	B, Chuquisaca	2	2	1	174	174	149	188	15
PI 597774	B, Chuquisaca	2	2	1	172	174	150	188	9
PI 473336	B, Potosi	2	2	1	172	174	149	188	7
PI 414152	B, Tarija	1	2	1	174/175	173	150	188	-
PI 458395	B, Tarija	2	2	1	174	175	150	188	19
PI 498290	B, Tarija	1	1	1	173	173	149	188	1
PI 217458	A, Salta	2	2	1	173	174	150	188	12
PI 275154	A, Salta	2	2	3	127	173	150	186	25
PI 414148	A, Salta	2	2	1	172	174	149	188	7
PI 442689	A, Salta	1	1	1	173	173	149	188	1
PI 458365	A, Salta	2	2	1	172	174	149	188	7
PI 473217	A, Salta	1	1	1	173	173	149	188	1
PI 473227	A, Salta	1	2	1	173	172	150	188	2
PI 498399	A, Salta	2	2	1	173	174	150	188	12
PI 500054	A, Salta	2	2	1	174/176	174	150	187	-
PI 558130	A, Salta	2	2	1	173	175	151	187	14
PI 217457	A, Salta	2	2	2	172	174	149	188	24
PI 414149	A, Salta	1	1	1	173	173	149	188	1
PI 414150	A, Salta	2	2	1	172	174	149	188	7
PI 458364	A, Salta	2	2	1	172	174	149	188	7
PI 458366	A, Salta	2	2	1	173	174	150	188	12
PI 472815	A, Salta	2	2	1	173	175	151	187	14
PI 473216	A, Salta	2	2	1	172	173	152	186	6
PI 473218	A, Salta	1	1	1	173	173	149	188	1
PI 473226	A, Salta	2	2	1	172	174	149	188	7
PI 473228	A, Salta	1	1	1	173	173	149	188	1

TABLE 1—*Continued.*

Accesssion	Origin[1]	Marker phenotype[2]							Haplo-type[3]
		H1	H2	H3	NTCP6	NTCP7	NTCP14	NTCP18	
PI 473245	A, Salta	1	1	1	173	173	149	188	1
PI 500043	A, Salta	2	2	1	173	175	151	187	14
PI 500055	A, Salta	2	2	1	173	175	151	187	14
PI 558129	A, Salta	2	2	1	173	175	151	187	14
PI 566799	A, Salta	2	2	1	172	173	152	186	6
PI 473219	A, Salta	2	2	1	172	174	149	188	7
PI 473220	A, Salta	2	2	1	173	175	151	187	14
PI 473221	A, Salta	2	2	1	173	175	151	187	14
PI 473222	A, Salta	2	2	1	173	175	151	187	14
PI 473223	A, Salta	2	2	1	173	174	150	188	12
PI 473224	A, Salta	2	2	1	172	174	149	188	7
PI 473225	A, Salta	1	1	1	173	173	149	188	1
PI 473229	A, Salta	1	1/2	1	174	173	149/150	188	-
PI 473230	A, Salta	1	2	1	174	173	150	188	4
PI 473231	A, Salta	1	1	1	173	173	149	188	1
PI 473232	A, Salta	1	1	1	173	173	149	188	1
PI 473233	A, Salta	1	2	1	174	173	149	188	3
PI 473234	A, Salta	2	2	1	173	174	150	188	12
PI 473235	A, Salta	2	2	1	173	174	150	188	12
PI 473236	A, Salta	2	2	1	173	174	150	188	12
PI 473237	A, Salta	2	2	1	173	174	150	188	12
PI 473238	A, Salta	2	2	1	173	174	150	188	12
PI 473239	A, Salta	1	1	1	173	173	149	188	1
PI 473240	A, Salta	2	2	1	172	174	149	188	7
PI 473241	A, Salta	2	2	1	172/174	174/175	149	188	-
PI 473242	A, Salta	1/2	2	1	174	173/174	150	188	-
PI 473243	A, Salta	1	1	1	173	173	149	188	1
PI 473244	A, Salta	1	1	1	173	173	149	188	1
S. berthaultii x S. tarijense hybrid									
PI 310927	B, Cochabamba	2	2	2	172	174	149	188	24
PI 473330	B, Cochabamba	1	1	1	173	173	149	188	1
PI 498094	B, Cochabamba	2	2	1	174	175	151	187	20
PI 498096	B, Cochabamba	2	2	1	175	174	149	188	21
PI 498097	B, Cochabamba	2	2	1	174	174	150	189	17
PI 498098	B, Cochabamba	2	2	1	173	175	151	187	14
PI 498099	B, Cochabamba	1	1	1	173	173	149	188	1
PI 498100	B, Cochabamba	2	2	2	172/175	174	149	188	-
PI 498105	B, Cochabamba	1	1	1	173	173	149	188	1
PI 545885	B, Cochabamba	1/2	1/2	1	173	173/175	149/151	187/188	-
PI 473333	B, Chuquisaca	2	2	1	172	174	149	188	7
PI 473334	B, Chuquisaca	2	2	2	172	174	149	188	24
PI 473339	B, Chuquisaca	2	2	1	174	175	150	187	18
PI 473340	B, Chuquisaca	2	2	1	172	174	149	188	7
PI 545886	B, Chuquisaca	2	2	1	175	174	149	188	21
PI 473337	B, Potosi	1	1	1	173	173	149	188	1
PI 473338	B, Potosi	2	2	2	172	174	149	188	24
PI 545890	B, Potosi	2	2	1	172	174	149	188	7
PI 310971	B, ?	2	2	1	172	174	149	188	7
PI 310981	B, ?	2	2	1	174	175	150	188	19
PI 320257	B, ?	2	2	1	172	174	149	189	7
PI 558035	B, ?	2	2	1	172	174	149	188	7
PI 558036	B, ?	2	2	1	173	175	150	187	13
PI 208881	A, ?	2	2	1	172	174	149	188	7
PI 283075	?, ?	2	2	1	174	174	150	187	16

[1]A, Argentina; B, Bolivia; C, Chile

[2]H1, H2 and H3 are represented by banding pattern types shown in Figure 1. NTCP6 to18 are microsatellite markers represented by size in base pair.

[3]Haplotypes were determined based on the phenotype combinations shown in Table 3.

TABLE 2—*Primer pairs used in this study to detect chloroplast DNA polymorphisms.*

Locus	Primers (5'-3')	Annealing temp. ° C	Location[1]
H1	GGAGGGGTTTTTTCTTGGTTG AAGTTTACTCACGGCAATCG	55	*ndhC/trnV* intergenic region (53221-53665)
H2	GCATCGAGCGTGTTGTTGGA AGTCCACCGCGAAGACATTC	55	*rbc*L (57851-58184)
H3	CAGGGGTCCATTCCCTTGAC AGAAAGAAATCCACCAGGGC	60	*ycf4* and *ycf10* (63082-63490)
NTCP6	GATTCTTTCGCATCTCGATTC GGTTCGAATCCTTCCGTC	55	*rps16/trnQ* intergenic region (7262-7437)
NTCP7	TGATCCCGGACGTAATCC CGAATCCCTCTCTTTCCG	55	*psbI/trnS* intergenic region (8475-8649)
NTCP14	AATCCGTAGCCAGAAAAATAAA CCGATGCATGTAATGGAATC	55	*psbM/trnD* intergenic region (31580-31730)
NTCP18	CTGTTCTTTCCATGACCCCTC CCACCTAGCCAAGCCAGA	55	*psbC/trnS* intergenic region (36872-37057)

[1]Locations are indicated according to the tobacco chloroplast DNA (the accession number Z00044 in the EMBL Nucleotide Sequence Database) (Wakasugi et al. 1998). First and last nucleotide numbers are given in parentheses.

except for a 176 bp NTCP6 fragment from *S. tarijense* PI 500054, were sequenced (Figure 2).

The H2 marker amplified a single fragment in all accessions in common, but restriction digestion with *Hae*III yielded type 1 with 193 and 141 bp fragments, while type 2 without fragmentation (Figures 1 and 2). The H3 marker provided four different types after *Dra*I digestion (Figure 1); a 1047 bp fragment in type 1, 747 and 300 bp fragments in type 2, 575 and 454 bp fragments in type 3, and 711 and 300 bp fragments in type 4 (although both ends of H3-amplified fragments were not completely sequenced, the entire lengths were estimated on the basis of the corresponding tobacco sequence) (Figure 2). Even after prolonged digestion with *Dra*I, re-annealed fragments were always observed faintly in types 2 to 4. A sequence analysis indicated that all the amplified products from H3

TABLE 3—*Chloroplast DNA haplotypes and their distribution.*

Haplo-	Marker phenotype (type or fragment length in bp)							No. of accessions				
type	H1	H2	H3	NTCP6	NTCP7	NTCP14	NTCP18	*ber*	hybrid	*tar*	*nrs*	Others
1	1	1	1	173	173	149	188		4	14		6*tbr*, 5*adg*, 1*stn*
2	1	2	1	173	172	150	188			1		
3	1	2	1	174	173	149	188			1		
4	1	2	1	174	173	150	188			1		
5	1	2	4	173	173	149	188				2	
6	2	2	1	172	173	152	186			2		
7	2	2	1	172	174	149	188	4	7	11		
8	2	2	1	172	174	149	189	2		1		
9	2	2	1	172	174	150	188	1		1		
10	2	2	1	172	175	149	188	1				
11	2	2	1	173	174	149	188	1				
12	2	2	1	173	174	150	188			9		
13	2	2	1	173	175	150	187		1			
14	2	2	1	173	175	151	187		1	8		
15	2	2	1	174	174	149	188	1		1		
16	2	2	1	174	174	150	187		1			
17	2	2	1	174	174	150	189		1			
18	2	2	1	174	175	150	187		1			
19	2	2	1	174	175	150	188		1	1		
20	2	2	1	174	175	151	187	1	1			
21	2	2	1	175	174	149	188		2			
22	2	2	1	175	174	151	187				3	
23	2	2	1	175	174	152	187					1*chc*
24	2	2	2	172	174	149	188	17	3	4		
25	2	2	3	127	173	150	186			2		1*tbr*, 1*phu*

Species abbreviation: *S. berthaultii* (*ber*), putative hybrid of *S. berthaultii* x *S. tarijense* (hybrid), *S. tarijense* (*tar*), *S. neorossii* (*nrs*), *S. tuberosum* ssp. *tuberosum* (*tbr*), ssp. *andigena* (*adg*), *S. stenotomum* (*stn*), *S. chacoense* (*chc*), *S. phureja* (*phu*).

H2

Type	Sequenced
1	*tbr* (cv. Konafubuki, CIP703254), *stn* (CIP704089), *tar* (PI 442689, PI 498290, PI 545922, PI 473228), hybrid (PI 473337)
2	*chc* (chc525-3), *nrs* (PI 473201, PI 473428), *tar* (PI 414152, PI 473227)
2*	*phu* (1.22)

Sequence

```
Tobacco     GCATCGAGCGTGTTGTTGGAGAAAAAGATCAATATATTGCTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCT
All         ....^.....................................................................C

Tobacco     GTTACCAACATGTTTACTTCCATTGTAGGTAACGTATTTGGGTTCAAAGCCCTGCGCGCTCTACGTCTGGAAGATCTGCG
1 and 2     ........T...C.....................................................................
2*          ........T.........................................................................

Tobacco     AATCCCTCCTGCTTATGTTAAAACTTTCCAAGGTCCGCCTCATGGGATCCAAGTTGAAAGAGATAAATTGAACAAGTATG
1           .......GT.....................^^.C.................................................
2           .......GT.........................................................................
2*          .......A..........................................................................

Tobacco     GTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGAGCCGTTTATGAATGT
All         ...................................................A.................T.............

Tobacco     CTTCGCGGTGGACT
All         ..............
```

H3

Type	Sequenced
1	*tbr* (cv. Konafubuki)
2	*ber* (PI 218215)
3	*phu* (1.22)
4	*nrs* (PI 473428)

Sequence

```
Tobacco     TCCACGAGAAATTGAACAAAAGGCTGCTGAATTAGCCTATTTCTTGCGTGTACCAATTGAAGTATTTTGAGAAATTGAGA
All         ..................................................................T.........

Tobacco     TATCAG**************************************************************************
1, 2 and 3  *.....AACGTTCATTCAATTAAAGAAAACGTATATGAAAGAACCATAAAACGAAACTCTTTTTATGGTCTTTATGCGTT
4           *.....AACGTTCATTCAATTAAAGAAAACGTATATGAAAGAACCATAAAACGAAACTCTTTTTATGGTCTTTATGCG**

Tobacco     **************************************************************************
1, 2 and 3  TTATGGTCTTTATGCGCACGCAATTCAATAACAAATAACAAATCGAAATAATAGATTCATCTCAGACGGCAAACCATTTC
4           ***************CACGCAATTCAATAACAAATAACAAATCGAAATAATAGATTCATCTCAGACGGCAAACCATTTC

Tobacco     **************************************************************************
All         GAAAGCAAGAATTTTTTTTAGTTCAATATTTGTTGCAATTCAATAACAAATAACAAATCGAAATAATAGATTCATCTCAG

Tobacco     **************************************************************************
All         ACGGCAAACCATTTCGAAAGCAAGAATTTTTTTTAGTTCAATATTTGTTGGAATTGACACTTTAGATCCAGATAGATCGT

Tobacco     **************************************************************************
1 and 2     ATCTTGTAAATTTGAAATTCTTTCTTTTGTATTCTTTAATGACCAACAATTGGATTTCTTAATTAAATTGGATTTCTTAA
3           ATCTTGTAAATTTAAATTCTTTCTTTTGTATTCTTTAATGACCAACAATTGGATTTCTTAATTAAAT**********
4           ATCTTGTAAATTTGAAATTCTTTCTTTTGTATTCTTTAATGACCAACAATTGGATTTCTTAATTAAAT**********
```

FIGURE 2.
Comparative sequences of PCR products showing length polymorphisms. Both ends of H3-amplified fragments were not completely sequenced because of the relatively large sizes. Types are represented by banding pattern types for H2 and H3 markers and by fragment sizes for microsatellite markers. Types shown with asterisks are those possessing base changes without length differences. The nucleotides homologous to the tobacco sequence are indicated by periods (.), and vacant positions are indicated by asterisks (*). Restriction sites that characterized the type difference are underlined. Primer sequences are shown in *Italic*. See the legend of Table 3 for species abbreviation.

FIGURE 2—*Continued.*

```
Tobacco     **********************************************************************************
1 and 2     TTAAATACTGAGAACTAGCCAATTTATCCTTTGCCAGTCATTTTTTTTTGATCATCCTAATATCTTTCCCTCAATTATTC
3           ******ACTGAGAACTAGCCAATTTATCCTTTGCCAGTCATTTTTTTTTGATCATCCTAATATCTTTCCCTCAATTATTC
4           ******ACTGAGAACTAGCCAATTTATCCTTTGCCAGTCATTTTTTTTTGATCATCCTAATATCTTTCCCTCAATTATTC

Tobacco     **********************************************************************************
1, 2 and 4  TATTCCTGACTATGGGTAATCAGTGAAATTTTTTCGAAATATTGGATATTTTGATAGCAAAGGAGTTCTTTCGTCTCAAA
3           TATTCCTGACTATGGGTAATCAGTGAAATTTTTTCTAAATATTGGATATTTTGATAGCAAAGGAGTTCTTTCGTCTCAAA

Tobacco     **********************************************************************************
1 and 3     ATCTGAATAATATTCATTACTTAAAGTGGTTCTTTCGATCATTCATCGAAAGGATACACTTTATTTTTAATTTGACCAAT
2 and 4     ATCTGAATAATATTCATTACTTAAAGTGGTTCTTTCGATCATTCATCGAAAGGATACACTTTATTTTAAATTTGACCAAT

Tobacco     *****TATCAGGAAACAATATTCTGAATTTCTTCATTCGAAGTGAATTCTTAGCTTTTTTCTGGATTCTTTCTAGATTCA
1, 2 and 4  TGAGA.....................A.............................C.A......T................
3           TGAGA....G..............A.............................C.A......T................

Tobacco     AAGACTAACCACAAAATCACAAAGAAAATAGATTCATTAGTCCGATACCTTGTATAAAACTCATGTGTGTAAGAAATATT
All         ....................................A...T.....................................

Tobacco     CGATCGCATAGAGTGTACGAATGGGTTGATTAACAATTCACAGATGAAAAAATGGCAAAAAAGAAAGCATTCACTCCTCT
All         ....................................T.....C.....................................

Tobacco     TTTCTA
All         ......
```

NTCP6

Type	Sequenced
175	*chc* (chc525-3), hybrid (PI 545886), *nrs* (PI 473201)
174	hybrid (PI 473339), *tar* (PI 458395)
173	*tbr* (cv. Konafubuki, CIP703254), *stn* (CIP704089), hybrid (PI 473337), *nrs* (PI 473428), *tar* (PI 217458)
172	*ber* (PI 218215), *tar* (PI 414148)
127	*phu* (1.22), *tar* (PI 275154, PI 195206), *tbr* (PI 245929)

```
Sequence
Tobacco     GATTCTTTCGCATCTCGATTCCGTTTTTGAAAAAAAAAAA*TGATTCATCGAAGAAAAAAATCAGAAACAACAATCACAT
175         ....................A..................AC.....................................
174         ....................A..................*C.....................................
173         ....................A.................**C.....................................
172         ....................A................***C.....................................
127         ....................A..................A.....................................*

Tobacco     TCCAGCTAACATTTCGATTTTAAACAGAACATTGTTAAAAAAGCAATCTATATTGTCATAGAATATATATATGTTCTGGG
Others      ...........................................................................**.........
127         *******************************************.....................**.........

Tobacco     ACGGAAGGATTCGAACC
All         .................
```

NTCP7

Type	Sequenced
175	*tar* (PI 458395)
175*	*ber* (PI 545852)
174	*chc* (chc525-3), *ber* (PI 218215), *nrs* (PI 473201), *tar* (PI 217458)
173	*phu* (1.22), *tbr* (cv. Konafubuki, CIP703254), *stn* (CIP704089), hybrid (PI 473337), *nrs* (PI 473428), *tar* (PI 275154)
172	*tar* (PI 473227)

```
Sequence
Tobacco     TGATCCCGGACGTAATCCTGGACGTGAAGAATAAAATAAAAAAGGTTTTTCCTTGCTTGATTTTCCAATTTTCTTATGA
All         ...........................................................A..........G..
175*        ..........................................G................A..........G..
```

FIGURE 2—*Continued.*

```
Tobacco       TTTGGTCTATTCCACACATTTAACTAAGAATAAGAACAAAGGATTTCGAAATTTGAAAAAAAAAAAATCAAGTCATCAAC
175 and 175*  .............T.....................................................................
174           .............T.....................................................*.............
173           .............T....................................................**.............
172           .............T...................................................***.............

Tobacco       GGAAAGAGAGGGATTCG
All           .................
```

NTCP14

Type	Sequenced
152	*chc* (chc525-3)
151	hybrid (PI 498141, PI 498098)
150*	*phu* (1.22)
150	*tar* (PI 458395)
149	*tbr* (cv. Konafubuki)

```
Sequence
Tobacco       AATCCGTAGCCAGAAAAATAAATTGTTTTTTTTTTT*GTTTTTTCTGGAAAGTATTTTCTTATATTAAATTTTGTATTG
152           ...................................T.............................C............
151           ...................................*.............................C............
150*          ........................T..........**.............................C............
150           ....................................**.............................C............
149           ...................................***.............................C............

Tobacco       GACAAGAAAGGAATTCCCCTTGTGTATGCGCGCCTCAAAAAGGTATAGTACTCGATTCCATTACATGCATCGG
All           .................TT......................A.............................
```

NTCP18

Type	Sequenced
189	hybrid (PI 498141)
188	*tbr* (Konafubuki)
187	*chc* (chc525-3), *nrs* (PI 473529)
186	*phu* (1.22)

```
Sequence
Tobacco       CTGTTCTTTTCCATGACCCCTCTTAATTGAGATGAGACAGGAGATCCAATGCTTGAATGAAGTAAAAATCACTTTGATTC
All           ............................T................................C....T..........

Tobacco       AATCATACATCTTGGAATCAGCCTAAGTATTCCTTTTTTGTATTCCTTTTTTCTTTTTTTTTTT*CAATTCATTT****
189           ......................T.............**********.............T...C......ATTT
188           ......................T.............**********.............*...C......ATTT
187           ......................T.............**********.............**...C......ATTT
186           ......................T.............**********............***...C......ATTT

Tobacco       TATCTAATTT**********ATTTTTCTGGCTTGGCTAGGTGG
All           ..........ATATCTAATCT......................
```

locus in potato had a 639 bp insertion into the corresponding tobacco sequence (Wakasugi et al. 1998), and within this inserted region an 18 bp deletion shared between types 3 and 4, an additional 18 bp deletion in type 4, a *Dra*I recognition site in the same position in types 2 and 4, but in a different position in type 3 were found.

Most of microsatellite marker bands differed by the repeated number in mononucleotide-repeated regions (Figure 2). However, the 127 bp band of NTCP6 contained an additional 48 bp deletion and one-base difference detected next to the mononucleotide-repeated region. Base changes without fragment size differences were also found in the H2 fragment from *S. phureja* 1.22, the 175 bp NTCP7 fragment from *S. berthaultii* PI 545852 and the 150 bp NTCP14 fragment from *S. phureja* 1.22 (Figure 2). In the following analysis, however, these base changes were neglected and only phenotypic differences observed on electrophoresed gels were used.

Most accessions showed either one of banding types or bands with each marker. However, two accessions of *S. berthaultii*, five accessions of *S. tarijense* and two accessions of *S. berthaultii-S. tarijense* hybrids showed mixed banding patterns with two bands or two types together (Table 1). This is probably because plant materials with different bands or types were bulked for DNA extraction, although a possibility of two different chloroplast DNA contained in one individual (heteroplasmy) could not be excluded as documented for one potato variety by Provan et al. (1999). Individually extracted DNA samples in a few such accessions showed single types (Hosaka 2002).

The accessions of *S. tuberosum* ssp. *andigena* and *S. stenotomum* previously known as T-type chloroplast DNA holders, five Chilean primitive potatoes and one Japanese advanced cultivar showed type 1 patterns in H1 locus, thus being T-type chloroplast DNA holders. All these accessions also showed the same polymorphism types in all the other markers (Table 1). Thus, this chloroplast DNA type was named haplotype 1. Likewise, a total of 25 haplotypes were distinguished by combination of marker phenotypes. The numbers of accessions in each species group, excluding accessions showing mixed bands or types, are tabulated for each haplotype in Table 3. The T-type chloroplast DNA was divided into five haplotypes (haplotypes 1 to 5). Haplotype 1 was found also in 14 accessions of *S. tarijense* and four accessions of *S. berthaultii-S. tarijense* hybrids. Twelve haplotype-1 accessions of *S. tarijense* were from Department of Salta, Argentina, while one accession each was from Departments of Chuquisaca and Tarija, Bolivia (Table 1). Three and one haplotype-1 accessions of natural hybrids were from Departments of Cochabamba and Potosi, Bolivia, respectively (Table 1). Haplotypes 2, 3, and 4 were found in one accession each of *S. tarijense*. Haplotype 5 was found only in two accessions of *S. neorossii*. *Solanum berthaultii* had eight haplotypes with a predominant haplotype 24 (61%), while *S. tarijense* had 14 haplotypes with frequent haplotypes 1 (25%), 7 (19%), 12 (16%), and 14 (14%). Only five haplotypes were shared by both species in common. Their hybrid accessions showed 11 haplotypes, where three were shared with *S. berthaultii*, five were shared with *S. tarijense*, and five were unique in the hybrids. *Solanum neorossii* had unique haplotypes 5 and 22.

DISCUSSION

Although the present high-resolution marker system could separate T-type chloroplast DNA into five haplotypes, all of the Chilean and Andean cultivated potatoes having T-type chloroplast DNA showed the same haplotype 1. Provan et al. (1999) found that all 151 European cultivars having T-type chloroplast DNA showed the same marker bands at seven NTCP loci including all four loci used in this study. These suggest that the cultivated potatoes having T-type chloroplast DNA share the same haplotype-1 chloroplast DNA and originated monophyletically. The haplotype 1 was found in some accessions of *S. tarijense* and its natural hybrids with *S. berthaultii*. *Solanum berthaultii* had neither haplotype 1 nor the other four haplotypes containing the 241 bp deletion, whereas *S. tarijense* possessed all five haplotypes. Therefore, I strongly suggest that some populations of *S. tarijense* initially conferred haplotype-1 chloroplast DNA to the cultivated potatoes. In a previous study (Hosaka 2002), *S. berthaultii* and *S. neorossii* were also suggested as possible candidates for a maternal ancestor because some accessions of these species possessed the 241 bp deletion in common. Although two accessions of *S. neorossii* did possess the 241 bp deletion, these were different from haplotype 1 particularly by a unique 18 bp deletion in H3 locus (Figure 2). *Solanum berthaultii* accessions previously determined as having T-type chloroplast DNA were all misclassified in the previous study (Hosaka 2002) and classified into the group of natural hybrids in this

study. This misclassification happened because "Inventory of Tuber-bearing *Solanum* Species" (Bamberg et al. 1996) listed all but one of *S. berthaultii-S. tarijense* natural hybrids under both *S. berthaultii* and putative hybrids. Consequently, involvement of *S. berthaultii* and *S. neorossii* in maternal ancestry of cultivated potato is now less likely. It has never been claimed that *S. tarijense* was involved in the origin of cultivated potatoes, because *S. tuberosum* and *S. tarijense* are morphologically distinct from each other and classified into different taxonomic series in tuber-bearing *Solanum* species (Correll 1962; Hawkes 1990; Ochoa 1990). Yet, *S. tarijense* is known as a superior parent in hybrid progenies between *S. tuberosum* haploids and diploid wild species for tuberization (Hermundstad and Peloquin 1985).

Hybrids between genotypes with different chloroplast genomes would show mixed phenotypes when DNA was extracted from bulked samples as they did in this study. However, the putative *S. berthaultii-S. tarijense* natural hybrids did not always show markers of mixed phenotypes, the frequency being 8.0%, which was almost equivalent to those for *S. berthaultii* (6.7%) and *S. tarijense* (8.1%). Furthermore, five of 11 haplotypes in the hybrids were unique. Thus, I cannot support that all of these accessions were of hybrid origin. It is rather likely that the three classifications tested here, *S. berthaultii*, *S. tarijense*, and hybrids, are part of a single large gene pool. Frequent natural hybridization has been reported between *S. tarijense* and *S. berthaultii* (Hawkes and Hjerting 1989). Extensive morphological overlaps between the two species (Spooner and van den Berg 1992) support that these materials classified into *S. berthaultii*, *S. tarijense* or their hybrids can be included into a large single species. If so, T-type chloroplast DNA of cultivated potatoes were likely conferred from some of *S. tarijense*-type variants in this large species. Molecular analysis of nuclear DNA in this large species would give us a clearer answer.

The Andean cultivated potatoes were likely domesticated in Peru, because four of five chloroplast DNA types (A, S, C, W and T) found in Andean cultivated tetraploid (*S. tuberosum* ssp. *andigena*) and diploid (*S. stenotomum*) potatoes were shared with a group of mostly Peruvian wild diploid species (Hosaka 1995). Chloroplast haplotype 1 was found mainly in the Bolivia-Argentine boundary area, and partly from central Bolivia. Thus, the haplotype-1 chloroplast DNA was likely introduced from some populations of *S. tarijense* to cultivated potatoes somewhere in this region. However, it still remains a mystery how wild populations having haplotype-1 chloroplast DNA of *S. tarijense* interacted with the Andean cultivated potatoes and finally became the Chilean ssp. *tuberosum*; possible events may be (1) *S. tarijense* was domesticated independently in Bolivia and became Bolivian *S. stenotomum*, (2) *S. tarijense* was naturally hybridized with *S. stenotomum* and became local varieties in Bolivia, or (3) *S. tarijense* was hybridized naturally with *S. tuberosum* ssp. *andigena* in the migration process towards Chile, as previously suggested by Grun (1990) who described a maternal ancestor as unknown species.

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