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Sukhotu, Thitaporn
Kamijima, Osamu
Hosaka, Kazuyoshi

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Nuclear and chloroplast DNA differentiation in Andean potatoes

Thitaporn Sukhotu, Osamu Kamijima, and Kazuyoshi Hosaka

Abstract: Over 3500 accessions of Andean landraces have been known in potato, classified into 7 cultivated species ranging from 2*x* to 5*x* (Hawkes 1990). Chloroplast DNA (ctDNA), distinguished into T, W, C, S, and A types, showed extensive overlaps in their frequencies among cultivated species and between cultivated and putative ancestral wild species. In this study, 76 accessions of cultivated and 19 accessions of wild species were evaluated for ctDNA types and examined by ctDNA high-resolution markers (ctDNA microsatellites and H3 marker) and nuclear DNA restriction fragment length polymorphisms (RFLPs). ctDNA high-resolution markers identified 25 different ctDNA haplotypes. The S- and A-type ctDNAs were discriminated as unique haplotypes from 12 haplotypes having C-type ctDNA and T-type ctDNA from 10 haplotypes having W-type ctDNA. Differences among ctDNA types were strongly correlated with those of ctDNA high-resolution markers (r = 0.822). Differentiation between W-type ctDNA and C-, S-, and A-type ctDNAs was supported by nDNA RFLPs in most species except for those of recent or immediate hybrid origin. However, differentiation among C-, S-, and A-type ctDNAs was not clearly supported by nDNA RFLPs, suggesting that frequent genetic exchange occurred among them and (or) they shared the same gene pool owing to common ancestry.

Key words: potato, chloroplast DNA, microsatellite markers, nuclear DNA RFLPs.

Résumé : Plus de 3500 accessions andéennes sont connues chez la pomme de terre, lesquelles sont classées en sept espèces cultivées dont la ploïdie varie de 2*x* à 5*x* (Hawkes, 1990). L'ADN chloroplastique (ctDNA), dont on distingue cinq types (T, W, C, S et A), montre d'importants chevauchements quant à la fréquence à laquelle on trouve ces types au sein des espèces cultivées et entre les espèces cultivées par rapport aux espèces ancestrales sauvages. Dans ce travail, 76 accessions d'espèces cultivées et 19 accessions d'espèces sauvages ont été examinées pour déterminer leur type d'ADN chloroplastique. Parallèlement, ces accessions ont été caractérisées à l'aide de marqueurs ctDNA à haute résolution (microsatellites chloroplastiques et le marqueur H3) ou de RFLP nucléaires. Les marqueurs chloroplastiques à haute résolution ont permis d'identifier 25 haplotypes différents au sein du ctDNA. Les types S et A ont été distingués au sein d'une collection de 12 haplotypes de type C. Des ADN chloroplastiques de type T ont été identifiés parmi 10 haplotypes de type W. Les différences entre types d'ADN chloroplastique étaient fortement corrélées avec les marqueurs à haute résolution (r = 0,822). La distinction entre les ADN chloroplastiques de type W et ceux des types C, S et A était supportée par les RFLP nucléaires chez la plupart des espèces à l'exception des hybrides récents. La différenciation au sein des types C, S et A n'était pas clairement supportée par les analyses RFLP nucléaires, ce qui suggère que des échanges génétiques fréquents se sont produits ou qu'ils partagent le même réservoir génique attribuable à un ancêtre commun.

Mots clés : pomme de terre, ADN chloroplastique, marqueurs microsatellites, ADN nucléaire.

[Traduit par la Rédaction]

Introduction

Potato has a relatively large genetic reservoir compared with other major crops. According to Hawkes (1990),[2] 7 cultivated species and 228 wild species in *Solanum* L. sect. *Petota* Dumort. have been described as the tuber-bearing *Solanum* species (potato and its relatives). Many of these wild as well as cultivated species have proven value in potato breeding as sources of resistance genes and other agronomic traits for cultivar improvement (Ross 1986; Hanneman 1989; Hawkes 1990).

Cultivated species consist of diploid (*Solanum stenotomum*, *Solanum phureja*, and *Solanum ajanhuri*), triploid (*Solanum chaucha* and *Solanum juzepczukii*), tetraploid (*Solanum tuberosum* subsp. *andigena* and *Solanum tuberosum* subsp. *tuberosum*), and pentaploid (*Solanum curtilobum*) species

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T. Sukhotu. Graduate School of Science and Technology, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan.
O. Kamijima. Faculty of Agriculture, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan.
K. Hosaka.[1] Food Resources Education and Research Centre, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan.

[1]Corresponding author (e-mail: hosaka@kobe-u.ac.jp).
[2]Hawkes' (1990) classification system is tentatively adopted throughout this text.

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(Hawkes 1990). All of these species are grown in the Andes of South America except *S. tuberosum* subsp. *tuberosum*, which is grown in southern Chile (referred to as Chilean subsp. *tuberosum*) and worldwide (referred to as the common potato). Species relationships among cultivated species have been morphologically investigated by various authors (Dodds 1962; Bukasov 1978; Hawkes 1990; Ochoa 1990), which, however, led to a serious controversy on the taxonomic treatment of cultivated species (reviewed in Huáman and Spooner 2002). In the latest taxonomic treatment by Huáman and Spooner (2002), all cultivated potatoes were classified into a single species, *S. tuberosum*, and divided under the same species name into nine cultivar groups.

There have long been arguments on the origin of the most primitive cultivated diploid species, *S. stenotomum*, and the most important Andean cultivated potato, *S. tuberosum* subsp. *andigena*. Hawkes (1958) suggested *Solanum leptophyes* and *Solanum canasense* as the ancestral species of *S. stenotomum* and, later, favored *S. leptophyes* (Hawkes 1988, 1990; Hawkes and Hjerting 1989). *Solanum stenotomum* is highly polymorphic (Hawkes 1956, 1990; Bukasov 1978; Ochoa 1990), and Ugent (1970) proposed its ancestor to be a single "superspecies, the "*Solanum brevicaule* complex", which included *S. brevicaule*, *Solanum bukasovii*, *S. canasense*, *Solanum coelestipetalum*, *Solanum gourlayi*, *S. leptophyes*, *Solanum multidissectum*, *Solanum multiinterruptum*, and *Solanum spegazzinii*. Most of these wild species are closely related to each other, and there are many controversies on their taxonomy (Correll 1962; Bukasov 1978; Hawkes 1990; Ochoa 1990). For the origin of *S. tuberosum* subsp. *andigena*, there are several different hypotheses: it originated via polyploidization from an intervarietal or interspecies cross within cultivated diploid potatoes (Swaminathan and Magoon 1961; Matsubayashi 1991; Hosaka 1995), from an interspecies cross between *S. stenotomum* and a wild diploid species, *Solanum sparsipilum* (Hawkes 1956, 1990; Cribb and Hawkes 1986), or from a wild species, *Solanum vernei* (Brücher 1964). There may be general acceptance on the origin of the other cultivated species. *Solanum phureja* was derived as a non-tuber-dormancy variant from *S. stenotomum* (Hawkes 1988, 1990). *Solanum ajanhuiri* originated from natural hybrids between *S. stenotomum* and a wild frost-resistant diploid species, *Solanum megistacrolobum* (Huamán et al. 1982). *Solanum chaucha* is a triploid hybrid between tetraploid *S. tuberosum* subsp. *andigena* and diploid *S. stenotomum* (Hawkes 1958; Jackson et al. 1977). The most frost-resistant species, *S. juzepczukii*, is a triploid hybrid between a wild frost-resistant tetraploid, *Solanum acaule*, and *S. stenotomum* (Hawkes 1958, 1962; Schmiediche et al. 1980; van den Berg et al. 1999). A pentaploid species, *S. curtilobum*, was originated by fertilization between a normal gamete from *S. tuberosum* subsp. *andigena* and a 2*n* gamete from *S. juzepczukii* (Bukasov 1939; Hawkes 1958, 1962; Schmiediche et al. 1980). Chilean subsp. *tuberosum* originated likely from *S. tuberosum* subsp. *andigena* by selection (Hawkes 1956, 1990; Brücher 1963; Hosaka and Hanneman 1988*a*).

Nuclear DNA (nDNA) restriction fragment length polymorphism (RFLP) analyses were performed for cultivated species and representative wild species in tuber-bearing *Solanum* species (Bonierbale et al. 1990; Debener et al. 1990, 1991) and disclosed close relationships among the cultivated species, *S. bukasovii* and *S. canasense*. Although over 3500 accessions of Andean landraces have been known (Huamán 1994), only a few accessions for each species were used in these studies. A large-scale evaluation of nDNA diversity in Andean landraces has never been conducted until recently. Ghislain et al. (1999) analyzed intraspecific variation of *S. phureja* by random amplified polymorphic DNA markers, and Raker and Spooner (2002) successfully used simple sequence repeat (or microsatellite) markers to separate Chilean subsp. *tuberosum* from *S. tuberosum* subsp. *andigena*.

In contrast, chloroplast DNA (ctDNA) RFLP analyses were extensively used to evaluate genetic diversity in Andean landraces (Hosaka et al. 1984; Buckner and Hyde 1985; Hosaka 1986, 1995; Hosaka and Hanneman 1988*a*, 1988*b*). Five basic ctDNA types (W, T, C, S, and A types) have been identified among cultivated potatoes (Hosaka 1986). None of the ctDNA types were species specific, but the frequencies were different among accessions of different species. *Solanum tuberosum* subsp. *andigena* had A-type ctDNA in many accessions, and also, four other ctDNA types with different frequencies varied from north to south of the Andes (Hosaka and Hanneman 1988*a*). The most primitive cultivated species, *S. stenotomum*, showed all five types, with the S type being the most frequent (Hosaka 1995). A considerable overlap in the ctDNA type frequencies between *S. stenotomum* and the wild diploid species *S. bukasovii*, *S. canasense*, *S. candolleanum*, *S. leptophyes*, and *S. multidissectum* suggested that all of these derived from the supposed "ancestral species complex" (Hosaka 1995).

As shown in Fig. 1*a*, these ctDNA types were distinguished by single differences detected on restriction fragment patterns of ctDNA (Hosaka 1986). The evolutionary directions between ctDNA types, i.e., primitive versus advanced (or derived), were determined by shared mutations (Fig. 1*a*). Recently, several high-resolution markers have become available to detect ctDNA variation. ctDNA microsatellite markers detect polymorphisms in the repeated number of mononucleotides in ctDNA (Provan et al. 2001), which revealed much higher levels of diversity than ctDNA RFLPs in potato (Bryan et al. 1999; Provan et al. 1999; Hosaka 2003). In this study, differences of ctDNA types in Andean cultivated potatoes and putative ancestral wild species as defined by restriction site analysis are compared with those of ctDNA microsatellites and with those of nDNA RFLPs. The nature of genetic differentiation in nDNA and ctDNA and its implication in species differentiation are discussed.

Materials and methods

Plant material and DNA isolation

Seventy-six accessions of seven cultivated species, 17 accessions of eight putative ancestral wild species, and two accessions of a distantly related wild species, *Solanum chacoense*, were used in this study (Table 1). Accessions with CIP numbers have all been clonally propagated in the International Potato Center, Lima, Peru, which were ob-

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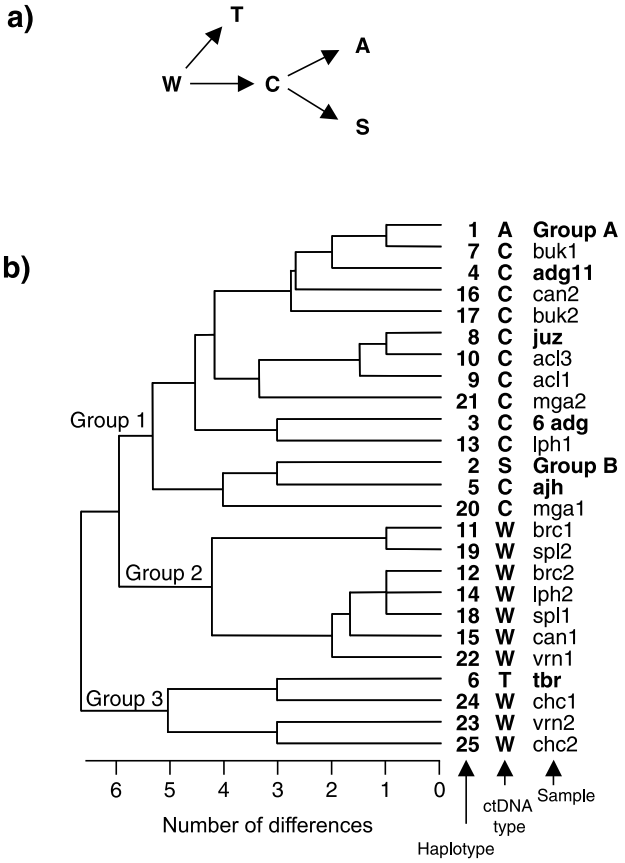
Fig. 1. ctDNA differentiation in cultivated potato species and their wild relatives as shown by (*a*) the relationships between ctDNA types (cited from Hosaka and Hanneman 1988*a*) and by (*b*) those between haplotypes. Corresponding ctDNA types and sample names are also denoted with haplotype numbers in Fig. 1*b*. Sample names are represented by codes or species abbreviations (see Table 1). Cultivated species are shown in bold. Group A contains cha, phu, stn, gon, adg, and tbr4, all having A-type ctDNA. Group B contains cha, cur, phu, stn, gon, and adg, all having S-type ctDNA.



tained as DNA samples. Accessions with PI numbers were obtained as seeds from the Potato Introduction Station (NRSP-6), Sturgeon Bay, Wisconsin, U.S.A. Total DNA was isolated from clones (chc 525-3, 1.22, and Konafubuki) or seedlings (many seedlings in each PI accession were bulked) by the method described in Hosaka and Hanneman (1998).

RFLP analysis of ctDNA and nDNA

To obtain restriction fragment patterns of ctDNA, total DNA was digested with restriction endonuclease *Bam*HI, *Hin*dIII, or *Pvu*II and entire ctDNA was used as probe DNA for Southern hybridization. Thirty-five single-copy probes were used to detect nDNA RFLPs: TG14, TG18, TG28, TG46, TG63, TG71, TG115, TG123, TG128, TG134, TG152, TG166, TG241, TG413, TG421, TG497, TG560, CT220, P101, P116, P215, P251, P335, P357, P368, P537, P695, P697, P769, P808, P845, P894, P948, P1069, and P1108. The probes prefixed with "TG" or "CT" were single-copy tomato probes obtained from Dr. S.D. Tanksley, Cornell University, Ithaca, N.Y., U.S.A. (Tanksley et al. 1992). The probes prefixed with "P" were random genomic clones from *S. phureja* clone 1.22 (Hosaka and Spooner 1992). Southern hybridization procedures are described in Nakagawa and Hosaka (2002). In the analysis of nDNA RFLPs, only visibly reliable and polymorphic (presence versus absence) bands were scored and converted to 1/0 type data.

ctDNA microsatellites and H3 marker

Seven ctDNA microsatellite markers, developed by Provan et al. (1999) from *Nicotiana tabacum* ctDNA (NTCP markers), were used (Table 2). Polymerase chain reaction (PCR), in a volume of 10 μL consisting of 10 ng of genomic DNA, 0.3 μM each of the primers, 1× PCR buffer attached to the enzyme, 200 μM each of dATP, dCTP, dGTP, and dTTP, and 0.25 U *Taq* DNA polymerase (AmpliTaq®; Applied Biosystems), was carried out in a thermal cycler (Gene Amp® PCR System 9700; Perkin Elmer) using the following parameters: (*i*) initial denaturation at 94 °C for 3 min, (*ii*) 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, and (*iii*) a final extension at 72 °C for 5 min. Amplification products were mixed with 10 μL of loading dye (95% formamide, 0.25% bromophenol blue, and 0.25% xylene cyanol). Five microlitres of the sample was separated by electrophoresis in 4.0% denaturating polyacrylamide gels (Sequi-Gen® GT Nucleic Acid Electrophoresis Cell; Bio-Rad) at 45 W constant power for 2 h and visualized by silver staining (Bassam et al. 1991). Fragment sizes were determined by visual comparison with 10 base pair (bp) ladder markers (30–330 bp AFLP DNA ladder; GIBCO-BRL) and with sequenced fragments.

The H3 marker (Table 2), developed by Hosaka (2003), was detected by PCR amplification using the same conditions as above, ethanol precipitation, digestion with restriction endonuclease *Dra*I, and separation in 1.6% agarose gels.

Data analysis

ctDNA types were determined based on the combination of restriction pattern types as defined by Hosaka and Hanneman (1988*b*). ctDNA microsatellites were scored as 1 or 0 for each fragment. Pairwise distances, shown as total character differences between accessions, were obtained separately for nDNA RFLP data and for ctDNA microsatellite data (including H3 marker data). The unweighted pair group method with arithmetic means (UPGMA) was used for clustering to produce dendrograms using PAUP 4.0b10.

Pearson correlation coefficients (*r*) between distance matrices of ctDNA types, ctDNA microsatellites, and nDNA RFLPs were calculated. Distances between ctDNA types were obtained as the number of arrows between ctDNA types (Fig. 1*a*), for example, three differences between T and A or T and S and two differences between S and A, W and A, or W and S. The Mantel (1967) test was performed using GenAlEx (Peakall and Smouse 2001) by which rows and columns in the distance matrix were randomly permutated 1000 times to test whether the original correlation occurred by chance.

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Table 1. *Solanum* species accessions used in this study and their ctDNA types, microsatellite marker phenotypes (in base pairs), H3 types, and haplotype numbers.

Code[a]	Accession	CtDNA type	Microsatellite marker (NTCP) phenotype							H3 type	Haplotype	Note[b]
			6	7	8	9	12	14	18			
Series *Yungasensa*												
***Solanum chacoense* Bitter (2*n* = 2*x* = 24) (chc)**												
chc1*	PI 537025	W	172	174	252	279	235	149	188	1	24	B, Chuquisaca
chc2*	chc 525–3	W	175	174	252	279	234	152	187	1	25	Inbred (Hosaka and Hanneman 1998)
Series *Megistacroloba*												
***Solanum megistacrolobum* Bitter subsp. *megistacrolobum* (2*n* = 2*x* = 24) (mga)**												
mga1*	PI 473356	C	173	173	249	289	237	152	186	1	20	B, Potosi
mga2*	PI 473361	C	175	174	250	288	237	150	186	1	21	B, La Paz
Series *Tuberosa*												
***Solanum brevicaule* Bitter (2*n* = 2*x* = 24) (brc)**												
brc1*	PI 498218	W	172	174	252	247	236	153	187	1	11	B, La Paz
brc2*	PI 545971	W	172	173	253	247	236	151	186	1	12	B, Cochabamba
***Solanum bukasovii* Juz. (2*n* = 2*x* = 24) (buk)**												
buk1*	PI 365304	C	173	174	250	289	237	151	186	2	7	P, Lima
buk2*	PI 458379	C	174	174	251	289	236	151	186	2	17	P, Apurimac
***Solanum canasense* Hawkes (2*n* = 2*x* = 24) (can)**												
can1*	PI 265865	W	173	173	253	247	236	151	186	1	15	B, La Paz
can2*	PI 473346	C	173	174	250	317	236	151	186	2	16	P, Puno
***Solanum leptophyes* Bitter (2*n* = 2*x* = 24) (lph)**												
lph1*	PI 473445	C	174	174	251	259	236	150	196	2	13	P, Cuzco
lph2*	PI 545994	W	172	173	253	247	236	150	186	1	14	B, Potosi
***Solanum sparsipilum* (Bitt.) Juz. et Buk. (2*n* = 2*x* = 24) (spl)**												
spl1*	PI 498305	W	172	173	253	247	236	152	186	1	18	P, Cuzco
spl2*	PI 498284	W	172	174	252	247	236	152	187	1	19	B, La Paz
***Solanum vernei* Bitt. et Wittm. subsp. *vernei* (2*n* = 2*x* = 24) (vrn)**												
vrn1*	PI 545884	W	172	173	253	247	237	152	186	1	22	B, Cochabamba
vrn2*	PI 473308	W	175	174	253	279	235	150	187	1	23	A, Tucuman
Cultivated												
***Solanum ajanhuiri* Juz. et Buk. (2*n* = 2*x* = 24) (ajh)**												
ajh	CIP 702677	C	174	173	249	289	237	151	186	2	5	B, La Paz, “Yari”
***Solanum chaucha* Juz. et Buk. (2*n* = 3*x* = 36) (cha)**												
cha1*	CIP 700431	A	174	174	250	289	237	151	186	2	1	P, “SPC-58”
cha2*	CIP 701013	A	174	174	250	289	237	151	186	2	1	P, “Huancaina Blanca”
cha3*	CIP 702551	S	127	173	251	289	239	150	186	2	2	B, Potosi, “Yana Achacana”
***Solanum curtilobum* Juz. et Buk. (2*n* = 5*x* = 60) (cur)**												
cur1*	CIP 702455	S	127	173	251	289	239	150	186	2	2	P, Puno, “Lukke O Pinoza”
cur2*	CIP 700273	S	127	173	251	289	239	150	186	2	2	P, “SC-1264”
***Solanum juzepczukii* Buk. (2*n* = 3*x* = 36) (juz)**												
juz1*	CIP 702443	C	174	174	250	317	236	150	186	1	8	P, Puno, “Parina”
juz2*	CIP 700895	C	174	174	250	317	236	150	186	1	8	P, “Zugucha”
***Solanum phureja* Juz. et Buk. (2*n* = 2*x* = 24) (phu)**												
phu1	CIP 703295	S	127	173	251	289	239	150	186	2	2	C, Valle, “Criolla Negra”

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Table 1 (*continued*).

Code[a]	Accession	CtDNA type	Microsatellite marker (NTCP) phenotype							H3 type	Haplotype	Note[b]
			6	7	8	9	12	14	18			
phu2*	CIP 703291	S	127	173	251	289	239	150	186	2	2	C, Cauca, "Rosca"
phu3*	CIP 703293	S	127	173	251	289	239	150	186	2	2	C, Cauca, "Conga"
phu4*	1.22	S	127	173	251	289	239	150	186	2	2	C, Nariño
phu5*	CIP 703308	S	127	173	251	289	239	150	186	2	2	P, Lambayeque, "Chaucha Amarilla"
phu6*	CIP 703274	S	127	173	251	289	239	150	186	2	2	P, Ayacucho
phu7*	CIP 703275	S	127	173	251	289	239	150	186	2	2	P, Puno, "Puca Papa"
phu8*	CIP 703276	S	127	173	251	289	239	150	186	2	2	P, Puno, "Maman Pecke (Rojo)"
phu9*	CIP 703272	A	174	174	250	289	237	151	186	2	1	B, La Paz, "Alcka Pinu"
***Solanum stenotomum* Juz. et Buk. (2*n* = 2*x* = 24) (stn)**												
stn1*	CIP 703317	S	127	173	251	289	239	150	186	2	2	P, Ancash, "Chingos"
stn2*	CIP 702464	S	127	173	251	289	239	150	186	2	2	P, Huanuco, "Natin Juito"
stn3	CIP 703313	A	174	174	250	289	237	151	186	2	1	P, Junin, "Jatun Huanca"
stn4	CIP 701165	S	127	173	251	289	239	150	186	2	2	P, Junin, "Calhua Rosada"
stn5*	CIP 701985	S	127	173	251	289	239	150	186	2	2	P, Cuzco, "Amayllo"
stn6	CIP 702199	S	127	173	251	289	239	150	186	2	2	P, Cuzco, "Chuco"
stn7	CIP 700235	S	127	173	251	289	239	150	186	2	2	P, "SA-2564"
stn8*	CIP 703286	S	127	173	251	289	239	150	186	2	2	B, La Paz, "Yana Pituwaya"
stn9*	CIP 703473	A	174	174	250	289	237	151	186	2	1	B, La Paz, "Phinu Rojo"
stn10*	CIP 702547	S	127	173	251	289	239	150	186	2	2	B, Potosi, "Espanol Papa"
***Solanum stenotomum* subsp. *goniocalyx* (Juz. et Buk.) Hawkes (2*n* = 2*x* = 24) (gon)**												
gon1	CIP 701830	A	174	174	250	289	237	151	186	2	1	P, Huanuco, "Garhuash Huayro"
gon2*	CIP 700304	S	127	173	251	289	239	150	186	2	2	P, "SS-467"
***Solanum tuberosum* L. subsp. *tuberosum* (2*n* = 4*x* = 48) (tbr)**												
tbr1	CIP 703252	T	173	173	252	279	235	149	188	1	6	Ch, "Clavela"
tbr2*	CIP 703254	T	173	173	252	279	235	149	188	1	6	Ch, "Darwin Potato (Low Bay)"
tbr3*	CIP 703610	T	173	173	252	279	235	149	188	1	6	Ch, Arch. Los Chonos, "Papa Cacho"
tbr4*	CIP 704165	A	174	174	250	289	237	151	186	2	1	Ch, "UA 11"
tbr5*	CIP 704168	T	173	173	252	279	235	149	188	1	6	Ch, "UA 22"
tbr6*	CIP 704171	T	173	173	252	279	235	149	188	1	6	Ch, "UA 28"
tbr7*	CIP 704172	T	173	173	252	279	235	149	188	1	6	Ch, "UA 31"
tbr8*	Konafubuki	T	173	173	252	279	235	149	188	1	6	Japan
***Solanum tuberosum* subsp. *andigena* Hawkes (2*n* = 4*x* = 48) (adg)**												
adg1*	CIP 704111	A	174	174	250	289	237	151	186	2	1	V, Trujillo
adg2*	CIP 703268	A	174	174	250	289	237	151	186	2	1	E, "Bolona"
adg3*	CIP 701304	A	174	174	250	289	237	151	186	2	1	P, Cajamarca, "Condorita Amarilla"
adg4*	CIP 701306	S	127	173	251	289	239	150	186	2	2	P, Cajamarca, "Alisa Maney"
adg5	CIP 703653	A	174	174	250	289	237	151	186	2	1	P, Cajamarca, "Huayro"
adg6	CIP 701278	A	174	174	250	289	237	151	186	2	1	P, La Libertad, "Shucuca"
adg7	CIP 701296	A	174	174	250	289	237	151	186	2	1	P, La Libertad, "Guayaba"
adg8	CIP 701463	A	174	174	250	289	237	151	186	2	1	P, Huanuco, "Manzana"

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Table 1 (*concluded*).

Code[a]	Accession	CtDNA type	Microsatellite marker (NTCP) phenotype							H3 type	Haplotype	Note[b]
			6	7	8	9	12	14	18			
adg9*	CIP 703682	A	174	174	250	289	237	151	186	2	1	P, Huancavelica
adg10*	CIP 700921	C	173	174	251	289	236	150	187	2	3	P, Cuzco, “Ccompis Rosada”
adg11*	CIP 701624	C	175	174	250	289	238	151	186	2	4	P, Cuzco, “Amaccaya”
adg12*	CIP 702453	A	174	174	250	289	237	151	186	2	1	P, Puno, “Huacanuno”
adg13*	CIP 700017	A	174	174	250	289	237	151	186	2	1	P, “SA-237”
adg14*	CIP 700045	A	174	174	250	289	237	151	186	2	1	P, “SA-450”
adg15*	CIP 700094	A	174	174	250	289	237	151	186	2	1	P, “SA-1012”
adg16*	CIP 700387	S	127	173	251	289	239	150	186	2	2	P, “3x-2682”
adg17*	CIP 700532	A	174	174	250	289	237	151	186	2	1	P, “DGV-117”
adg18*	CIP 700598	C	173	174	251	289	236	150	187	2	3	P, “EE-693”
adg19*	CIP 700616	A	174	174	250	289	237	151	186	2	1	P, “EE-971”
adg20*	CIP 700652	C	173	174	251	289	236	150	187	2	3	P, “EE-1737”
adg21*	CIP 700696	A	174	174	250	289	237	151	186	2	1	P, “EE-1817”
adg22*	CIP 700767	A	174	174	250	289	237	151	186	2	1	P, “EE-2013”
adg23*	CIP 700771	S	127	173	251	289	239	150	186	2	2	P, “EE-2020”
adg24	CIP 700787	C	173	174	251	289	236	150	187	2	3	P, “EE-2057”
adg25	CIP 700790	A	174	174	250	289	237	151	186	2	1	P, “EE-2060”
adg26*	CIP 700863	C	173	174	251	289	236	150	187	2	3	P, “Llangapoga Obrera”
adg27*	CIP 700877	C	173	174	251	289	236	150	187	2	3	P, “Carganaca Amarilla”
adg28	CIP 700882	A	174	174	250	289	237	151	186	2	1	P, “Shucuca”
adg29*	CIP 700960	S	127	173	251	289	239	150	186	2	2	P, “Juana Blanca”
adg30*	CIP 701021	S	127	173	251	289	239	150	186	2	2	P, “Cusi”
adg31*	CIP 701546	A	ND	ND	ND	ND	ND	ND	ND	ND	ND	P
adg32	CIP 702535	A	174	174	250	289	237	151	186	2	1	B, Potosi, “Sipancachi”
adg33	CIP 702698	A	174	174	250	289	237	151	186	2	1	B, Potosi, “Atacama”
adg34*	CIP 703474	A	174	174	250	289	237	151	186	2	1	B, Potosi
adg35*	CIP 704082	S	127	173	251	289	239	150	186	2	2	B, Potosi, “Malula”
adg36*	CIP 701065	A	174	174	250	289	237	151	186	2	1	B, “Ccillo Acoto”
adg37*	CIP 701067	A	174	174	250	289	237	151	186	2	1	B, “Sale”
adg38*	CIP 701074	S	127	173	251	289	239	150	186	2	2	B, “Yurac Rumpus”
adg39*	CIP 704152	A	174	174	250	289	237	151	186	2	1	A, Salta, “Runa Bola”
Series *Acaulia*												
***Solanum acaule* Bitter (2*n* = 4*x* = 48) (acl)**												
acl1	CIP 761143	C	174	174	251	289	236	150	186	1	9	P, Ayacucho
acl2*	CIP 761144	C	ND	ND	ND	ND	ND	ND	ND	ND	ND	P, Ayacucho
acl3*	PI 210030	C	174	174	250	289	236	150	186	1	10	B, Potosi

Note: ND, not determined.
[a]Abbreviations of species names follow Hawkes (1990). Samples used in the nDNA RFLP analysis are marked with asterisks.
[b]Locality: A, Argentina; B, Bolivia; C, Colombia; Ch, Chile; E, Ecuador; P, Peru; V, Venezuela; vernacular in quotations.

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Table 2. Primer pairs used in this study to detect ctDNA polymorphisms.

Locus	Primers (5′–3′)	Annealing temperature (°C)	Location	Size (bp)
NTCP6	GATTCTTTCGCATCTCGATTC	55	*rps16*/*trnQ* intergenic region	176
	GGTTCGAATCCTTCCGTC		(7262–7437)	
NTCP7	TGATCCCGGACGTAATCC	55	*psbI*/*trnS* intergenic region	175
	CGAATCCCTCTCTTTCCG		(8475–8649)	
NTCP8	ATATTGTTTTAGCTCGGTGG	55	*trnG* intron (9895 – 10 145)	251
	TCATTCGGCTCCTTTATG			
NTCP9	CTTCCAAGCTAACGATGC	55	*trnG*/*trnR* intergenic region	237
	CTGTCCTATCCATTAGACAATG		(10 220 – 10 456)	
NTCP12	CCTCCATCATCTCTTCCAA	55	*rps*2/*rpoC*2 intergenic region	126
	ATTTATTTCAGTTCAGGGTTCC		(16 892 – 17 017)	
NTCP14	AATCCGTAGCCAGAAAAATAAA	55	*psbM*/*trnD* intergenic region	151
	CCGATGCATGTAATGGAATC		(31 580 – 31 730)	
NTCP18	CTGTTCTTTCCATGACCCCTC	55	*psbC*/*trnS* intergenic region	186
	CCACCTAGCCAAGCCAGA		(36 872 – 37 057)	
H3	CAGGGGTCCATTCCCTTGAC	60	*ycf4* and *ycf10*	409
	AGAAAGAAATCCACCAGGGC		(63 082 – 63 490)	

Note: Locations are indicated according to the tobacco ctDNA (Wakasugi et al. 1998; the accession number Z00044 in the EMBL Nucleotide Sequence Database), where the first and last nucleotide numbers are given in parentheses. The size of amplified fragments is the one expected from tobacco ctDNA.

Results

ctDNA types

ctDNA types were assigned to all of the accessions (Table 1). Frequencies of different ctDNA types within each cultivated species were almost similar to those of previous studies (Hosaka and Hanneman 1988*a*, 1988*b*; Hosaka 1995): in *S. phureja*, A type (11.1% in this study versus 15.4% in a previous study) and S type (88.9% versus 84.6%); in *S. stenotomum*, A type (20.0% versus 18.5%) and S type (80.0% versus 72.2%); in *S. tuberosum* subsp. *andigena*, A type (64.1% versus 61.9%), S type (17.9% versus 12.3%), and C type (17.9% versus 14.2%). Wild species had either C- or W-type ctDNA, whereas those derived from the ancestral species complex (Hosaka 1995) were prone to have C-type ctDNA. These ctDNA type frequencies in wild species mostly fit those reported earlier (Hosaka and Hanneman 1988*b*; Hosaka 1995; Nakagawa et al. 2000; Nakagawa and Hosaka 2002).

ctDNA microsatellites and H3 marker

Seventy-five accessions of cultivated species and 16 accessions of wild species were examined by seven ctDNA microsatellites and H3 marker (Table 1). Since DNA from most accessions was extracted from individual plants, single fragments or types were detected with each microsatellite marker or H3 marker in all of the accessions.

Overall, 33 fragments were detected using ctDNA microsatellites. Not all necessarily resulted from polymorphisms in the repeated number in mononucleotide-repeated regions that were usually expected for ctDNA microsatellites (Provan et al. 2001). It has been shown that the 127-bp fragment of NTCP6 contains a 48-bp deletion (Hosaka 2003). The NTCP9 fragments involved repeat number differences of one base as well as 30 bases and additionally a nine-base insertion/deletion (Bryan et al. 1999; our unpublished data). In the following study, however, only total fragment sizes were measured. The H3 marker provided two banding patterns, types 1 and 2 (corresponding to types 1 and 3, respectively, in Hosaka 2003) (Fig. 2). Their difference was considered as one phenetic difference in the analysis, although they were different with an 18-bp deletion/insertion and a single-base change (Hosaka 2003).

Out of 33 microsatellite fragments and two H3 types, 23 were shared between cultivated and wild species. Nine were specifically found in wild species, while three were found in cultivated species. The 127-bp fragment of NTCP6 and 239-bp fragment of NTCP12 were perfectly associated with S-type ctDNA (Table 1). The 289-bp fragment of NTCP9 and type 2 of H3 (Fig. 2) were particularly interesting because these were perfectly correlated and found in a few wild species accessions and in all cultivated species except *S. tuberosum* subsp. *tuberosum* having T-type ctDNA and *S. juzepczukii* (Table 1).

The combination of a total of 35 fragments and types distinguished 25 different ctDNAs (or haplotypes), among which seven were only found in cultivated species (Table 1). Haplotypes 1, 2, and 6 corresponded exclusively to A-, S-, and T-type ctDNA, respectively. Twelve and 10 haplotypes were identified within C- and W-type ctDNA, respectively. Differences between haplotypes are shown by a UPGMA dendrogram in Fig. 1*b*. These haplotypes were classified into three groups (Fig 1*b*). Group 1 haplotypes consisted of A-, C-, and S-type ctDNAs and included all cultivated accessions except those of *S. tuberosum* subsp. *tuberosum* having T-type ctDNA. Group 2 and 3 haplotypes consisted of W- and T-type ctDNAs and were clearly different from Group 1 haplotypes. This dendrogram seems to indicate that A- and

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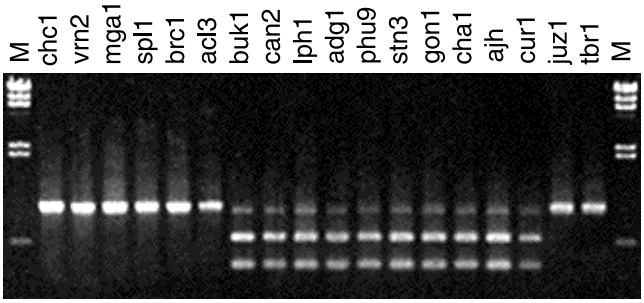
Fig. 2. Type 1 (single-banded) and type 2 (double-banded) patterns by H3 marker. See Table 1 for accession codes. M, *Hin*dIII-digested lambda DNA.



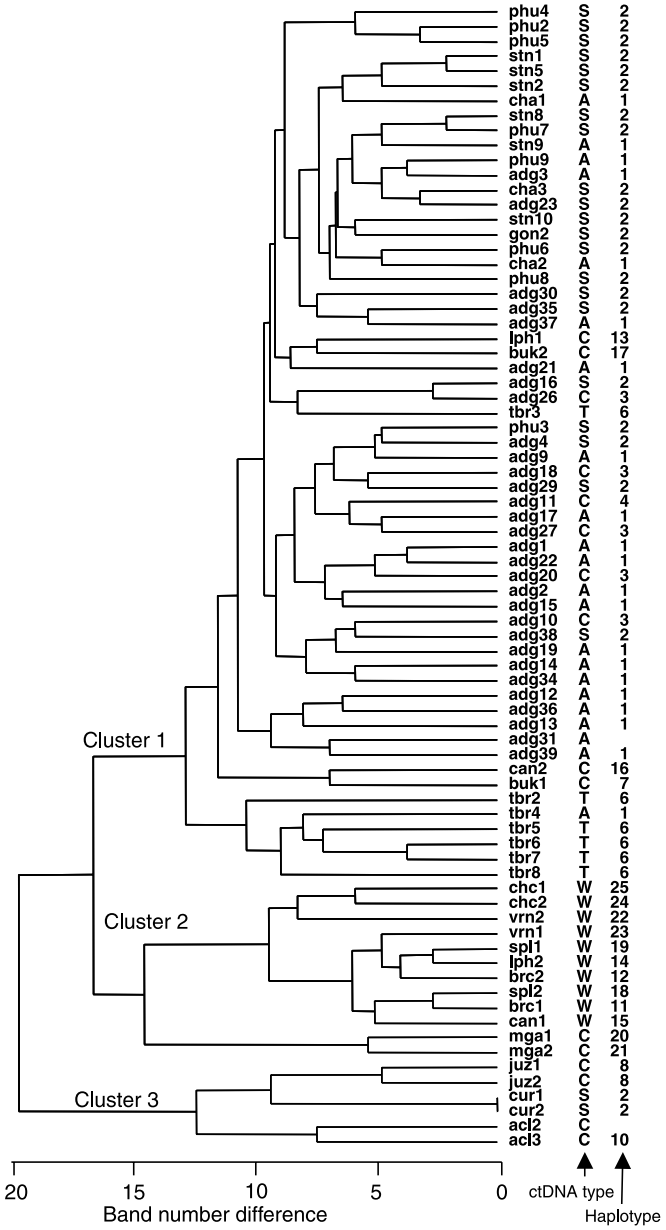
S-type ctDNAs were separated independently from C-type ctDNA and these three ctDNAs and T-type ctDNA were separated independently from W-type ctDNA, which is in agreement with the relationships between ctDNA types shown by Hosaka and Hanneman (1988*a*) (Fig. 1*a*).

nDNA RFLP analysis

Seventy-seven accessions of six cultivated and nine wild species (Table 1) were analyzed by using 35 single-copy RFLP probes. A total of 111 polymorphic bands were scored, of which nine were unique to single accessions. Seven bands were uniquely shared between *S. juzepczukii*, *S. curtilobum*, and *S. acaule* and these and an additional two bands between these three species and *S. megistacrolobum*. No band was unique to *S. stenotomum*, *S. phureja*, *S. chaucha*, or *S. tuberosum* subsp. *andigena*, while one band was specific to *S. tuberosum* subsp. *tuberosum*. An average of 24.0 bands were different between accessions, which could distinguish all accessions except two accessions of *S. curtilobum*. The difficulty in distinguishing the two accessions of *S. curtilobum* is probably due to the fact that *S. curtilobum* has only two morphotypes in which one is a somatic mutant for tuber color from the other one (Schmiediche et al. 1980).

A UPGMA dendrogram was constructed that identified three large clusters (clusters 1, 2, and 3) (Fig. 3). The most distant cluster (cluster 3) consisted of *S. juzepczukii*, *S. curtilobum*, and *S. acaule*. Cluster 2 was formed exclusively by wild species accessions having W-type ctDNA and *S. megistacrolobum* having C-type ctDNA. The remaining cultivated species, one accession each of *S. leptophyes* (lph1) and *S. canasense* (can2) and two accessions of *S. bukasovii* (buk1 and buk2), were classified into cluster 1 with C-, S-, A-, or T-type ctDNA. In this cluster, none of the species, ctDNA types, or haplotypes were uniquely distinguished as subclusters, although the accessions of *S. tuberosum* subsp. *tuberosum* showed interesting classification; five accessions having T-type ctDNA and one accession having A-type ctDNA formed a unique subcluster, whereas one accession having T-type ctDNA (tbr3) was clustered with *S. tuberosum* subsp. *andigena*. Interestingly, accessions of *S. leptophyes* and *S. canasense* were separately clustered into clusters 1 and 2; those in cluster 1 (lph1 and can2) had C-type ctDNA, whereas those in cluster 2 (lph2 and can1) had W-type ctDNA.

Fig. 3. UPGMA dendrogram constructed using nDNA RFLPs. See Table 1 for accession codes. The ctDNA type and haplotype number are also denoted for each accession.



Correlation between distance matrices derived from nDNA and ctDNA differences

Correlation coefficients were calculated between distance matrices of ctDNA types, ctDNA microsatellites, and nDNA RFLPs, each with a total of 2775 accession pairs from 75 samples that completed all analyses. The ctDNA type distance matrix was positively and strongly correlated with that of ctDNA microsatellites (r = 0.822), while the nDNA RFLP distance matrix was correlated with much lower coefficients with those of ctDNA types (r = 0.217) and ctDNA microsatellites (r = 0.415). The Mantel test indicated that the correlations did not occur by chance.

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Discussion

ctDNA differentiation

The present high-resolution marker system using ctDNA microsatellites and H3 marker could support relationships among ctDNA types with a high correlation coefficient (r = 0.822). According to the UPGMA dendrogram shown in Fig. 1*b*, C-type ctDNA was differentiated into various haplotypes from which A- and S-type ctDNAs were clearly distinguished as single haplotypes. W-type ctDNA was also differentiated into various haplotypes, which could be expected because several derived types (W1, W2, and W3) have been found (Hosaka and Hanneman 1988*b*). T-type ctDNA was derived as a single haplotype within a group of haplotypes having W-type ctDNA, which was distantly related from haplotypes of C-, S-, or A-type ctDNA. This distant relationship of T-type ctDNA from the haplotypes of Andean cultivated species could be supported if T-type ctDNA of the cultivated potato was initially introduced from some populations of a wild species, *Solanum tarijense* (Hosaka 2003), because *S. tarijense* is morphologically distinct and classified into a different taxonomic series from that of Andean cultivated potatoes (Correll 1962; Hawkes 1990; Ochoa 1990). Therefore, it can be concluded that W- and C-type ctDNAs were differentiated diversely within and between them, and T-type ctDNA and S- and A-type ctDNAs were clearly distinguished from the group of haplotypes having W- and C-type ctDNAs, respectively.

nDNA and ctDNA differentiation

Hybridization results in hybrid plants with maternal ctDNA and intermediate nDNA between parental nDNAs. Subsequent hybridization can modify the initial hybrids into various variants depending on types and frequencies of hybridization and extent of selection. In this study, nDNA RFLP data did not clearly support differences among S-, C-, and A-type ctDNAs in cultivated potatoes (Fig. 3). Consequently, only weak correlation was found between nDNA differentiation and ctDNA differentiation (r = 0.415 with ctDNA microsatellites or r = 0.217 with ctDNA types). This suggests that frequent genetic exchange occurred through hybridization between accessions with different ctDNAs in cultivated species.

Chilean subsp. *tuberosum* could be a derived form from Andean highland potatoes, *S. tuberosum* subsp. *andigena* (Hawkes 1956; Brücher 1963; Hosaka and Hanneman 1988*a*). Regardless of ctDNA types in initial *S. tuberosum* subsp. *andigena* materials brought to Chile (probably A- or T-type ctDNA), subsequent differentiation evolved Chilean subsp. *tuberosum*, which were distinguished by nDNA RFLPs as a separate subcluster from the Andean cultivated potatoes (Fig. 3). Distinctiveness of nDNA between the two subspecies was also supported by an nDNA microsatellite analysis (Raker and Spooner 2002). Thus, one accession of Chilean subsp. *tuberosum* (tbr3), which had T-type ctDNA, but clustered with subsp. *andigena*, might be an immediate hybrid between *S. tuberosum* subsp. *andigena* and Chilean subsp. *tuberosum*, showing Chilean subsp. *tuberosum* like morphology with T-type ctDNA but more *S. tuberosum* subsp. *andigena* like nDNA.

The triploid and pentaploid cultivated species *S. juzepczukii* and *S. curtilobum* had C- or S-type ctDNA, and likewise for the other Andean cultivated species. However, these together with *S. acaule* formed the most distant, unique cluster (Fig. 3), strongly supporting common ancestry of these species (Hawkes 1962; Schmiediche et al. 1980; van den Berg et al. 1999). The contradictory differentiation between nDNA and ctDNA could be due to sterility caused by their odd-numbered polyploidy, which prevented subsequent hybridization and modification from the original genetic constitution of initial hybrids.

Wild species having W-type ctDNA were highly differentiated in both ctDNA and nDNA from accessions (mostly from cultivated species) having A-, S-, or C-type ctDNA. This differentiation can be supported by a previous study (Nakagawa and Hosaka 2002) using a larger set of wild species to elucidate the origin of *S. acaule*, where various wild species mostly from Bolivia and Argentina having W-type ctDNA were clearly separated by nDNA RFLPs from those (including cultivated species) mostly from Peru having C- or S-type ctDNA. Therefore, it can be concluded that, excluding immediate hybrids, ctDNA and nDNA concordantly differentiated into two groups: (*i*) cultivated species and putative ancestral species having S-, C-, or A-type ctDNA mostly from Peru and (*ii*) wild species having W-type ctDNA mostly from Bolivia and Argentina. Considering the composed taxa in each group, the two differentiated groups could correspond to two clearly separate clades on the phylogram constructed using AFLP markers by Groendijk-Wilders et al. (1999) and to the two morphological groups in the *S. brevicaule* complex identified by van den Berg et al. (1998).

Phylogenetic implications

Each of the Andean cultivated species *S. stenotomum*, *S. phureja*, *S. chaucha*, and *S. tuberosum* subsp. *andigena* was not uniquely identified by either ctDNA or nDNA analysis. This suggests that these species share the same gene pool. Huamán and Spooner (2002) examined morphologically a large collection of cultivated species and found that most morphological characters overlap extensively with those of the other species. Considering proposed hypotheses on the origin of these species (reviewed in the Introduction), the shared gene pool is attributed primarily to the common ancestry of these species from the most primitive cultivated diploid species, *S. stenotomum*. Subsequent genetic exchange through hybridization within the gene pool undoubtedly occurred, as evidenced by the frequent appearance of a triploid species, *S. chaucha*, which arose by a cross between tetraploid *S. tuberosum* subsp. *andigena* and diploid *S. stenotomum* (Hawkes 1958; Jackson et al. 1977) and other natural hybrids with wild species (Brush et al. 1981; Johns and Keen 1986; Hawkes 1990; Huamán and Spooner 2002). However, involvement of a wild species, *S. sparsipilum* (Cribb and Hawkes 1986; Hawkes 1990) or *S. vernei* (Brücher 1964), into this gene pool to originate *S. tuberosum* subsp. *andigena* could hardly be adopted by the present nDNA RFLP analysis, as previously shown by ctDNA analysis (Hosaka 1995).

The wild species clustered with cultivated species in Fig. 3 are particularly interesting to elucidate the origin or

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domestication of cultivated potatoes. In a previous study (Hosaka 1995), multiple ctDNA types were found within *S. bukasovii* (A, S, C, and W), *S. canasense* (S and C), *S. multidissectum* (S and C), *S. leptophyes* (C and W), and *S. candolleanum* (S and C). The shared nature of ctDNA types with cultivated species suggested successive domestication of potato and parallel differentiation of these wild species from the ancestral species complex (Hosaka 1995). In this study, accessions of *S. bukasovii*, *S. canasense*, and *S. leptophyes* having C-type ctDNA showed close nDNA similarity to cultivated potatoes (Fig. 3). Thus, the common ancestry of these species with cultivated species could be supported. The C-type ctDNA was divided into 12 haplotypes, among which four haplotypes were not found in any wild species but only in the cultivated species (Fig. 1*b*). A future survey for a large number of accessions of these wild species would disclose which species shared these cultivated species-specific haplotypes.

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