Nuclear and cytoplasmic genome composition of *Solanum bulbocastanum* (+) *S. tuberosum* somatic hybrids

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Abstract: Somatic hybrids between the wild incongruent species *Solanum bulbocastanum* ($2n = 2x = 24$) and *S. tuberosum* haploids ($2n = 2x = 24$) have been characterized for their nuclear and cytoplasmic genome composition. Cytologic observations revealed the recovery of 8 (near-)tetraploid and 3 hexaploid somatic hybrids. Multicolor genomic in situ hybridization (GISH) analysis was carried out to study the genomic dosage of the parental species in 5 somatic hybrids with different ploidy. The GISH procedure used was effective in discriminating parental genomes in the hybrids; most chromosomes were unambiguously colored. Two (near-)tetraploid somatic hybrids showed the expected 2:2 cultivated-to-wild genomic dosage; 2 hexaploids revealed a 4:2 cultivated-to-wild genomic dosage, and 1 hexaploid had a 2:4 cultivated-to-wild genomic dosage. Characterization of hybrid cytoplasmic genomes was performed using gene-specific primers that detected polymorphisms between the fusion parents in the intergenic regions. The analysis showed that most of the somatic hybrids inherited the plastidial and mitochondrial DNA of the cultivated parent. A few hybrids, with a rearranged mitochondrial genome (showing fragments derived from both parents), were also identified. These results confirmed the potential of somatic hybridization in producing new variability for genetic studies and breeding.

Key words: potato, wild species, multicolor GISH, genome dosage, organelle DNA.

Résumé : La composition des génomes nucléaire et cytoplasmique d'hybrides somatiques entre l'espèce sauvage *Solanum bulbocastanum* ($2n = 2x = 24$) et le *S. tuberosum* ($2n = 2x = 24$) a été caractérisée. Des observations cytologiques ont révélé l'obtention de 8 hybrides somatiques (quasi-) tétraploïdes et de 3 hexaploïdes. Des analyses GISH multicolores ont été réalisées afin de déterminer le dosage génomique de l'espèce parentale chez 5 hybrides somatiques de ploïdies différentes. Le protocole GISH employé ici a permis de distinguer les génomes parentaux chez les hybrides puisque la plupart des chromosomes étaient colorées sans équivoque. Deux hybrides somatiques (quasi-) tétraploïdes montraient le dosage génomique attendu (2 génomes cultivés : 2 génomes sauvages); deux hexaploïdes affichaient un dosage 4 cultivés : 2 sauvages et un hexaploïde avait un dosage 2 cultivés : 4 sauvages. La caractérisation des génomes cytoplasmiques chez les hybrides a été effectuée à l'aide d'amorces spécifiques pour certains gènes et qui révélaient des polymorphismes entre les parents au sein de régions intergéniques. L'analyse a montré que la plupart des hybrides somatiques avaient hérité l'ADN plastidique et mitochondrial du parent cultivé. Quelques hybrides montrant un génome mitochondrial (avec des fragments dérivés des deux parents) ont également été identifiés. Ces résultats confirment le potentiel de l'hybridation somatique pour la production d'une nouvelle variabilité pour des fins d'analyse génétique et de sélection.

Mots-clés : pomme de terre, espèces sauvages, GISH multicolore, dosage génomique, ADN des organites.

[Traduit par la Rédaction]

Introduction

The characterization of the genome composition of interspecific somatic hybrids is an important prerequisite to efficiently exploit protoplast fusion. Because somatic hybridization generates novel variability, not only at the nuclear level but also in cytoplasmic DNA, studies on the inheritance of mitochondria (mt) and chloroplast (cp) DNA and the cytoplasmic complement of hybrids are very important. The most common approach used for cytoplasm characterization is Southern analysis with specific mt and cp probes (Lössl et al. 1999). However, this method is compa-

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ratively expensive and time-consuming. Recently, several "universal primers" homologous to conserved sequences of cpDNA and mtDNA and amplifying highly variable regions have been designed and used on many land plants (Bastia et al. 2001; Scotti et al. 2003).

Cytogenetics has often complemented molecular tools in somatic hybrid analysis. It provides insight into aspects such as aneuploidy, the dosage between parental genomes, and their effect on hybrid morphology and fertility. The expected chromosome number of a symmetric somatic hybrid should be the sum of the chromosomes of the fusion parents. However, somatic hybrids with different ploidy have often been described (Orczyk et al. 2003). Cytogenetic characterization of somatic hybrids relies on chromosome differentiation between parental karyotypes. When chromosomes of donor parents are small and poorly diversified, classic banding techniques do not allow reliable chromosome identification (Dong et al. 2000). Genomic in situ hybridization (GISH) provides a direct and visual method with which to study the genomic composition of the somatic hybrids and their derivates (Dong et al. 1999; Tek et al. 2004). GISH is complementary to molecular markers in following the introgression of whole chromosomes, recombination events, and chromosomal rearrangements in progenies derived from somatic hybrids.

Interspecific somatic hybridization has been used in potato to exploit the genetic variability of related wild species. Tuber-bearing species are included in section *Petota*, whereas all nontuber-bearing species, including tomato and related species, are in the sections *Etuberosum*, *Juglandifolium*, and *Lycopersicum* (Spooner and Castillo 1997). Although somatic hybridization has been extensively exploited in potato, comparably few reports involving GISH analysis are available. As far as we know, GISH analysis of somatic hybrids and their derivates has been applied only on taxonomically distant species (Dong et al. 1999, 2000, 2005; Gavrilenko et al. 2003).

Recently, somatic hybrids of *Solanum bulbocastanum* and *S. tuberosum* have been produced in our laboratories (Savarese et al. 2005). In this paper, we report on the characterization of the nuclear and cytoplasmic genomes of these hybrids. To achieve a detailed characterization of the genome constitution of the somatic hybrids, multicolor GISH (mcGISH) was used. As far as we know, this represents the first report of successful GISH discrimination of *Solanum* species belonging to section *Petota*.

Materials and methods

Plant material

Eleven *S. tuberosum* (+) *S. bulbocastanum* somatic hybrids and their fusion parents were included in this study. Somatic hybrids were regenerated from different calli after protoplast fusion between a late-blight-resistant *S. bulbocastanum* clone of PI 275190 (blb1C) and *S. tuberosum* haploid DEI-23 (fusion combination coded HF9, including 7 hybrids); a late-blight-resistant *S. bulbocastanum* clone of PI 275188 (blb2C) and DEI-23 (fusion combination coded HF6, including 3 hybrids); and blb2C and *S. tuberosum* haploid V2I-59 (1 hybrid called HF5A). All somatic hybrids were produced through electrofusion and were identified by inter-simple sequence repeat (ISSR) markers (Savarese et al. 2005). All the regenerated shoots had been clonally propagated for approximately 30 months before this study. *Solanum bulbocastanum* accessions were kindly provided by Dr. J. Bamberg (University of Wisconsin–Madison). DEI-23 and V2I-59 were developed at DiSSPA (Department of Soil, Plant and Environmental Sciences, University of Naples Federico II, Portici, Italy) during previous potato-breeding programs. They were obtained from $4x \times 2x$ crosses of *S. tuberosum* and an *S. phureja* haploid inducer, clone IVP35.

Cytogenetic characterization of nuclear genomes

Determination of ploidy level

Ploidy level of somatic hybrids was determined after chromosome counts in root-tip metaphase cells. Root tips about 1–2 cm long were harvested from young plants grown in vitro. Root-tip treatments and slide preparation followed published protocols (Dong et al. 2000, 2001). Slides were checked under a phase-contrast microscope, and those with appropriate metaphase chromosome spreads were stored at room temperature in dry conditions until needed. Chromosome counts were generally based on 5 well-spread metaphases, using 4′,6-diamidino-2-phenylindole (DAPI) for chromosome staining. Slides were also used for in situ hybridization.

Multicolor genomic in situ hybridization

mcGISH experiments were carried out to analyze the chromosomal constitution and the genome ratio (cultivated vs. wild) of 5 somatic hybrids. Included in the analysis were the tetraploid hybrid HF5A from blb2C (+) V2I-59, the 49-chromosome hybrid HF9AP, and 3 hexaploids: HF9B, HF9R, and HF9AI (all from blb1C (+) DEI-23). Parental genomic DNA was extracted from the young leaves of plants grown in a temperature-controlled greenhouse, using a cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). *Solanum tuberosum* and *S. bulbocastanum* DNA were labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively, using nick translation kits (Roche Diagnostics). Both labeled genomes were used, in a 1:1 ratio, as genomic probes on the same slide. Procedures for hybridization, post-hybridization washes, and signal detection were essentially as described by Dong et al. (2001). Minor modifications were used to decrease the extent of cross-hybridization between the genomic DNA of the 2 species. Briefly, the chromosomes on the slide were denatured in 70% formamide at 80 °C for 1.5 min, and then dehydrated in a –20 °C ethanol series (70%, 90%, and 100%). The hybridization mixture (25 μL for each slide), containing the labeled probes, was denatured at 85 °C for 10 min and then added to the slides. The hybridization mixture consisted of 3–5 ng/μL of each labeled genomic probe, 50% deionized formamide, 10% dextran sulfate, 2× standard saline citrate (SSC) (1× SSC: 0.15 mol/L NaCl, 0.015 mol/L sodium citrate), and 1 μg/μL of sheared salmon sperm DNA. After overnight incubation at 37 °C and a 20 min post-hybridization washing in 2× SSC at 42 °C, *S. tuberosum* genomic probe was detected with 1% fluorescein isothiocyanate (FITC)-conjugated anti-biotin antibody

Table 1. Primer pairs specific for chloroplast (cp) DNA and mitochondria (mt) DNA regions used to characterize the *Solanum* genotypes included in this study (for each primer pair, the annealing temperature is given).

Primer pair (code)[a]	Genomic region	Annealing temperature (°C)	Reference for sequence
cpDNA			
ALc1/3	*atpE*	44	Lössl et al. 1999
pucA	*trnH-trnK*	55	Demesure et al. 1995
pucB	*trnD-trnT*	55	Demesure et al. 1995
pucC	*psbC-trnS*	55	Demesure et al. 1995
pucD	*trnS-trnfM*	55	Demesure et al. 1995
pucE	*trnS-trnT*	57.5	Demesure et al. 1995
pucF	*trnM-rbcL*	55	Demesure et al. 1995
pucG	*trnK*	55	Demesure et al. 1995
pucH	*trnC-trnD*	55	Demesure et al. 1995
pucI	*psaA-trnS*	55	Demesure et al. 1995
pucJ	*trnF-trnV*	57.5	Dumolin-Lapègue et al. 1996
pucK	*trnV-rbcL*	57.5	Dumolin-Lapègue et al. 1996
pucL	*trnQ-trnR*	57.5	Dumolin-Lapègue et al. 1996
pucM	*trnT-psbC*	55	Dumolin-Lapègue et al. 1996
pucN	*rbcL-aacD*	55	Petit et al. 1998
pucO	*trnT-trnF*	55	Petit et al. 1998
pucP	*rbcL-psaI*	55	Petit et al. 1998
pucQ	*rpoC2-rpoC1*	55	Petit et al. 1998
pucR	*trnK-trnQ*	47.5	Dumolin-Lapègue et al. 1996
pucS	*rpoC1-trnC*	47.5	Dumolin-Lapègue et al. 1996
pucT	*trnfM-psaA*	47.5	Dumolin-Lapègue et al. 1996
mtDNA			
Alm4/5	Ψ*cob-rps10*	57	Lössl et al. 1999
NSm1	*rpl5-rps14*	58	Scotti 2002
pumD	*rps14-cob*	57.5	Demesure et al. 1995

Note: Primers generating polymorphisms (in bold) were used for the characterization of somatic hybrid cytoplasm.

[a]Primer codes as in Bastia et al. (2001) and Scotti et al. (2003).

(Roche Diagnostics), and *S. bulbocastanum* genomic probe was detected with 1% rhodamine-conjugated anti-digoxygenin antibody (Roche Diagnostics). Chromosomes were counterstained with DAPI in ProLong Gold antifade solution (Invitrogen).

Image capturing and processing

Slides were examined under a Zeiss Axiovert 135 fluorescence microscope equipped with separate excitation filter sets for DAPI, FITC, and rhodamine. All images were captured digitally with a charge-coupled device camera (AxioCam MRm, Zeiss). The camera was controlled using MRGrab software (Zeiss). Gray-scale images were acquired for each color channel and merged using Adobe Photoshop. The final images were adjusted in Adobe Photoshop for contrast and background optimization.

PCR analysis of cytoplasmic genomes

As for the characterization of cytoplasmic genomes, DNA from fusion parents and somatic hybrids was extracted from young leaves of greenhouse-grown plants, using the QIAGEN DNeasy Plant Mini Kit in accordance with the manufacturer's instructions. Genomic DNA (25 ng) was used in PCR with gene-specific primers for cpDNA (21 primer pairs) and mtDNA (3 primer pairs) (Table 1). Primers were first used to detect polymorphisms between the fusion parents in the intergenic regions. Amplification reactions (25 μL) consisted of 0.4 μmol/L of each primer, 200 μmol/L of each deoxynucleoside triphosphate (dNTP), 0.2 units of *Taq* DNA polymerase, and 1× reaction buffer supplied by the manufacturer (Perkin Elmer Cetus). Amplifications were performed in a DNA thermal cycler, programmed as follows: 4 min at 94 °C; 30 cycles of 45 s at 94 °C, 1 min at the annealing temperature (Table 1), and 3 min at 72 °C; and a final extension of 10 min at 72 °C. Amplification products were directly analyzed under UV light by electrophoresis in 1% agarose gel and staining with ethidium bromide. Differentiation between blb2C and V2I-59 cpDNAs was based on *S. tuberosum* and *S. bulbocastanum* cpDNA sequences available in GenBank (accession Nos. DQ386163 and DQ347958, respectively). Following PCR of blb2C and V2I-59 with the pucI primer pair, amplified fragments were digested with *Eco*RV before separation in agarose gel. Primer pairs (primers/enzyme) detecting polymorphisms between parental organelle genomes were used to characterize the cytoplasm of the somatic hybrids.

Results and discussion

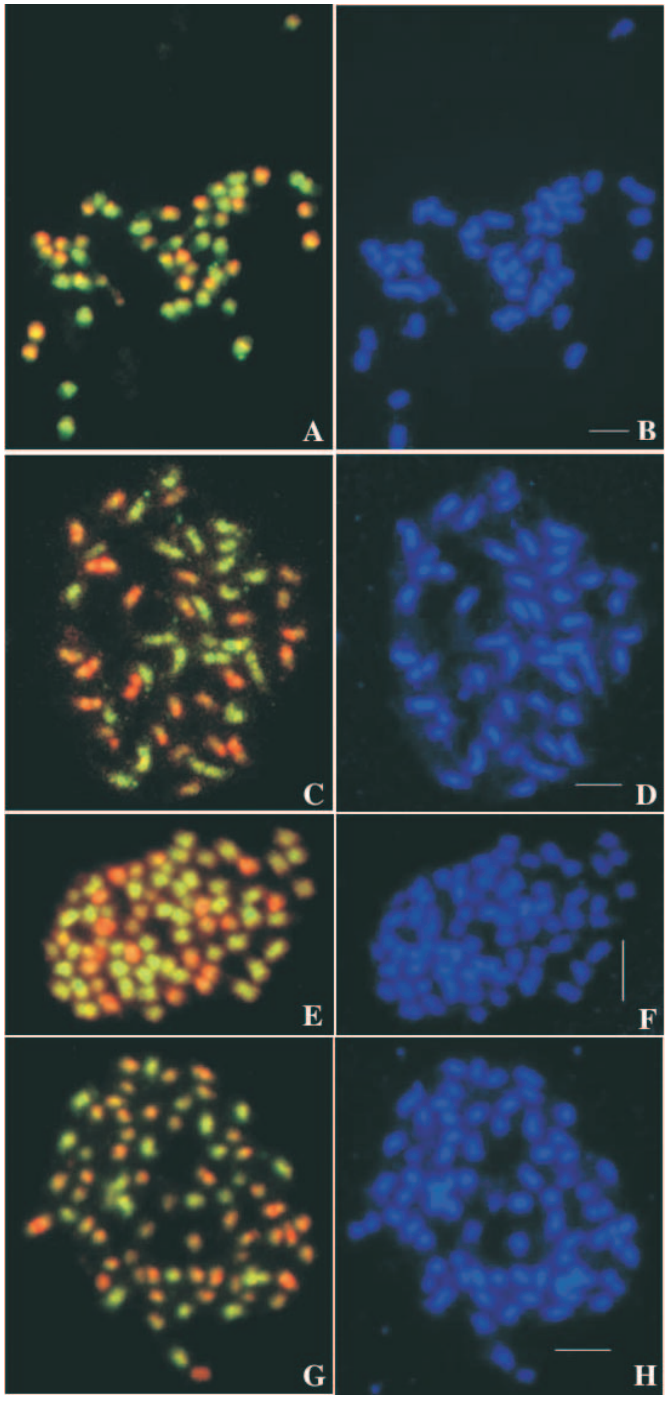
Cytogenetic characterization of nuclear genomes

Cytologic observations were first carried out to assess the

ploidy level of the somatic hybrids produced. Chromosome counts revealed the occurrence of 7 hybrids with 48 chromosomes (HF5A, HF6A, HF6E, HF6G, HF9I, HF9M, and HF9AQ). The recovery of tetraploid genotypes was expected after the binary fusions of two 24-chromosome species. We also found 1 hybrid with 49 chromosomes (HF9AP, Fig. 1D) and 3 with 72 chromosomes (HF9B, HF9R, HF9AI). Multiple fusions, fusion of protoplasts at different phases of the cell cycle, and protoclonal variation might explain this range of chromosome counts. Chromosome elimination and structural chromosome changes have also been reported for somatic hybrids (Pijnacker et al. 1987; McGrath and Helgeson 1998). To investigate the chromosome constitution and the genomic dosage (cultivated vs. wild) of 5 somatic hybrids with different ploidy, mcGISH analysis was applied. Results are summarized in Table 2 and Fig. 1. By adjusting the time of post-hybridization rinses, most parental chromosomes were well differentiated by color. This enabled a direct and unambiguous identification of their origin. HF5A is a somatic hybrid with 48 chromosomes. GISH confirmed the expected genomic ratio (*S. tuberosum* haploid/wild species) of 2:2 in HF5A hybrid (Figs. 1A and 1B). Expected genomic dosage (2:2) was also observed in HF9AP, an aneuploid somatic hybrid with 49 chromosomes (Fig. 1C). GISH revealed different chromosome constitutions among the 3 hexaploid hybrids obtained. HF9B (not shown) and HF9R (Figs. 1E and 1F) showed a predominance of *S. tuberosum* chromosomes and a genomic dosage of 4:2. The opposite was observed for the hexaploid HF9AI, which displayed a 2:4 cultivated-to-wild genomic dosage (Figs. 1G and 1H). *Solanum* species differ in ploidy and endosperm balance number (EBN) (Johnston et al. 1980). *Solanum bulbocastanum* is 1EBN, whereas *S. tuberosum* haploids are 2EBN. Because the EBN value of hybrids is expected to be the sum of the parents' EBN, we expect that hybrids with a 2:2 genome ratio are 3EBN, whereas hexaploids might have a different EBN (either 4 or 5), depending on their cultivated-to-wild genome ratio (Ehlenfeldt and Hanneman 1984). Carputo et al. (1998) found variability in the fertility of hexaploid somatic hybrids between 2*x*(2EBN) *S. tuberosum* and 2*x*(1EBN) *S. commersonii*. Differences might be due to different cultivated-to-wild genome ratios. Research is in progress to test whether our hexaploids demonstrate phenotypic differences that can be correlated to different genomic ratios and EBN values.

GISH has proven to be an excellent tool with which to investigate the nuclear genome of somatic hybrids at the chromosome level. Indeed, species of the Solanaceae family, including potato and its wild relatives, have karyotypes characterized by small and relatively poorly differentiated chromosomes (Camadro et al. 2004) that cannot be readily distinguished with conventional cytologic methods. GISH, combined with fluorescent in situ hybridization (FISH) using chromosome-specific potato bacterial artificial chromosome (BAC) clones, is a powerful approach that has been used to highlight chromosome rearrangements and the introgression of alien chromosomes in progenies derived from *S. brevidens*–*S. tuberosum* somatic hybrids (Tek et al. 2004; Dong et al. 2005). However, since the establishment of in situ hybridization using total genomic DNA probes (Schwarzacher et al. 1989), analysis in *Solanum* has been limited to inter-

Fig. 1. Genomic constitution of *Solanum tuberosum* (+) *Solanum bulbocastanum* somatic hybrids revealed by multicolor genomic in situ hybridization (mcGISH). Parental genomic probes were detected with fluorescein isothiocyanate (FITC)-conjugated antibody (*S. tuberosum*, yellow-green signal) and with rhodamine-conjugated antibody (*S. bulbocastanum*, red signal). The left column shows mcGISH signals from *S. tuberosum* and *S. bulbocastanum* genomic probes. In the right column, the corresponding somatic chromosome spreads counterstained with 4′,6-diamidino-2-phenylindole (DAPI) are presented. (A and B) Hybrids HF5A (2*n* = 48) and (C and D) HF9AP (2*n* = 49), showing a 2:2 cultivated-to-wild genomic dosage; (E and F) HF9R (2*n* = 72) with a 4:2 cultivated-to-wild genomic dosage; (G and H) HF9AI (2*n* = 72) with a 2:4 cultivated-to-wild genomic dosage. Bars = 5 μm.



generic hybrids, such as *S. tuberosum* (+) *S. lycopersicon* and *S. etuberosum* (+) *S. lycopersicon* (Garriga-Calderé et al. 1997; Gavrilenko et al. 2001). It has also been successfully applied to somatic hybrids between species belonging to different sections, such as *S. brevidens* (+) *S. tuberosum* (Dong et al. 2001; Gavrilenko et al. 2002) and *S. etuberosum* (+) *S. tuberosum* (Dong et al. 1999; Gavrilenko et al. 2003). *Solanum brevidens* and *S. etuberosum* belong to the section *Etuberosum*, which includes non-tuber-bearing species distantly related to *S. tuberosum* (Spooner and Castillo 1997). As far as we know, this is the first report of GISH-based genome discrimination between 2 *Solanum* species belonging to the same section.

PCR analysis of cytoplasmic genomes

To determine the cytoplasmic composition of our *S. tuberosum* (+) *S. bulbocastanum* somatic hybrids, we first detected polymorphisms between the parental cpDNAs and mtDNAs, using a set of gene-specific primers. Low levels of cpDNA polymorphisms between *S. tuberosum* and *S. bulbocastanum* were observed in this study. Of the 21 cpDNA primer pairs tested, 18 gave clear and repeatable amplifications (Table 3). Among these, 2 primer pairs (pucJ and ALc1/3) generated inter- and intraspecific fragment length polymorphisms among the fusion parents (Fig. 2). Both pucJ and ALc1/3 differentiated DEI-23 (T cpDNA type) from blb1C and blb2C (non-T cpDNA type). The main difference between T and all other cpDNA types is a 241 bp deletion detected by both primer pairs in the *trnV–ndhC* intergenomic region (Scotti et al. 2003). Therefore, pucJ and ALc1/3 were used to analyze the cpDNA of the HF6 and HF9 fusion combinations. However, these primers gave monomorphic bands between *S. tuberosum* haploid V2I-59 and blb2C. Haploid V2I-59 derived from clone V2 is an *S. tuberosum* subsp. *andigena* genotype from CIP; thus, it contains "andigena" cytoplasm. A similar amplification pattern was reported in *S. tuberosum* subsp. *andigena* clone 7XY.1 (Scotti et al. 2003). Discrimination between V2I-59 and blb2C cpDNAs was achieved by digesting the fragment amplified by the pucI primer pair with *Eco*RV restriction enzyme (Fig. 3). The pucI/*Eco*RV combination gave a 2.7 kb fragment and a 0.9 kb fragment in V2I-59, and 1 fragment of 3.60 kb in blb2C. This allowed discrimination between V2I-59 and blb2C at the *psaA/trnS* intergenic region.

As for the distribution of the parental cpDNA among the somatic hybrids from the fusion combinations HF6 and HF9, 80% of them showed the cpDNA amplification pattern of DEI-23 (Fig. 2, Table 4). HF5A showed the *S. bulbocastanum* restriction pattern using PucI/*Eco*RV (Fig. 3). Overall, only 3 hybrids (HF9R, HF9I, and HF5A) showed the *S. bulbocastanum* cpDNA, suggesting a nonrandom transmission of parental chloroplasts. However, because of the limited number of hybrids, the results are not statistically significant. Both random and biased chloroplast segregation have been reported in *Solanum* spp. interspecific somatic hybrids (Orczyk et al. 2003). Chloroplasts were preferentially sorted in *S. tuberosum* (+) *S. commersonii* hybrids (Cardi et al. 1999) and in asymmetric hybrids between *S. tuberosum* and *S. brevidens* (Perl et al. 1990). However, random transmission was observed in asymmetric hybrids between *S. tuber-*

Table 2. Somatic chromosome number and genomic ratio of 5 *Solanum tuberosum* (+) *S. bulbocastanum* somatic hybrids, based on genomic in situ hybridization (GISH) analysis.

Somatic hybrid	Somatic chromosome No.	Estimated genomic ratio, cultivated (T) : wild (B)
HF5A	48	2T:2B
HF9AP	49	2T:2B
HF9B	72	4T:2B
HF9R	72	4T:2B
HF9AI	72	2T:4B

Table 3. Amplification patterns of cpDNA and mtDNA in *Solanum* genotypes used for somatic hybridization.

	Genotypes			
Primer pairs[a]	blb1C	blb2C	DEI-23	V2I-59
cpDNA				
ALc1/3	A[b]	A	B	A
PucJ	A	A	B	A
mtDNA				
Alm4/5	—	—	B	A
PumD	—	—	B	A

Note: blb1C, *S. bulbocastanum* PI 275190; blb2C, *S. bulbocastanum* PI 275188; DEI-23 and V2I-59, *S. tuberosum* haploid clones; —, no amplification.

[a]Other primer pairs, as reported in Table 1, were not polymorphic.

[b]For each primer pair, the same letter indicates the same amplification pattern in the tested genotypes.

Fig. 2. PCR analysis of parental genotypes and their somatic hybrids using the chloroplast (cp) DNA primer pair ALc1/3. M, DNA size marker.

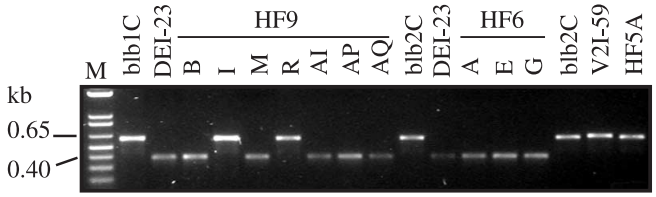


Fig. 3. cpDNA banding pattern amplified with universal primer pair pucI, before (u) and after (d) digestion with *Eco*RV. M, DNA size marker.

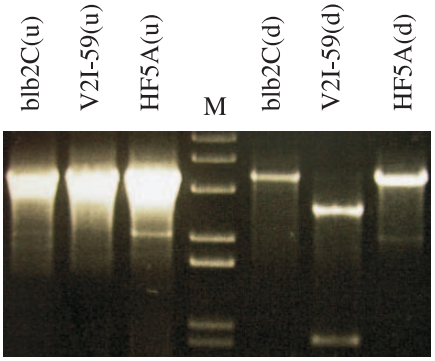


Table 4. Cytoplasmic genome analysis of 11 *S. tuberosum* (+) *S. bulbocastanum* somatic hybrids with primer pairs specific for cpDNA and mtDNA regions.

	Somatic hybrid										
Primer pair	HF5A	HF6A	HF6E	HF6G	HF9B	HF9I	HF9M	HF9R	HF9AI	HF9AP	HF9AQ
cpDNA											
ALc1/3	—[a]	tbr	tbr	tbr	tbr	blb	tbr	blb	tbr	tbr	tbr
pucJ	—	tbr	tbr	tbr	tbr	blb	tbr	blb	tbr	tbr	tbr
mtDNA											
ALm4/5	tbr	tbr	tbr	tbr	tbr	tbr	tbr	tbr	blb	tbr	blb
pumD	tbr	tbr	tbr	tbr	tbr	tbr	tbr	tbr	tbr	tbr	tbr

Note: HF5A, blb2C (+) V2I-59; HF6, blb2C (+) DEI-23; HF9, blb1C (+) DEI-23.

[a]Primer pairs listed did not differentiate between blb2C and V2I-59; HF5A showed the blb cpDNA pattern after *Eco*RV digestion of the PCR fragment amplified using pucI.

osum and *S. chacoense* (Perl et al. 1990). Southern-based analysis and (or) analysis of the segregation pattern of other molecular markers, such as the highly polymorphic cpDNA SSRs (Bryan et al. 1999), might be useful to investigate the cpDNA composition of our somatic hybrids. However, because chloroplast DNA rearrangements are rare events (Orczyk et al. 2003), novel plastome configurations are not expected.

As for the mitochondrial genome, variability in gene organization between *S. bulbocastanum* and *S. tuberosum* was detected in 2 mtDNA genomic regions using 2 primer pairs (pumD and ALm4/5) (Table 3, Fig. 4). No intraspecific variation was found. The primer pair pumD amplifies the intergenic region between the *rps14* and *cob* genes, revealing a possible linkage between these 2 genes. Several plant mitochondrial genomes, including those of *Arabidopsis thaliana*, *Brassica napus*, *Nicotiana tabacum*, and *S. tuberosum*, show a conserved arrangement of the *rpl5–rps14–cob* gene cluster (Scotti et al. 2004; Sugiyama et al. 2005). Amplification of DEI-23 and V2I-59 DNA with pumD gave a fragment of about 1.5 kb, confirming the *rps14–cob* linkage. In contrast, the lack of PCR products in blb1C and blb2C suggests the absence of association (within the size limits detectable by our PCR conditions) between the 2 genes in *S. bulbocastanum*. Lack of association between *rps14* and *cob* was also found in *Solanum commersonii* (Bastia et al. 2001; Scotti et al. 2003). According to Lössl et al. (1999) and Scotti et al. (2003), both *S. bulbocastanum* and *S. commersonii* cytoplasmic genomes belong to the same group. The presence/absence of PCR products was also observed using the primer pair ALm4/5, which revealed rearrangements at the intergenic region between Ψ*cob* and *rps10*. *Solanum tuberosum* has 2 divergent copies of the *cob* gene, 1 of which is a pseudogene located upstream of the *rps10–coxI* gene cluster (Scotti et al. 2003). The presence of a PCR fragment (of about 1.65 kb) amplified with ALm4/5 confirmed the linkage between the 2 genes in *S. tuberosum* haploids DEI-23 and V2I-59. However, no amplification was obtained in blb1C and blb2C, indicating a lack of Ψ*cob–rps10* linkage. Similarly, no Ψ*cob–rps10* linkage was observed in *S. commersonii* (Scotti et al. 2003).

As for the distribution of parental mtDNA in the somatic hybrids, after PCR with pumD, all the somatic hybrids showed the *S. tuberosum* pattern (presence of the fragment,

Fig. 4. PCR analysis of parental genotypes and their somatic hybrids using mtDNA primer pairs (A) pumD and (B) ALm4/5. blb1C and blb2C are *S. bulbocastanum* PI 275190 and PI 275188, respectively; DEI-23 and V2I-59 are *S. tuberosum* haploids; HF9B, HF9I, HF9M, HF9R, HF9AI, and HF9AQ are blb1C (+) DEI-23 somatic hybrids; HF6A, HF6E, and HF6G are blb2C (+) DEI-23 somatic hybrids; and HF5A is a blb2C (+) V2I-59 somatic hybrid. M, DNA size marker.

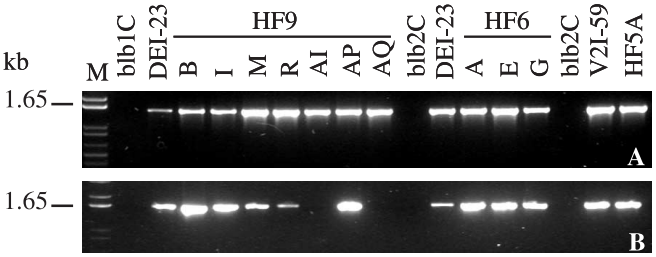


Fig. 4A). As for the *rps10* upstream region (amplified with Alm4/5), 2 hybrids (HF9AQ and HF9AI) did not show any amplification product, like *S. bulbocastanum* (Fig. 4B). The remaining hybrids showed the amplification product, as did the *S. tuberosum* parent. Although the type of markers used does not rule out the presence of both parental mtDNAs, this possibility is a rather rare event in regenerated somatic hybrids (Earle 1995). In addition, organelle DNA segregation patterns detected with the same PCR markers used in this study and with restriction fragment length polymorphism (RFLP) markers were largely coincident (Bastia et al. 2001). Preferential inheritance of *S. tuberosum* mtDNA was observed in several interspecific *Solanum* spp. somatic hybrids and cybrids (Perl et al. 1991; Yamada et al. 1997; Cardi et al. 1999). Our analysis showed that at least 2 hybrids had non-parental rearranged mitochondria. Somatic hybrids showing recombination, amplification of the substoichiometric mtDNA molecules, and partial addition of parental mtDNA fragments have been often described (Lössl et al. 1999; Scotti et al. 2004). Considering the results for cpDNA and mtDNA altogether, somatic hybrids were clustered into 3 groups as a consequence of the rearrangement of the parental mitochondrial genomes and the assortment of chloroplasts and mitochondria.

In summary, our work underlines the power of somatic

hybridization in generating novel nuclear and cytoplasmic variability. GISH is confirmed to be a valuable tool that can be used to detect such variability at the chromosome level, even in somatic hybrids derived from 2 closely related species. Hybrids with different ploidies, genomic ratios, and novel cytoplasmic constitutions were detected. These hybrids are currently being evaluated for disease resistance, fertility, and several agronomic traits.

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