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Organelle DNA and male fertility variation in *Solanum* spp. and interspecific somatic hybrids

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Abstract Novel and potentially useful genetic variation in cytoplasmic genomes can be induced by interspecific somatic hybridization in plants. To evaluate such variability and correlate it with nuclear-cytoplasmic interactions leading to male sterility in *Solanum* spp., we examined progeny of male-sterile and male-fertile somatic hybrids between *Solanum tuberosum* (*tbr*), the common potato, and *S. commersonii* (*cmm*), a wild species showing sexual incongruity with *tbr*, for fertility and organelle DNA composition. Uniform male-fertile and male-sterile progenies were obtained by selfing the male-fertile hybrid and crossing the male-sterile ones, indicating maternal inheritance of the fertility phenotype. The two fusion partners were only slightly differentiated in the plastidial genome. MtDNA polymorphism between the species was greater, although its extent varied with the genomic region investigated. All somatic hybrids had non-parental organelle genomes, with reassorted organelles and/or rearranged mitochondria (i.e., *cmm*-specific bands for some regions and *tbr*-specific bands for others). Mitochondria reassorted independently from chloroplasts. Most hybrids showed the *cmm* cpDNA hybridization pattern, indicating non-random transmission of chloroplasts. Most male-sterile hybrids showed preferential inheritance of *tbr* mtDNA fragments. The male-fertile somatic hybrid clone had predominantly *cmm* mtDNA fragments. This result suggests that a *tbr*-derived region involved in nuclear-cytoplasmic incompatibility and male sterility has been lost by rearrangement; however, no clear correlation between a specific mitochondrial region and male sterility has been found so far.

Key words Interspecific hybridization · *Solanum* · Male fertility · Chloroplasts · Mitochondria

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Introduction

The genetic basis of the cultivated potato (*Solanum tuberosum* spp. *tuberosum*) (*tbr*) is very narrow. On the other hand, the *Solanum* genus includes many species useful as a source of genetic variability for the improvement of common potato. New breeding approaches generally based on the manipulation of ploidy level have helped to exploit wild germplasm and enlarge genetic variability in the cultivated gene-pool (Ortiz 1998). Such approaches, however, have been mainly directed to the manipulation of nuclear genomes, with little attention being given to the cytoplasmic organelles (i.e., chloroplasts and mitochondria). Nevertheless, the cytoplasm can play an important role in plant performance, and genetic uniformity at the cytoplasmic level can have disastrous consequences, as exemplified by the Texas-CMS (cytoplasmic male sterility) maize case (Levings 1990).

Cytoplasmic genes, or their interactions with nuclear genes, are involved in the control of several morphological, physiological, and agronomic traits in potato (Hoopes et al. 1980; Kaul 1988; Perl et al. 1991; Lössl et al. 1994; Gounaris 1996; Frei et al. 1998). Information about the genetic basis of such control, however, is generally very limited. One or few nuclear genes can interact with incompatible cytoplasms to cause male sterility in interspecific *Solanum* hybrids (Kaul 1988; Ortiz 1998). Several cytoplasmic 'factors' were hypothesized (Grun 1979; Kaul 1988), but no relevant organellar genes could be identified because of the limited cytoplasmic variability induced by conventional procedures of hybridization or mutagenesis.

The chloroplast DNA (cpDNA) of *tbr* and other related species has been studied extensively (Hosaka et al. 1984, and later studies by the same author; Heinhorst et al. 1988; Perl et al. 1991). Comparison of chloroplast DNA in several potato species suggests that the expression of CMS in *Solanum* spp. is not controlled by cpDNA (Buckner and Hyde 1982), as also noted in other species. The mitochondrial genome probably plays a key role in the induc-

tion of alloplasmic male sterility, but only recently has the organization of the mitochondrial genome been examined in common potato (Zanlungo et al. 1991; Dell'Orto et al. 1993; Binder et al. 1994; Zanlungo et al. 1994; Quiñones et al. 1995; Giese et al. 1996; Lössl et al. 1999). Scanty information is available for other *Solanum* species (Kemble et al. 1986; Perl et al. 1991; Xu et al. 1993; Yamada et al. 1997; Lössl et al. 1999).

The interactions between nuclear and cytoplasmic genes of different species could be applied to the one-step conversion of fertile potato cultivars to CMS parents useful for F_1 hybrid True Potato Seed (TPS) production (Perl et al. 1990). In addition, (Grun 1979) used the type of male sterility and degree of flower malformation observed in interspecific hybrid progenies to establish phylogenetic relationships in the *Solanum* genus. On the other hand, tetraploid and dihaploid clones of common potato are usually male-sterile because of gene mutations and/or chromosome abnormalities and cannot be used as male parents. Thus, CMS male sterility of F_1 hybrids between them and wild species used as pollen donors can create a partial sterility barrier against interspecific gene flow, limiting the utilization of *tbr* and some wild species in breeding (Vilaró et al. 1989).

Somatic hybridization is a powerful method to create new nuclear-cytoplasmic organelle combinations and to induce variability in the organelle genomes (Earle 1995; Hanson et al. 1995). Somatic hybrids between different genotypes of dihaploid *tbr* and the sexually incompatible diploid South American species *S. commersonii* (*cmm*) were found to be male-sterile (Cardi et al. 1993a; Carotenuto and Bastia 1995; Nyman and Waara 1997). On the other hand, when a clone with *S. stoloniferum* cytoplasm was combined with *cmm* by protoplast fusion, male-fertile hybrids were produced (Nyman and Waara 1997). The male-sterile hybrids showed a 'pollen-less' phenotype and various abnormalities during tapetum development, meiosis-II and cytokinesis (Conicella et al. 1997). A similar phenotype was seen in triploid sexual hybrids between dihaploid *tbr* as female and tetraploid *cmm* as male, in that they did not shed any pollen grain and had meiosis blocked at early stages (Novy and Hanneman 1991). In contrast, hybrids showed good pollen production and stainability when tetraploid *cmm* was used as female (Carputo et al. 1995). Hence, as in other species combinations, the male sterility of *cmm*(+)*tbr* somatic hybrids is probably due to incompatibilities between nuclear and cytoplasmic (mitochondrial?) genes of the wild and cultivated species, respectively. An exceptional male-fertile somatic hybrid was found within an otherwise completely male-sterile population (Cardi et al. 1993a). It can be hypothesized that this fertile somatic hybrid either contained the intact mitochondrial genome from *cmm* or that the *tbr* cytoplasmic factors involved in such interactions were eliminated by mitochondrial recombination.

The variation observed in male fertility and organelle DNA of *cmm*, *tbr,* and their somatic hybrids can help in our understanding of the genetic and molecular bases of nuclear-cytoplasmic interactions in *Solanum* spp. Such information could make it possible to identify specific genes involved in such interactions and to constitute genotypes with improved performance resulting from novel compositions of organelles. In this paper we report on the genetic characterization of the male sterility observed in the somatic hybrids and on the molecular analysis of variability in the organellar genomes of parental and hybrid genotypes.

Materials and methods

Plant material

The *S. commersonii* clone (coded hereafter as Cmm1) was isolated from seed-derived plants of the accession PI 243503 and micropropagated *in vitro.* Seeds were kindly provided by Dr. J.B. Bamberg, Inter-Regional Introduction Station, Sturgeon Bay, Wis., USA. The *S. tuberosum* dihaploid clone DH 81–7-1463 (coded SVP11) was kindly provided by Dr. K.J. Puite, DLO-CPRO, Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands. It was obtained from the tetraploid clone W 72–22–492 (K.J. Puite, personal communication). Somatic hybrids between Cmm1 and SVP11 were produced as previously described (Cardi et al. 1993b). The two parental genotypes, 24 tetraploid or near-tetraploid, and 8 hexaploid or near-hexaploid hybrids were included in the present study.

Genetic analysis

To characterize the segregation pattern of male sterility observed in regenerated hybrids, we crossed the male-sterile genotypes with the fertile somatic hybrid SH9A, and selfed the latter. Progenies were grown in an air-conditioned greenhouse (18°–30°C) under natural daylight, in an aphid-proof screenhouse, or in open field. When most of the plants had flowered, pollen production was analyzed by shaking anthers with a battery-powered vibrator. Stainability of pollen collected was determined with 1% acetocarmine.

Molecular analysis

Total DNA was isolated from leaves of parental and hybrid genotypes using either the procedure of Dellaporta et al. (1983) or the procedure of Doyle and Doyle (1990), both modified as reported in Cardi and Earle (1997).

Total DNA (2.5–5 µg) was digested with a number of restriction enzymes, electrophoresed for about 20 h in 1×TBE at 1.2–1.4 V cm^{-1}, and blotted on either neutral or positively charged nylon membrane. In the former case, blots were prepared and analyzed by a chemioluminescent method as previously described (Cardi and Earle 1997), while in the latter case they were prepared under alkaline conditions (0.4 *N* NaOH) and analyzed by a radioactive method after washing one to two times at 65°C (20 min each) with 0.1×SSC, 0.1% SDS.

The probes listed in Table 2 were used for analysis of cpDNA and mitochondrial DNA (mtDNA) polymorphism. They were kindly provided by Dr. S. Heinhorst, University of Southern Missouri, USA, Dr. C.J. Leaver, Oxford University, UK, Dr. C.S. Levings, North Carolina State University, USA, Drs. C.A. Sutton, M.R. Hanson, and D.B. Stern, Cornell University, USA, and Dr. P.F. Fransz, Wageningen Agricultural University, The Netherlands.

Statistical analysis

Based on the presence/absence of species-specific bands seen after restriction fragment length polymorphism (RFLP) analysis of organellar DNA, parental and hybrid genotypes were grouped by a Hierarchic Cluster Analysis procedure using the "Normalized Percent Disagreement" index to calculate distances among clusters and the Average Linkage Method as clustering criterion (Wilkinson et al. 1992). Deviations from expected segregation ratios of RFLP bands were tested by means of $\chi 2$ analysis. Co-segregation of parental bands in the somatic hybrid population was

analyzed by computing the simple matching dichotomy coefficient of similarity S4=(a+d)/(a+b+c+d), where a and d are the cases where the values of both variables agree (i.e., two bands are both present or absent, respectively), and b and c are those in which they disagree (Wilkinson et al. 1992).

Results

Genetic analysis

Pollen production of parental genotypes, hybrids, and their progenies is reported in Table 1. *S. commersonii* and the somatic hybrids flowered profusely in all environments. About 70–80% of the progenies flowered in the greenhouse, and almost all of them formed flowers in two independent experiments in a screenhouse or open field. The *S. tuberosum* dihaploid clone showed little vigor and flowering both in the greenhouse and the screenhouse. Some male-sterile hybrids and their progenies showed flowers with deformed anthers. Male-sterile hybrids with normal anthers generally gave progenies with the same flower phenotype.

Virtually all flowering plants of Cmm1, SH9A, and the progenies derived by selfing SH9A were fully fertile with abundant production of highly stainable pollen. One genotype derived by selfing SH9A did not produce pollen, but when the analysis was repeated in tuber-derived progenies, the latter were all male-fertile. On the other hand, no pollen production was observed in the somatic hybrid SH9B and in the progenies derived by crossing it and other male-sterile genotypes with SH9A. The flowers occasionally produced by SVP11 were not fully developed and shed very little pollen.

Table 1 Flowering and pollen production in *S. commersonii* (Cmm1) and *S. tuberosum* (SVP11) parents, male-fertile (SH9A) and male-sterile (SH9B) somatic hybrids, and progenies obtained by selfing or by crossing a sample of male-fertile and male-sterile hybrids, respectively

Environment	Genotype[a]	No. of plants	
		w/ flowers[b]	w/ pollen
Greenhouse	Cmm1	3	3
	SH9A	3	3
	SH9B	3	0
	SH9A×SH9A	30	30
	SH9B×SH9A	27	0
Screenhouse	Cmm1	15	15
	SVP11	4	0
	SH9A	23	23
	SH9B	16	0
	SH9A×SH9A	386	385
	SH9B×SH9A	98	0
Field	SH9A×SH9A	44	44
	SH9B×SH9A	37	0
	SH1A×SH9A	37	0
	SH2B×SH9A	5	0
	SH3A×SH9A	22	0
	SH5A×SH9A	8	0
	SH7A×SH9A	43	0
	SH8A×SH9A	9	0
	SH10C×SH9A	8	0
	SH12A×SH9A	11	0
	SH13A×SH9A	32	0
	SH14A×SH9A	2	0
	SH15D×SH9A	43	0
	SH16A×SH9A	32	0
	SH20A×SH9A	31	0
	SH22A×SH9A	27	0
	SH25A×SH9A	35	0

[a] In previous experiments (Cardi et al. 1993a) all somatic hybrids, except for SH9A, did not produce any pollen grains
[b] SVP11 either did not form flowers in the greenhouse or differentiated flowers not fully developed in the screenhouse. The other genotypes generally flowered profusely in all environments

Organellar DNA polymorphism between parental genotypes

After restriction of total DNA of Cmm1 and SVP11 with eight or nine enzymes and probing with cpDNA probes, polymorphism was only found with the potato-derived probe pStB153 and *Bam*HI. A single fragment of about 12.3 kb or two fragments of 2.3 and 10 kb were seen in the *tbr* clone and the wild parent, respectively. No polymorphism was seen with the *Petunia*-derived probes pPCY20–1, pPCY64, and S8 (Table 2).

For mtDNA polymorphism analysis, total DNA was digested with four to ten (mostly 4) enzymes and probed with the 14 heterologous mtDNA fragments reported in Table 2. Polymorphism was found with *cox1*, *cox2*, *cob*, *atp6*, *atp9*, *rrn18-rrn5*, *rps12*, *nad1bc*, *nad3*, and pSpom1, but not with the other probes tested. Species-specific gene fragments for both *cmm* and *tbr* were observed for *cox1, atp6, rrn18-rrn5*, *rps12* and *nad3*. In other cases, each parent showed a band not present in the other genotype. Thus, in comparison with SVP11, Cmm1 showed an additional band for *cox2* (3.0 kb) and *atp9* (6.0 kb), and lacked one band for *cob* (6.5 kb), and *nad1* (3.1 kb). Single-band restriction patterns were obtained for *cox1*, *atp6*, *rps12*, and *nad3* in both species, and also for *nad1* in Cmm1 and for *cox2* in SVP11 (Table 3).

Organellar DNA profiles of somatic hybrids

The two parents and a sample of 32 hybrids were included in RFLP analysis of organellar DNA with one plastidial (pStB153) and nine mitochondrial (*cox1*, *cox2*, *cob*, *atp6*, *atp9*, *rrn18-rrn5*, *rps12*, *nad1bc* and *nad3*) probes (Fig. 1). Except for clone SH8B, which showed a bi-parental hybridization pattern in the *cox1*, *atp6* and *rrn18-rrn5* loci, hybrids usually displayed only one of the parental patterns at each locus. Although some hybrids showed both *rps12* and *nad3* parental bands, the *cmm*-specific signals were much fainter than *tbr* ones, the genes from the wild species being probably present in substoichiometric amounts. For classification purposes in further analyses, those genotypes were scored as "*tuberosum*" for the *rps12* and *nad3* loci.

Table 2 Polymorphism between parental *Solanum commersonii* and *S. tuberosum* clones after RFLP analysis of cpDNA and mtDNA with various enzyme/probe (E/P) combinations

Probe[a]	Species	Fragment (kb)	E/P combinations (no.)	
			Tested	Polymorphic
cpDNA				
pStB153	*Solanum tuberosum*	*Bam*HI (12.3)	9	1
pPCY20–1	*Petunia hybrida*	*Eco*RI-*Hin*dIII (1.8)	8	0
pPCY64	*Petunia hybrida*	*Bam*HI (2.4)	8	0
S8	*Petunia hybrida*	*Pst*I (21.0)	8	0
mtDNA				
cox1	*Zea mays*	*Ecor*RI-*Bam*HI (3.95)	4	2
cox2	*Zea mays*	*Eco*RI (2.4)	5	2
cox3	*Oenothera berteriana*	*Ecor*RI-*Pst*I (1.1)	10	0
cob	*Zea mays*	*Eco*RI-*Hin*dIII (0.68)	4	2
atp6	*Zea mays*	*Hin*dIII (2.7)	8	1
atp9	*Petunia hybrida*	*Pst*I-*Bgl*I (1.5)	5	3
rrn18-rrn5	*Zea diploperennis*	*Bam*HI (6.0)	5	3
rrn26	*Zea mays*	*Sma*I (4.8)	8	0
rps3-rpl16	*Petunia hybrida*	*Xba*I (4.2)	8	0
rps12	*Petunia hybrida*	*Bam*HI-*Xho*I (0.7)	4	1
nad1 (exons *bc*)	*Petunia hybrida*	*Sst*I-*Xho*I (0.23)	4	3
nad3	*Petunia hybrida*	*Bam*HI-*Xho*I (0.9)	4	1
D23	*Raphanus sativus*	*Eco*RI-*Bam*HI (0.65)	8	0
pSpom1	*Spirodela oligorhiza*	*Pst*I (18.0)	4	4

[a] pPCY20–1 (de Haas et al. 1987), pPCY64 (de Haas et al. 1986), S8 (Palmer et al. 1983), pStB153 (Heinhorst et al. 1988), *cox1* (Isaac et al. 1985), *cox2* (Fox and Leaver 1981), *cox3* (Hiesel et al. 1987), *cob* (Dawson et al. 1984), *atp6* (Dewey et al. 1985), *atp9* (Young et al. 1986), *rrn18-rrn5* (Gwynn et al. 1987), *rrn26* (Stern et al. 1982), *rps3-rpl16* (Conklin and Hanson 1993), *rps12* (Hanson et al. 1995), *nad1bc* (Conklin et al. 1991), *nad3* (Hanson et al. 1995), D23 (Temple et al. 1992), pSpom1 (de Heij et al. 1985)

Table 3 Restriction fragments (kb) seen by Southern analysis of some polymorphic mitochondrial genome regions in parental clones of *Solanum commersonii* (Cmm1) and *S. tuberosum* (SVP11)

Probe	Enzyme	Cmm1	SVP11
cox1	*Eco*RI	4.6	5.1
cox2	*Hin*dIII	3.0; 2.4	2.4
cob	*Dra*I	3.0; 2.8	6.5; 3.0; 2.8
atp6	*Bgl*I	25.3	15.1
atp9	*Hin*dIII	9.0; 6.0; 2.3	9.0; 2.3
rrn18-rrn5	*Eco*RI	5.9; 4.6; 3.1[a]; (2.0)[b]	5.9; 5.0; 3.1[a]; (2.7)[b]
rps12	*Dra*I	11.8	17.1
nad1bc	*Dra*I	6.0	6.0; 3.1
nad3	*Dra*I	11.8	17.1

[a] This band derives from cross-hybridization to chloroplast sequences (Wolters et al. 1995).
[b] ( ), Weak bands

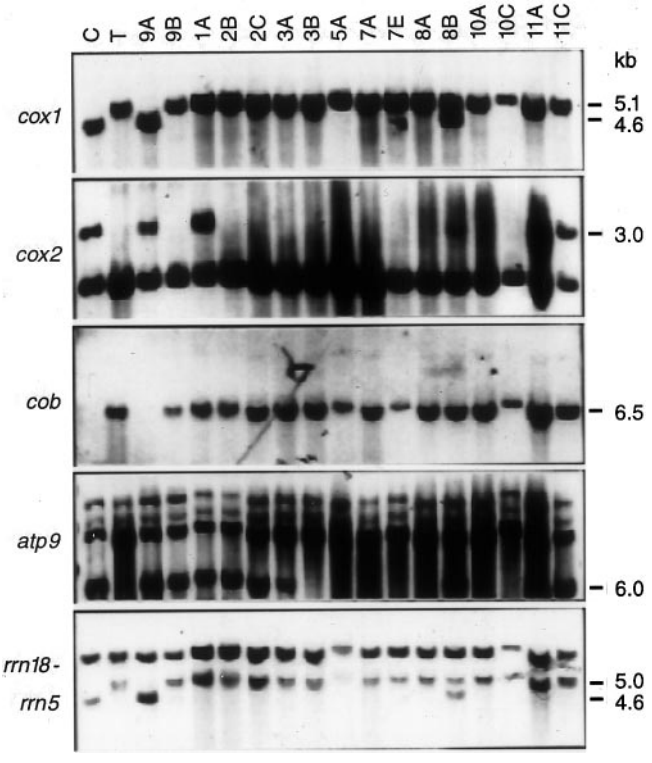

Fig. 1 Closeup of hybridization patterns in parental genotypes and a sample of somatic hybrids after Southern analyses with various probes for mtDNA. The molecular weights of the polymorphic bands are indicated. *C S. commersonii*, *T S. tuberosum*, *9A* male-fertile hybrid SH9A, *9B–11C*, male-sterile hybrids

Based on the presence/absence of nine *cmm*-specific and eight *tbr*-specific bands, parental and hybrid genotypes could be placed in 16 groups (A-R) (Fig. 2 and Table 4). All hybrid genotypes were non-parental, with reassorted organelles and/or rearranged mitochondria (i.e., *cmm*-specific bands for some regions and *tbr*-specific bands for others). The relative presence of the parental fragments allowed clustering of the basic groups into larger ones (Fig. 2). Eventually, two big clusters were obtained, a larger one containing SVP11 and 23 hybrids with a '*tbr*-like' cytoplasm and a smaller one containing Cmm1 and the remaining 9 hybrids with a '*cmm*-like' cytoplasm. The fertile clone SH9A belonged to the latter group and was closest to the tetraploid clones 13A and 13B and to the hexaploid clones regenerated from calli 14 and 25.

Overall, 88% of the hybrids showed the cpDNA hybridization pattern *cmm*, indicating nonrandom transmission of chloroplasts ($P<0.01$) (Table 5). On the other hand, in the hybridizations with the mitochondrial

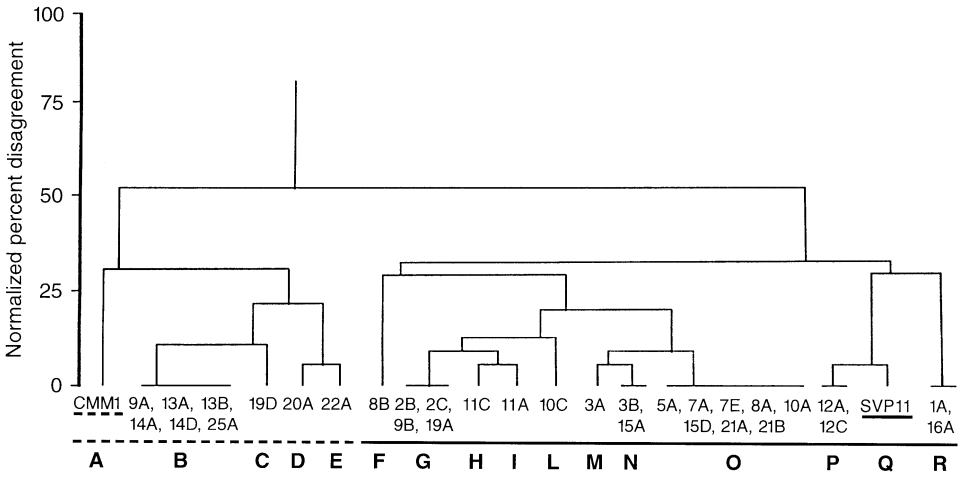

Fig. 2 Dendrogram obtained by cluster analysis of parental and hybrid genotypes based on presence/absence of seven mtDNA and two cpDNA *commersonii*-specific, and seven mtDNA and one cpDNA *tuberosum*-specific restriction fragments. Groups A-E and F-R include clones with *cmm*- or *tbr*-like cytoplasms, respectively

Table 4 Organelle DNA composition of parental (Cmm1 and SVP11) and hybrid clones (1A–25A) based on Southern analyses with one plastidial and nine mitochondrial probes[a]

Group	Clones[b]	mtDNA									cpDNA
		cox1	*cox2*	*cob*	*atp6*	*atp9*	*rrn18-rrn5*	*rps12*	*nad1-bc*	*nad3*	pStB153
A	Cmm1	C[c]	C	C	C	C	C	C	C	C	C
B	9A, 13A, 13B, 14A, 14D, 25A	C	C	C	C	C	C	T	C	T	C
C	19D	C	T	C	C	C	C	T	T	T	C
D	20A	C	T	C	T	C	C	T	C	T	C
E	22A	C	T	C	T	T	C	T	C	T	C
F	8B	C+T	C	T	C+T	C	C+T	T	T	T	C
G	2B, 2C, 9B, 19A	T	T	T	C	C	T	T	T	T	C
H	11C	T	C	T	C	C	T	T	C	T	C
I	11A	T	C	T	C	C	T	T	T	T	C
L	10C	T	T	T	C	T	T	T	C	T	C
M	3A	T	T	T	T	C	T	T	C	T	C
N	3B, 15A	T	T	T	T	T	T	T	C	T	C
O	5A, 7A, 7E, 8A, 10A, 15D, 21A, 21B	T	T	T	T	T	T	T	T	T	C
P	12A, 12C	T	T	T	T	T	T	T	C	T	T
Q	SVP11	T	T	T	T	T	T	T	T	T	T
R	1A, 16A	T	C	T	C	C	T	T	T	T	T

[a] For mtDNA analysis, total plant DNA was restricted with the enzymes indicated in Table 3. For cpDNA analysis, it was restricted with *Bam*HI

[b] The same number followed by different letters indicates two clones regenerated from the same callus

[c] C and T=*S. commersonii*- and *S. tuberosum*-specific hybridization patterns, respectively

probes, more than 70% of the hybrids were either identical to *tbr* (25%) or more similar to the cultivated than to wild species (47%). Statistical evidence that transmission of parental organellar genes was not random ($P<0.05$) was also obtained when the segregation ratios of *cox1*, *cob*, *rrn18-rrn5*, *rps12*, and *nad3* were analyzed individually. For all of these and also for *cox2* where statistical significance was not attained, the *tbr*-specific fragments were preferentially transmitted to the hybrids. Depending on the probe used, 66–100% of the hybrids showed the hybridization pattern of *tbr* for these genes. In contrast, 44–53% of the hybrids showed the *tbr* pattern for *atp6*, *atp9*, and *nad1bc*, indicating random transmission of parental fragments for these genes (Table 5).

The hexaploid hybrids displayed *cmm* fragments at a higher frequency than the tetraploid ones (Table 5). Interestingly, some correlation was found between general morphology and flowering behavior of hexaploid clones and their mtDNA restriction profile: genotypes late in flowering and more similar to SVP11 showed the *tbr* hybridization pattern in all nine mitochondrial loci analyzed, whereas those with good flowering and other traits more similar to Cmm1 showed the *cmm* pattern (data not shown).

No polymorphism was found in the cpDNA of hybrid clones regenerated from the same callus, whereas within-callus polymorphism for mtDNA was seen in about one-third of calli analyzed (data not shown).

Table 6 presents the similarity coefficients relative to the co-segregation of parental bands in the somatic hybrids. Besides the expected co-segregation of the two plastidial bands C8 and C9 from *cmm*, perfect co-segre-

Table 5 Ratio of hybrid clones showing *S. commersonii*/*S. tuberosum*-specific hybridization patterns[a]

Group[b]	mtDNA									cpDNA
	cox1	*cox2*	*cob*	*atp6*	*atp9*	*rrn18-rrn5*	*rps12*	*nad1-bc*	*nad3*	pStB153
F (4*x*)	1/0	1/0	1/0	1/0	1/0	1/0	0/1	1/0	0/1	1/0
S (4*x*)	5/18	4/19	5/18	10/13	11/12	5/18	0/23	10/13	0/23	19/4
S (6*x*)	3/4	6/2	3/5	5/2	6/2	3/4	0/8	4/4	0/8	8/0
Total S	8/22	10/21	8/23	15/15	17/14	8/22	0/31	14/17	0/31	27/4
Overall	9/22	11/21	9/23	16/15	18/14	9/22	0/32	15/17	0/32	28/4
$\chi^2_{1:1}$	5.5*	3.2 ns	6.2*	0.1 ns	0.5 ns	5.5*	32.0**	0.2 ns	32.0**	18.0**

*, P<0.05, **, P<0.01, ns not significant. χ^2 for overall 1:1 segregation

[a] Overall, 32 hybrids were analyzed for mtDNA and cpDNA composition. Clone SH8B, showing both parental *cox1*, *atp6* and *rrn18-rrn5* fragments (see Table 4), was not considered in segregation analysis for these probes

[b] F (4*x*), Male-fertile tetraploid hybrid SH9A; S (4*x*), male-sterile eu- or hypotetraploid hybrids; S (6*x*), male-sterile eu- or hypohexaploid hybrids

Table 6 Similarity coefficients[a] for analysis of co-segregation of *Solanum commersonii*- (upper triangle) and *S. tuberosum*-specific (lower triangle) restriction fragments in the somatic hybrids

tuberosum-specific fragments (T)[b]	mtDNA						cpDNA		*commersonii*-specific fragments (C)
	C2	C3	C4	C5	C6	C7	C8	C9	
	0.78	0.66	0.69	1.00	0.69	0.69	0.44	0.44	C1
		0.81	0.78	0.78	0.66	0.66	0.34	0.34	C2
T2	1.00								
			0.91	0.66	0.47	0.47	0.53	0.53	C3
T3	0.66	0.66							
				0.69	0.44	0.44	0.56	0.56	C4
T4	1.00	1.00	0.66						
					0.69	0.69	0.44	0.44	C5
T5	0.72	0.72	0.50	0.72					
						1.00	0.13	0.13	C6
T6	0.75	0.75	0.53	0.75	0.53				
							0.13	0.13	C7
T7	0.72	0.72	0.50	0.72	1.00	0.53			
								1.00	C8
T8	0.41	0.41	0.50	0.41	0.13	0.47	0.13		
	T1	T2	T3	T4	T5	T6	T7		
	mtDNA						cpDNA		

[a] S4, (a+d)/(a+b+c+d) (Wilkinson et al. 1992). See Materials and methods for details

[b] Fragments obtained by Southern analysis as reported in Table 3. Related probes and MWs (kb) as follows:

C1, *cox1*/4.6; C2, *cox2*/3.0; C3, *atp6*/25.3; C4, *atp9*/6.0; C5, *rrn18-rrn5*/4.6; C6, *rps12*/11.8; C7, *nad3*/11.8; C8, pStB153/10.0; C9, pStB153/2.3

T1, *cox1*/5.1; T2, *cob*/6.5; T3, *atp6*/15.1; T4, *rrn18-rrn5*/5.0; T5, *rps12*/17.1; T6, *nad1bc*/3.1; T7, *nad3*/17.1; T8, pStB153/12.3

gation in the hybrid population was also obtained for C1 (*cox1*) and C5 (*rrn18-rrn5*), and for C6 (*rps12*) and C7 (*nad3*) bands from the wild parent; similarly, it was evident for T1 (*cox1*), T2 (*cob*), and T4 (*rrn18-rrn5*), and for the T5 (*rps12*) and T7 (*nad3*) fragments from *tbr.* A relatively high coefficient was also found for the C3 (*atp6*) and C4 (*atp9*) fragments from *cmm.* The lowest similarity coefficients were found between plastidial and mitochondrial fragments in both parents, while intermediate values were obtained in other cases.

Discussion

In this study, the cytoplasmic organelle DNA composition of the two incongruous species *S. tuberosum* and *S. commersonii* and of somatic hybrids between them was analyzed with multiple probes and restriction enzymes. The extent of interspecific and protoplast fusion-induced variability was thus determined. In addition, an attempt was made to correlate variation in male fertility and organelle DNA in order to discover the molecular bases of nuclear-cytoplasmic interactions leading to male sterility and flower malformations in interspecific *Solanum* hybrids (Kaul 1988).

Genetic analysis of progenies derived by selfing male-fertile hybrids and crossing male-sterile ones demonstrated the maternal inheritance of the male fertility phenotype in our material. Since one or few dominant nuclear genes are reported to be involved in the induction of alloplasmic male sterility in *Solanum* spp. (Kaul 1988; Ortiz 1998), it could be hypothesized that the *cmm* parent was heterozygous for a major *Ms* gene and that its mutation *in vitro* was responsible for the expression of male fertility in the SH9A hybrid. But, due to the simplex condition of the *Ms* locus (*Ms ms ms ms*) in the sterile somatic hybrids, a high frequency of fertile segregants would be expected in their progenies after a cross with SH9A (*ms ms ms ms*). On the other hand, assuming that *cmm* was homozygous *Ms Ms* it is unlikely that SH9A in the duplex condition (*Ms Ms ms ms*) reverted to fertility as a result of nuclear mutations. Similar considerations would apply if more than one nuclear gene was involved in interactions with the sensitive cytoplasm. Hence, a change in the mitochondrial genome of SH9A is probably responsible for the expression of male fertility. The fact that no male-fertile genotypes were observed in the progenies of male-sterile hybrids suggests that no segregation occurred in the *Ms* locus due to disomic inheritance of the parental chromosomes in the somatic hybrids and/or the presence of multiple *Ms* genes.

In our conditions, the wild species used in fusion experiments showed profuse flowering and high fertility. We were not able to obtain good estimates of male fertility in the *tbr* dihaploid clone SVP11 due to low vigor and difficulties in obtaining good flowering. In other somatic hybridization studies, the same clone produced pollen with 5–15% stainability and either male-sterile or male-fertile progenies, depending on the fusion partner used (Puite and Mattheij 1989; Mattheij et al. 1992; Pijnacker et al. 1992; Conicella et al. 1997). The latter results and those derived from sexual and somatic hybridizations with different *cmm* and *tbr* genotype combinations (Novy and Hanneman 1991; Carotenuto and Bastia 1995; Carputo et al. 1995) suggest that the hypothetical mitochondrial factors derived from SVP11 are involved in nuclear-cytoplasmic interactions leading to CMS, rather than directly carrying mutations for male sterility.

Relatively low cpDNA polymorphism between *cmm* and *tbr* was observed in this study. Phylogenetic studies by Hosaka et al. (1984) showed that the plastidial genomes of the two species are slightly differentiated. The pStB153 probe maps on the large single-copy region of the potato plastidial genome and contains the *trnV* (UAC) and part of the *atpE* genes (Heinhorst et al. 1988; du Jardin 1990). A strong bias in favor of the segregation of the parental wild species chloroplasts was observed in the somatic hybrids. Further studies are necessary to establish whether the bias was due to a selective advantage of *cmm* chloroplasts in comparison with the *tbr* ones or to a preferential sorting out of plastids derived from the bleached fusion partner. As a matter of fact, random transmission of chloroplasts was obtained in *cmm*(+)*tbr* fusion combinations in which neither parent were pretreated with the herbicide (Bastia et al. 1998). In experiments involving *S. phureja* and bleached SVP11 protoplasts, however, no negative effect of the bleaching treatment on the transmission of plastids in the fusion products was detected (Puite and Mattheij 1989). Only 5% of the *tbr*(+)*cmm* cybrids with the nucleus of *S. tuberosum* inherited the chloroplasts from *S. commersonii*, and they usually showed abnormal pigmentation, suggesting some nuclear-chloroplast incompatibility between the two species (Perl et al. 1991). No evident abnormalities were found in any of our hybrids, probably because they contained the complete sets of nuclear chromosomes of both parental species. Co-transmission of *cmm* nuclear and chloroplast DNA was also found in fusion experiments with albino tomato (Derks et al. 1992). Since the chloroplast type of the hybrids regenerated from the same callus never varied, the parental plastids probably sorted out soon after protoplast fusion.

A higher variability was found between the mitochondrial genomes of *cmm* and *tbr* than between their plastidial ones. The extent of polymorphism varied in different mtDNA regions; some of them, such as *cox3*, *rrn26*, and *rps3-rpl16*, were highly conserved, whereas *cox1*, *atp9*, *rrn18-rrn5*, *nad1*, and others were more variable. The degree of variability did not seem to be influenced by the number of copies of each gene. In *tbr*, *rrn26*, *cox1*, and *atp6* are present in single copy (Binder et al. 1994; Quiñones et al. 1995; Lössl et al. 1999), while one gene and one pseudogene, two genes, and two genes and one pseudogene are reported for *atp9*, *rrn18-rrn5*, and *cob*, respectively (Zanlungo et al. 1991; Dell'Orto et al. 1993; Zanlungo et al. 1994; Giese et al. 1996). Further, based on the independent segregation of restriction fragments in a population of somatic hybrids between tomato and potato, two copies of the *cox2* gene were hypothesized in the latter (Wolters et al. 1995). To our knowledge, no data are available in potato for the other genes we analyzed; nevertheless, since *cox3*, *rps3-rpl16*, *rps12*, *nad1bc*, and *nad3* generally gave single-band hybridization patterns with various restriction enzymes, they are probably present in single copy in the potato mitochondrial genome. pSpom1, which is known to hybridize to a repetitive sequence originally derived from the weed *Spirodela oligorhiza* (de Heij et al. 1985), gave a more complex hybridization pattern, suggesting the presence of multiple copies in *tbr* as well (data not shown).

In most cases, the same gene copy number found or hypothesized in common potato could be also supposed for *cmm*. For *cox2* and *atp9*, however, the wild species showed a band in addition to those present in *tbr*, suggesting the presence of other relevant homologous sequences elsewhere in the mtDNA genome. For *cob*, two common bands of 2.8 and 3.0 kb were found in both species, while an additional one of 6.5 kb was found only in SVP11. Since the two copies of *cob* in *tbr* are reported to diverge in the 5' region and an internal *Dra*I site is present in the coding region of the two genes (Zanlungo et al. 1991), it can be assumed that either one or two, but not divergent, copies of *cob* are present in *cmm*.

When the two species were used as fusion parents in somatic hybridization experiments, much variability at the cytoplasmic level was apparent among the somatic hybrids. By using seven mtDNA and two cpDNA *cmm*-specific, and seven mtDNA and one cpDNA *tbr*-specific fragments, 16 groups were obtained as a consequence of the rearrangement of the parental mitochondrial genomes and the reassortment of chloroplasts and mitochondria. Mitochondria were reassorted independently from chloroplasts in the fusion products, as generally reported in previous interspecific or intergeneric fusion combinations involving potato (Xu et al. 1993; Sidorov et al. 1994; Wolters et al. 1995; Yamada et al. 1997). Seventy-five percent of the somatic hybrids showed a non-parental mtDNA restriction pattern, due to new arrangements of parental fragments in the mithondrial genome. Similar figures were also obtained in other fusion-derived *Solanum* populations (Kemble et al. 1986; Xu et al. 1993; Lössl et al. 1994), although in the latter some new non-parental bands were also noted. Since the tuber-bearing *cmm* is closer to common potato than the non-tuber bearing *S. brevidens* used in other experiments (Kemble et al. 1986; Xu et al. 1993), the genetic distance between the fusion partners could be one cause of the different frequency in the occurrence of new non-parental bands. Similar results were reported for fusions between tomato and potato and between tomato and *Nicotiana* spp. (Wolters et al. 1995). In other interspecific fusion combinations within the *Solanum* genus (Sidorov et al. 1994; Yamada et al. 1997) either very low or null frequencies of mtDNA rearrangements were found, but in both cases a relatively low number of hybrids and/or probes was tested.

The variability induced at the cytoplasmic level in the present study can be used in future investigations to correlate agronomic performance with specific nuclear-organellar arrangements. Previously, either specific mtDNA regions or the degree of homogeneity of the mitochondrial genome were associated with yield parameters in potato somatic hybrids (Lössl et al. 1994). The strongly biased segregation of most of the mitochondrial genes investigated may indicate some selective advantage related to mtDNA composition. It is possible that hybrid cells that had inherited some mtDNA fragments from *tbr* were more viable during growth and regeneration *in vitro* than those with *cmm*-specific fragments. Preferential inheritance of *tbr* mtDNA was also obtained in *tbr*(+)*cmm* cybrids (Perl et al. 1991) as well as in other interspecific somatic hybrids in *Solanum* spp. (Xu et al. 1993; Sidorov et al. 1994; Yamada et al. 1997).

Some parental mtDNA fragments co-segregated in the somatic hybrids, suggesting either physical linkage on the mitochondrial chromosome or physiological requirements to have gene products from the same source in the hybrid cell (Wolters et al. 1995). Unfortunately, a physical map of the potato mitochondrial genome is not yet available to discriminate between these possibilities. However, *rps12* and *nad3* genes, which were always jointly derived from the *tbr* fusion partner, are closely linked in other potato genotypes as well as in *Petunia* and other species (Hanson et al. 1995; Lössl et al. 1999).

Some variability for mtDNA composition among shoots regenerated from the same callus was observed, suggesting that the sorting out of rearranged mitochondria was not complete in all calli at the regeneration stage (Kemble et al. 1986; Perl et al. 1990) and that it was slower than for cpDNA (Earle 1995). Interestingly, the largest intra-callus variability, a possible indication of more extensive rearrangements of the parental genomes, was found in callus 9, from which the single male-fertile clone SH9A was regenerated along with a male-sterile one (SH9B).

The prevalence of *cmm* fragments in the fertile clone SH9A is consistent with the hypothesis that the *tbr*-derived region involved in nuclear-cytoplasmic incompatibility and induction of male sterility could have been sorted out; however, no clear correlation between any specific mitochondrial region and male sterility has been found so far. On the other hand, the presence of the same parental fragments in SH9A and some male-sterile hybrids suggests that alterations in the organization of these regions are not involved in alloplasmic male sterility in *Solanum* spp. More detailed genetic and molecular analyses, looking at the restoration of CMS and segregation of nuclear genes as well as at the organization and expression of mitochondrial genomes in various fusion combinations, are necessary.

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