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Organelle DNA variation in parental *Solanum* spp. genotypes and nuclear-cytoplasmic interactions in *Solanum tuberosum* (+) *S. commersonii* somatic hybrid-backcross progeny

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Abstract Nuclear-cytoplasmic interactions can influence fertility and agronomic performance of interspecific hybrids in potato as well as other species. With the aim of assessing the potential value of a novel recombinant cytoplasm derived by interspecific somatic hybridization, backcross progeny were produced by crossing a somatic hybrid between *Solanum tuberosum* (tbr) and the wild incongruous species *S. commersonii* (cmm) with various potato clones. BC_1 clones were evaluated for male fertility and other agronomic traits. Male fertility clearly depended on the cross direction and the cytoplasm source. Genotypes with cytoplasms sensitive to nuclear genes derived from *Solanum commersonii* and inducing male sterility showed identical mtDNA composition, as based on mtDNA analyses with various PCR-based and RFLP markers. On the other hand, genotypes with cytoplasms not inducing male sterility in the presence of the cmm nuclear genes showed a different mtDNA organisation. Analysis of cpDNA confirmed similarity of cytoplasmic composition in CMS-inducing genotypes and clear differences with the others. Genotypes with recombinant cytoplasm induced by somatic hybridization generally showed similar agronomic performances in reciprocal hybrids with tbr cytoplasm, suggesting that the novel cytoplasm can be used in potato breeding.

Keywords Interspecific hybridization · *Solanum* · Male fertility · Chloroplasts · Mitochondria

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Introduction

Wild tuber-bearing *Solanum* species are an important source of genetic variation for the improvement of the common potato, *Solanum tuberosum* ssp. *tuberosum*, concerning both the nuclear and cytoplasmic genomes (Hawkes 1990; Perl et al. 1991). Nuclear-cytoplasmic interactions and other genetic factors, however, are known to influence the morphology, physiology and agronomic performance of reciprocal interspecific hybrids (Sanford and Hanneman 1979, 1982; Hoopes et al. 1980; Hilali et al. 1987; Maris 1989). Such interactions can limit, or even prevent, the full exploitation of available genetic variability, as discussed by the authors cited above. In some instances, however, they provide an advantage, as in the case of CMS (cytoplasmic male sterility), a useful trait for TPS (true potato seed) breeding (Perl et al. 1990). Furthermore, interactions between hypothetical nuclear genes and cytoplasmic factors, leading to alterations in male fertility and flower development in various species combinations, formed the bases for evolutionary studies in *Solanum* spp. (Grun 1979), as well as for the first attempts to understand genetic and molecular mechanisms underlying CMS in this genus (Hanson and Conde 1985).

Early studies suffered from the lack of methods to induce and characterize genetic variability in cytoplasmic genomes. Somatic hybridization by protoplast fusion has allowed the creation of novel cellular genome configurations by combining sexually incongruent species. Further, the availability of molecular markers has permitted the tracing of either natural or artificially induced genetic-variability in plastids and mitochondria (Hosaka and Hanneman 1988; Earle 1995; Cardi et al. 1999; Lössl et al. 1999).

Somatic hybrids between *S. tuberosum* (tbr) and the incongruous species *Solanum commersonii* (cmm) were recently synthesized in our and other laboratories (see Cardi 2001 for a review). Somatic as well as sexual hybrids with tbr cytoplasm, the latter produced by ploidy manipulation approaches in the wild species (Novy and

Hanneman 1991), were mostly male-sterile (Cardi 2001). Male-sterile hybrids showed meiosis blocked at the early stages and also an early tapetal degeneration (Novy and Hanneman 1991; Conicella et al. 1997). An analysis of organellar genome composition, carried out with nine mtDNA gene probes in cmm (+) tbr somatic hybrids and parental species (Cardi et al. 1999), indicated that 75% of hybrids had a non-parental mitochondrial genome, with most male-sterile hybrids showing a preferential inheritance of tbr mtDNA fragments. On the other hand, an exceptional male-fertile hybrid showed cpDNA from *S. commersonii*, and a rearranged mtDNA mostly derived from the wild parent. When somatic hybrids with different cytoplasms were tested in the field, a negative effect on agronomic performance was hypothetically attributed to the presence of wild *S. commersonii* mtDNA (Cardi et al. 2001).

In order to correlate molecular variation in cytoplasmic genomes with the agronomic performance of potato genotypes, extensive analyses with molecular markers were recently carried out in intra- and inter-specific somatic hybrids, cultivars and wild species, and reciprocal crosses (Lössl et al. 1994, 1999, 2000). However, *S. commersonii*, for which limited molecular data are available in the literature, was not included in those studies; further, correlative analyses of cytoplasmic genome-composition and male fertility were carried out in cultivars, but not in reciprocal hybrids derived by controlled crossings and with the same nuclear-genetic background.

In the present study, BC_1 progenies obtained by reciprocally crossing a *S. commersonii* (+) *S. tuberosum* somatic hybrid with various potato genotypes were analysed for male fertility and agronomic performance, while cytoplasmic genomes of parental genotypes were characterized at the molecular level. A good correlation was obtained between the mtDNA molecular data and the nuclear-mitochondrial genome interaction leading to male sterility. In addition, male fertility and agronomic data suggested that useful genetic variation at the cytoplasmic level was introduced in potato through interspecific somatic hybridization, and that the novel recombinant cytoplasm available in the somatic hybrid can be used in potato breeding.

Materials and methods

Plant material

The *S. commersonii* clone used in this study (Cmm1) was isolated from seed-derived plants of the accession PI243503, while the *S. tuberosum* di-haploid clone DH 81-7-1463 (SVP11) was derived from the tetraploid clone W 72-22-492 (Cardi et al. 1999). The male-fertile somatic hybrid SH9A was obtained by protoplast fusion between SVP11 and Cmm1 (Cardi et al. 1993). Carmine, Tollocan, LT-7, LT-5 and 7XY.1 are tetraploid *S. tuberosum* clones and were kindly provided by Dr. E. Chujoy, International Potato Center (CIP), Lima, Peru. Backcross progenies were produced by crossing the five tetraploid *S. tuberosum* clones with the somatic hybrid SH9A.

Phenotypic analyses

Field experiments were carried out under insect-proof tunnels in Camigliatello Silano, Italy. For each genotype, generally five tuber-derived plants were analysed during growth and at harvest. When most plants had flowered, flowers were collected and pollen was harvested by shaking anthers with a battery powered vibrator. Pollen stainability was determined with 1% aceto-carmine. Differences in pollen production were evaluated statistically by a χ^2 test. The following traits were also recorded on every plant and then averaged for each genotype: plant vigour, plant pigmentation, degree of flowering, vine maturity 90 days after sowing, stolon length, tuber shape, eye depth, skin colour, flesh colour, non-marketable and marketable plant-tuber yield (number and weight of tubers smaller or bigger than 35 mm in diameter, respectively). The mean tuber weights (non-marketable and marketable) were derived by dividing the respective yields in weight by the number of tubers. The total plant-tuber yields (tuber number and weight) were derived by adding the non-marketable and marketable figures. The percentage of marketable tuber yield was derived by dividing the marketable tuber yield by the total yield. Mean figures for each family, i.e. cross combination, were compared by the Analysis of Variance (Wilkinson et al. 1992).

Molecular analyses

Total DNA was isolated from fresh or frozen leaves as previously reported (Cardi et al. 1999). Twenty five nanograms of genomic DNA was used in PCR amplifications with primers for cp and mtDNA (Lössl et al. 1999, 2000; Bastia et al. 2001). Amplification reactions were generally performed in volumes of 25 μl containing 0.4 μM of each primer, 200 μM each of dATP, dCTP, dGTP and dTTP, 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, 3 min at 72°C, and a final extension of 10 min at 72°C.

Total DNA (2.5–3 μg) digested with different restriction enzymes was used in Southern analyses with various mitochondrial probes (Cardi et al. 1999; Lössl et al. 1999). The digested DNA was electrophoresed for about 20 h in 1×TAE agarose gel (0.8% w/v) at 1.2–1.4 V/cm, blotted onto a positively charged membrane under alkaline conditions (0.4 N NaOH), hybridized under high-stringency conditions with 40% formamide, and analysed by a radioactive method after washing one to three times at 42°C (10 min each wash) with 0.1×SSC, 0.1% SDS as a final wash.

Based on the presence/absence of 27 species-specific mitochondrial fragments detected by molecular analyses, the analysed genotypes were grouped by a Hierarchic Cluster Analysis procedure using the "Normalized Percent Disagreement" index to calculate distances among clusters, with the "Average Linkage" method as a clustering criterion (Wilkinson et al. 1992). Distances between genotypes were analysed 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., the same band is either present or absent in two genotypes, respectively), and b and c those in which they disagree (Wilkinson et al. 1992).

Results

Phenotypic analysis

A high variability among backcross progenies was evident for pollen production (Table 1). In different families (i.e. cross combinations) the percentage of clones producing pollen ranged from 15.2% in Carmine×SH9A

Table 1 Percentage of clones showing pollen production in progenies derived by crossing the *S. commersonii* (+) *S. tuberosum* somatic hybrid SH9A with five *S. tuberosum* genotypes. The statistical significance of differences between selected percentages is also reported

Cross	Pollen production %
(A) LT-7×SH9A	17.9[a] (28)[b]
(B) SH9A×LT-7	70.7 (41)
(C) Tollocan×SH9A	23.5 (81)
(D) SH9A×Tollocan	100.0 (53)
(E) Carmine×SH9A	15.2 (79)
(F) SH9A×Carmine	96.4 (56)
(G) 7XY.1×SH9A	100.0 (26)
(H) LT-5×SH9A	99.0 (100)
χ^2b. crosses (*df*=7)	289.36***
χ^2A vs B (*df*=1)	16.55***
χ^2C vs D (*df*=1)	72.45***
χ^2E vs F (*df*=1)	83.33***
χ^2b. A, C and E (*df*=2)	1.80
χ^2G vs H (*df*=1)	0.26
χ^2(A+C+E) vs (G+H) (*df*=1)	190.34***

*** $P<0.001$

[a] The data of two experiments were pooled since χ^2 for heterogeneity between experiments was not significant

[b] No. of observations is reported in parentheses

to 100% in SH9A×Tollocan and 7XY.1×SH9A. The pollen produced was generally highly stainable.

The percentage of male-fertile clones was significantly different in the reciprocal crosses between SH9A and LT-7, Tollocan and Carmine, always being higher when the somatic hybrid was used as a female. On the other hand, no significant differences were obtained by comparing families with the cytoplasms of the same tbr genotypes (A, C and E in Table 1). By contrast, a highly significant difference was found when pooled results of A, C and E families were compared with those of G and H. The latter had the cytoplasms of 7XY.1 and LT-5, respectively, and included approximately 100% of male-fertile clones. All clones used as parents in backcrosses were male-fertile (this study; Ortiz et al. 1993; Carputo et al. 1998).

The families derived by the reciprocal crosses between SH9A and LT-7, Tollocan and Carmine were also compared for some agronomic traits (Table 2). Based on statistically significant differences, however, results were only partially consistent across the tbr genotype used. LT-7×SH9A progenies outyielded those of SH9A×LT-7 for marketable and total tuber yield/plant. Contrasting results were obtained in SH9A×Tollocan vs Tollocan×SH9A families; the former also showed a higher degree of flowering and reduced eye depth. In comparison with Carmine×SH9A progenies, SH9A×Carmine genotypes showed a slight improvement in tuber yield, since they had a significant lower number of non-marketable tubers per plant and a higher proportion of marketable tubers; in addition, they were more vigorous with an earlier vine maturity, and showed a higher degree of flowering and reduced stolon length.

Table 2 Mean differences for some agronomic traits between reciprocal progenies derived by crossing the SH9A somatic hybrid with LT-7, Tollocan and Carmine *S. tuberosum* clones

Trait[b]	Crosses[a]					
	SH9A×LT-7 vs LT-7×SH9A		SH9A×Tollocan vs Tollocan×SH9A		SH9A×Carmine vs Carmine×SH9A	
	X_1-X_2	(n_1/n_2)	X_1-X_2	(n_1/n_2)	X_1-X_2	(n_1/n_2)
Plant vigour	0.2	(30/23)	0.2	(41/64)	0.5**	(59/101)
Degree of flowering	−0.5	(28/21)	0.8**	(37/57)	0.7**	(52/90)
Vine maturity	0.4	(30/23)	0.2	(39/59)	−0.4**	(56/91)
Stolon length	0.0	(29/21)	−0.2	(38/61)	0.3**	(57/97)
Tuber shape	0.5*	(29/21)	−0.1	(38/61)	−0.1	(57/97)
Eye depth	−0.3	(29/21)	0.4**	(38/61)	0.0	(57/97)
Not-marketable plant tuber yield (no.)	−0.5	(30/23)	−0.4	(41/65)	−1.0**	(61/101)
Marketable plant tuber yield (no.)	−1.6*	(30/23)	1.7*	(41/65)	0.2	(61/101)
Marketable plant tuber yield (g)	−141.6*	(30/23)	181.7*	(41/65)	−23.7	(61/101)
Total plant tuber yield (no.)	−2.1*	(30/23)	1.3	(41/65)	−0.9	(61/101)
Total plant tuber yield (g)	−150.4**	(30/23)	178.4*	(41/65)	−32.0	(61/101)
Marketable tuber yield (% of total)	−20.3	(29/21)	13.7**	(38/60)	9.1*	(57/98)

*=$P<0.05$; **=$P<0.01$

[a] For each cross combination and trait, X_1-X_2 indicates the mean difference between families with SH9A or tbr cytoplasm, respectively. The number of genotypes analysed in each family is reported in parentheses (n_1/n_2)

[b] Only traits for which at least a significant contrast was obtained are reported. Plant vigour: 1=very weak, 5=very vigorous; Degree of flowering: 0=no buds, 7=profuse; Vine maturity: 1=very early, 5=very late; Stolon length: 1=long, 3=short; Tuber shape: 1=round, 3=elongated; Eye depth: 1=deep, 3=shallow. Comparisons for other traits analysed (see Materials and methods) were never significant and thus were not included in the Table

[c] marketable yield = tuber ∅>35 mm; not-marketable tuber yield = tuber ∅<35 mm

Table 3 Amplification patterns of cpDNA and mtDNA in different *Solanum* spp. genotypes

Primer pairs	Genome	Genomic region	Genotypes							
			Cmm1[c]	SVP11	SH9A	Carmine	Tollocan	LT-7	LT-5	7XY.1
pucJ[a]	cpDNA	*trn*F-*trn*V	1[d]	2	1	2	2	2	1	1
ALc1/3[b]	cpDNA	*atp*E	1	2	1	2	2	2	1	1
pumD[a]	mtDNA	*rps14-cob*	0	1	0	1	1	1	1	1
ALm1/3[b]	mtDNA	*atp6*	0	0	0	0	0	0	1	0
ALm4/5[b]	mtDNA	*cob-rps10*	0	1	0	1	1	1	2	1
ALm6/7[b]	mtDNA	*cob*	1	1	1	1	1	1	0	0

[a, b] Primer codes as in Bastia et al. 2001 and Lössl et al. 2000, respectively
[c] Cmm1 = *S. commersonii* PI 243503; SVP11 = *S. tuberosum* di-haploid clone DH 81-7-1463; SH9A = Cmm1 (+) SVP11 somatic hybrid; Carmine, Tollocan, LT-7, LT-5 and 7XY.1 = *S. tuberosum* clones
[d] For each primer pair, the same number indicates that the amplification pattern was identical in the tested genotypes; 0 = no amplification

Table 4 Hybridization patterns of different *Solanum* spp. genotypes in Southern analyses with various mtDNA gene probes

mtDNA probes	DNA for blot cut with	Genotypes							
		Cmm1[a]	SVP11	SH9A	Carmine	Tollocan	LT-7	LT-5	7XY.1
rpl2	*Eco*RI	1[b]	2	3	2	2	2	4	5
rps10-cox1	*Eco*RI	1	2	1	2	2	2	3	2
rps14	*Eco*RI	1	2	1	2	2	2	3	2
nad1 bc	*Dra*I	1	2	1	2	2	2	1	1
cob	*Eco*RI	1	1	1	1	1	1	2	1
rrn 18-5	*Eco*RI	1	2	1	2	2	2	3	2
rps10	*Hin*dIII	1	2	1	2	2	2	3	2

[a] Cmm1 = *S. commersonii* PI 243503; SVP11 = *S. tuberosum* di-haploid clone DH 81-7-1463; SH9A = Cmm1 (+) SVP11 somatic hybrid; Carmine, Tollocan, LT-7, LT-5 and 7XY.1 = *S. tuberosum* clones
[b] For each mtDNA probe, the same number indicates that the hybridization pattern was identical in the tested genotypes

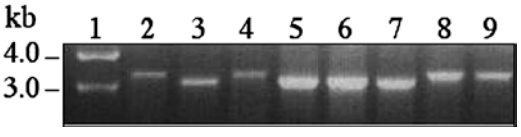

Fig. 1 PCR analysis of different *Solanum* spp. genotypes using the cpDNA primer pair pucJ. (1) DNA size marker, (2) Cmm1, (3) SVP11, (4) SH9A, (5) Carmine, (6) Tollocan, (7) LT-7, (8) LT-5, (9) 7XY.1

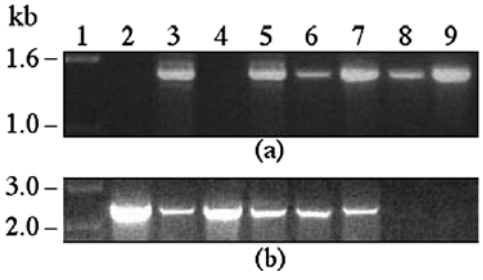

Fig. 2 PCR analysis of different *Solanum* spp. genotypes using the mtDNA primer pairs pumD (*a*) and ALm6/7 (*b*). In both photos: (1) DNA size marker, (2) Cmm1, (3) SVP11, (4) SH9A, (5) Carmine, (6) Tollocan, (7) LT-7, (8) LT-5, (9) 7XY.1

Molecular analyses

In order to investigate cpDNA and mtDNA variability among tested genotypes and to detect correlations between cytoplasm composition and phenotypic data, parental genotypes of the somatic hybrid SH9A and of the backcross progenies were analysed for their organellar types.

Both pucJ and Alc1/3 primer pairs revealed polymorphism among genotypes (Table 3 and Fig. 1), and allowed the arrangement of the analysed genotypes into two groups: one containing chloroplasts similar to *S. commersonii* (SH9A, LT-5 and 7XY.1), and one containing the *S. tuberosum* chloroplast type (SVP11, LT-7, Tollocan and Carmine).

Four primer combinations and seven probes were used in PCR and RFLP analyses of mtDNA, respectively (Tables 3 and 4). All DNA markers showed high variability in the tested genotypes (Tables 3 and 4, and Figs. 2 and 3). The male-fertile somatic hybrid SH9A revealed either the amplification/hybridization pattern of the wild parent or novel RFLP fragments absent in both parental genotypes. Such fragments, of the same size of some already present in 7XY.1, were apparent with the *rpl2* gene probe after digestion with *Eco*RI (Fig. 3c), as well as with other enzymes (data not shown). The three *S. tuberosum* clones LT-7, Tollocan and Carmine showed the same mtDNA configuration observed in the original *S. tuberosum* clone used for protoplast fusion. On the other hand, LT-5 and 7XY.1 had patterns different to the other *S. tuberosum* genotypes and *S. commersonii* in many loci.

Cluster analysis, based on the presence/absence of 27 species-specific mitochondrial fragments detected by

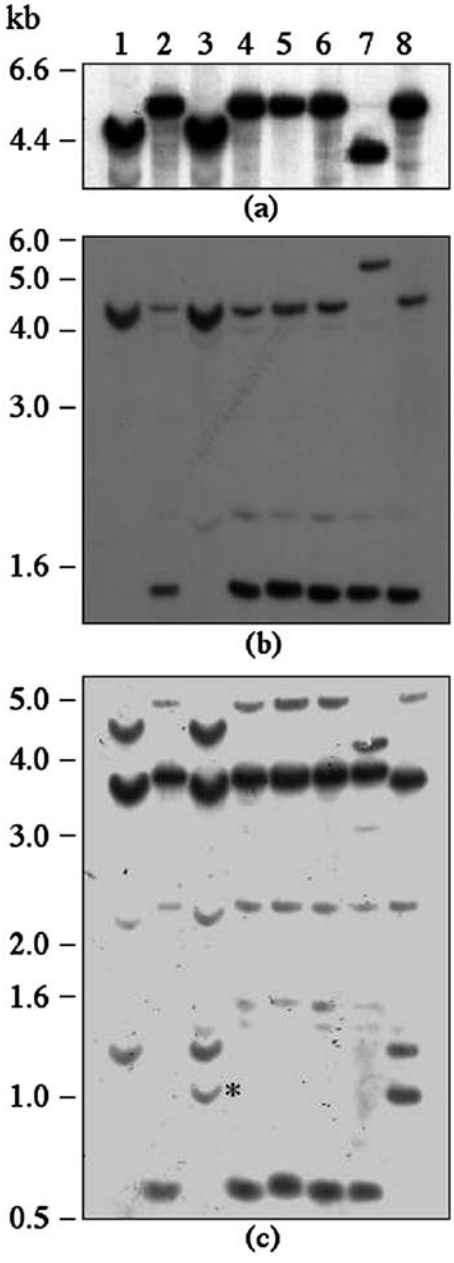

Fig. 3a–c Southern analysis of different *Solanum* spp. genotypes with *rps10-cox1* (**a**), *rps14* (**b**) and *rpl2* (**c**) mt genes as probes. DNA for blot cut with *Eco*RI. In all photos: (1) Cmm1, (2) SVP11, (3) SH9A, (4) Carmine, (5) Tollocan, (6) LT-7, (7) LT-5, (8) 7XY.1. A novel band present in the somatic hybrid SH9A after hybridization with the *rpl2* probe is marked by an asterisk

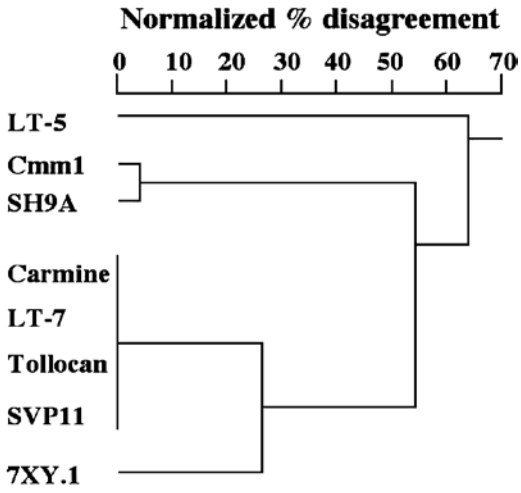

Fig. 4 Dendrogram obtained by cluster analysis based on 27 specific mtDNA fragments detected by molecular analyses

molecular analyses, allowed the analysed genotypes to be arranged into four groups (Fig. 4). One group contained the wild cmm species and the somatic hybrid SH9A, whilst the second included SVP11, Tollocan, Carmine and LT-7. Genotypes 7XY.1 and LT-5 remained isolated, although the former was more similar to the "tbr group" than the latter.

Results of mtDNA similarity coefficient analysis among genotypes (Table 5) confirmed the identities of SVP11, Carmine, Tollocan and LT-7. The similarity coefficients between them and Cmm1, SH9A, LT-5 and 7XY.1 were 0.481, 0.444, 0.407 and 0.741, respectively. With respect to the wild species cmm, the similarity coefficient was 0.333 for LT-5 and 0.444 for 7XY.1. The latter two genotypes showed a 0.296 coefficient between them.

Discussion

Male sterility is a common phenomenon in somatic hybrids between cmm and tbr (Cardi 2001). Further, when sexual hybrids between the two species were produced by means of ploidy manipulation, male sterility was associated with the cross direction and the tbr cytoplasm (Novy and Hanneman 1991; Carputo et al. 1995). Hence, it was hypothesized that male sterility was due to an incompat-

Table 5 Similarity coefficients[a] between genotypes based on the presence or absence of genotype-specific mtDNA fragments

Genotype	Cmm1	SVP11	SH9A	Carmine	Tollocan	LT-7	LT-5
SVP11	0.481						
SH9A	0.963	0.444					
Carmine	0.481	1.000	0.444				
Tollocan	0.481	1.000	0.444	1.000			
LT-7	0.481	1.000	0.444	1.000	1.000		
LT-5	0.333	0.407	0.296	0.407	0.407	0.407	
7XY.1	0.444	0.741	0.481	0.741	0.741	0.741	0.296

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

ibility between nuclear and mitochondrial genes of the wild and cultivated species, respectively. The exceptional male fertility of SH9A (Cardi et al. 1993) was most likely derived from the recombination of the unidentified mtDNA genomic regions involved in such interactions. This assumption was supported by the maternal inheritance of male sterility in crosses between somatic hybrids, and by the finding that SH9A had a rearranged mitochondrial genome largely derived from the wild species *S. commersonii* (Cardi et al. 1999; Scotti 2002).

The results obtained in the present study further corroborate the hypothesis mentioned above, since the male sterility of most clones of the A, C and E families can be explained by the interaction of cmm nuclear dominant genes present in SH9A and "sensitive" mitochondrial genes present in LT-7, Tollocan and Carmine cytoplasms. The occurrence of some fertile genotypes in the same BC_1 families (from 15.2 to 23.5%) is consistent with the segregation of a single nuclear gene involved in such an interaction and either random chromosome or random chromatid segregation (for pooled data: χ^2=0.83 and χ^2=0.51, P>0.05, respectively) (Allard 1999). The "sensitive" mitochondrial genes are obviously absent not only in the cytoplasm of SH9A, as expected, but also in those of 7XY.1 and LT-5. The former is a selection from a *S. tuberosum* ssp. *andigena* population and thus contains an adg cytoplasm, whereas the latter derives from a cross, tbr×neo tbr, and contains the cytoplasm of the cv Snow Flake (E. Chujoy, International Potato Center, personal communication; Ortiz et al. 1993). The latter authors suggested the presence of a dominant male fertility restorer gene both in LT-7 and Tollocan. However, due to the relatively low number of male-fertile BC_1 clones obtained, it seems that such a gene does not restore fertility in our system by counteracting the effect of the *Ms* gene derived from cmm and SH9A.

A good correlation was found between male fertility and organellar DNA data. Interestingly, the four genotypes which produced male-sterile hybrids also showed a very high degree of similarity in their mtDNA. Although differences in the mtDNA regions not analysed cannot be ruled out, they were identical in several genomic regions as highlighted by PCR and hybridization data. Comparison of our data with those published by Lössl et al. (1999, 2000), suggests that their cytoplasm resembles the β type. The two genotypes used in backcrosses and not inducing male sterility, i.e. LT-5 and 7XY.1, appear quite distant from the other genotypes. A cytoplasmic divergence between adg and tbr was already demonstrated by Grun and coworkers, based on fertility and the flower phenotype of hybrids (Grun 1979; Hanson and Conde 1985). On the basis of molecular data, Lössl et al. (1999) attributed the ε type to adg. The results obtained with 7XY.1 allow us to classify it as a ε type based on the *rpl2* hybridization pattern, but it resembled the β pattern in other loci. Previously, great ctDNA diversity, distributed according to a geographical cline, and large variation in morphological and physiological traits were found in the *andigena* subspecies (Hosaka and Hanneman 1988). LT-5 showed a rather dissimilar chondriome not only in comparison with the tbr group, but also with cmm and 7XY.1. Unfortunately, although its mtDNA resembles the α type of Lössl (1999) in most loci, we are not aware of the source of its cytoplasm. Recent results obtained by mtDNA analysis of a larger set of genotypes pointed out some similarities between LT-5 and a number of wild species, such as *Solanum tarijense, Solanum acaule, Solanum sparsipilum* and *Solanum infundibuliforme* (Scotti 2002). Interestingly, the first three were previously classified as α type, whilst the fourth was not analysed by Lössl and collaborators (1999).

In this study, variability in gene organization between genotypes was found in various mtDNA genome regions. In particular, *S. tuberosum* has two distinct full copies of the *cob* gene, one of which is located downstream of the *rpl5–rps14* gene cluster, diverging in the 5′ non-coding region, and one pseudogene (ψcob) upstream of the *rps10–cox1* genes (Zanlungo et al. 1991, 1995; Quiñones et al. 1996). Based on amplification and hybridization results reported in this study, both 7XY.1 and LT-5 seem to have only the *cob* copy downstream of *rpl5–rps14*, and the *cob–rps10–cox1* gene cluster; but the latter is rearranged in LT-5, since the intergenic region between the first two genes is longer than in tbr. *S. commersonii*, whose patterns are related to the Delta (W/δ) group of Lössl et al. (1999), lacks both the *rpl5–rps14–cob* and *cob–rps10–cox1* clusters, and seems to have only the second *cob* arrangement of tbr (this study; Cardi et al. 1999; Bastia et al. 2001).

In a set of German cultivars, male sterility was found associated with the γ mtDNA type (Lössl et al. 2000), whilst in our system the cmm nuclear gene(s) interacted negatively with the β mtDNA type to produce male-sterile hybrids. Nuclear cytoplasmic interactions in the tested genotypes affect only male fertility, since when the same BC_1 genotypes used in this study were used as female in crosses with potato clones, seeds were obtained in all cases indicating that they were female-fertile (Cardi et al. 2002).

SVP11, LT-7, Tollocan and Carmine showed a T cpDNA type, whereas the other four genotypes had a non-T cpDNA (Lössl et al. 2000; Hosaka 2002). The main difference between T and all other cpDNA types is the presence of a 241-bp deletion, highlighted by both primer combinations used in this study, in the *trnV–ndhC* intergenic region of *S. tuberosum* (Kawagoe and Kikuta 1991; Hosaka 2002). Further analyses, however, are needed to discriminate among the various possible cpDNA types (Hosaka and Hanneman 1988). In any case, although a direct role of cpDNA in the control of male sterility can be ruled out (Buckner and Hyde 1985; Hosaka et al. 1988), these data further indicate a higher similarity among the four genotypes with the CMS-inducing cytoplasm than with the others.

Analysis of the cytoplasmic genomes in our genotypes confirm the occurrence of a correlative pattern between cp and mt types, as already found by Frei et al. (1998) and Lössl et al. (1999, 2000). The *S. tuberosum* cytoplasm

was characterized by the T cp type and the β-like mt type combination, whereas the W cp type was combined with several mt types.

In crosses carried out in *Solanum* spp., reciprocal differences for tuber yield and other field parameters have been reported in a number of studies, and attributed to maternal effects, cytoplasmic inheritance, dauermodification, genetic imprinting or gametophytic selection (Sanford and Hanneman 1979, 1982; Hoopes et al. 1980; Staub et al. 1982; Hilali et al. 1987; Amoah et al. 1988; Maris 1989). In most cases, whatever the genetic and physiological basis, hybrids with tbr cytoplasm were more productive than reciprocals with other cytoplams. In a comparison of various *S. tuberosum* ssp. *tuberosum* and ssp. *andigena* accessions containing different cpDNA types, a dramatically higher tuber yield was evident for the T type (Hosaka and Hanneman 1993), but the same authors questioned whether the apparent difference in yield was due to the cpDNA type or to the latitude of origin of the genotypes. More recently, however, genetic and molecular studies showed that while cultivars with T cpDNA and β mtDNA are optimized for stable yields, the 'wild-type' cytoplasms Wα and Wγ have a significant advantage over other cytoplamic types (Tβ, Wδ, Sε) as far as starch content is concerned (Lössl et al. 2000). Differences in yield and other traits were also found associated to specific cp or mtDNA configurations or to the degree of mtDNA rearrangements in potato somatic hybrids (Lössl et al. 1994, 2000).

Our data do not allow any conclusions to be drawn about the genetic mechanism involved in the differences between BC_1 progenies for agronomic traits, and repeated trials are needed in more genetic backgrounds and environments. However, the novel SH9A cytoplasm shows some interesting features both as male fertility and agronomic performance are concerned. On the basis of results obtained, it can be envisaged that, at least with some nuclear genetic backgrounds, the mitochondrial and plastidial genes derived from *S. commersonii* as well as the relatively high degree of mtDNA rearrangements present in the somatic hybrid either are not associated with negative effects, as found in other fusion combinations (Lössl et al. 1994), or present some advantages. This is important considering the need to enlarge and maintain genetic diversity at the cytoplasmic level in potato and other crop plants (Hoopes et al. 1980; Levings 1990).

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