Theor Appl Genet (1995) 90: 285–293 © Springer-Verlag 1995

A. M. A. Wolters · H. C. H. Schoenmakers · M. Koornneef

Chloroplast and mitochondrial DNA composition of triploid and tetraploid somatic hybrids between *Lycopersicon esculentum* and *Solanum tuberosum*

Received: 3 March 1994 / Accepted: 10 June 1994

Abstract The chloroplast (cp) DNA type and mitochondrial (mt) DNA composition of 17 somatic hybrids between a cytoplasmic albino tomato and monoploid potato (A7-hybrids) and 18 somatic hybrids between a nitrate reductase-deficient tomato and monoploid potato (C7-hybrids) were analyzed. Thirteen A7-hybrids and 9 C7-hybrids were triploids (with one potato genome); the other hybrids were tetraploid. As expected, all A7-hybrids contained potato cpDNA. Of the C7-hybrids 7 had tomato cpDNA, 10 had potato cpDNA and 1 hybrid contained both tomato and potato cpDNA. The mtDNA composition of the hybrids was analyzed by hybridization of Southern blots with four mtDNA-specific probes. The mtDNAs in the hybrids had segregated independently from the cpDNAs. Nuclear DNA composition (i.e. one or two potato genomes) did not influence the chloroplast type in the C7-hybrids, nor the mtDNA composition of A7- or C7-hybrids. From the co-segregation of specific mtDNA fragments we inferred that both tomato and potato mtDNAs probably have a *cox*II gene closely linked to 18S+5S rRNA genes. In tomato, *atpA*, and in potato, *atp6* seems to be linked to these mtDNA genes.

Key words Tomato · Potato · Somatic hybrids
Chloroplast DNA · Mitochondrial DNA

Introduction

By somatic hybridization it is possible to bring the nuclei, chloroplasts and mitochondria from two different species together in one cell. When the two species involved in the protoplast fusion are closely related, the resulting somatic hybrids generally contain the nuclear DNA complements of both parents (e.g. Wijbrandi et al. 1990; Daunay et al. 1993; Taguchi et al. 1993). However, when the fusion parents are distantly related, uni- or biparental chromosome elimination may occur (Gilissen et al. 1992; Wolters et al. 1993b). The heteroplasmic state of two types of chloroplasts is unstable, and sorting out of the chloroplasts usually occurs rapidly (Akada and Hirai 1986), resulting in somatic hybrid plants that contain either one or the other parental chloroplast type (e.g. Belliard et al. 1978; Pehu et al. 1989; San et al. 1990). Similarly, the segregation of mitochondrial DNA (mtDNA) molecules takes place. The mtDNA composition of somatic hybrids can range from purely parental to a combination of some, but not all, mtDNA fragments from both parents (e.g. Kemble et al. 1986; Wachocki et al. 1991). New, non-parental mtDNA fragments are frequently present, most likely resulting from recombination (Rothenberg et al. 1985; Rothenberg and Hanson 1987). To obtain viable somatic hybrid plants, organelle segregation must result in cells in which the remaining chloroplasts and mitochondrial genes can function well with genes encoded by the hybrid nucleus.

We have been investigating the possibilities of somatic hybridization between tomato and other more or less related Solanaceous species, such as potato, tobacco and *Nicotiana plumbaginifolia*,and have studied nucleo-cytoplasmic interactions in the resulting hybrids. Symmetric somatic hybrid plants between tomato and potato were easily obtained (Schoenmakers et al. 1993), whereas it was much more difficult to produce shoot-regenerating hybrids between tomato and *N. tabacum* or *N. plumbaginifolia* (Wolters et al. 1993b). In the *Lycopersicon* (+) *Nicotiana* hybrids, but also in *Solanum tuberosum* (+) *N. plumbaginifolia* somatic hybrids (Wolters et al. 1993a), which showed an extensive elimination of the chromosomes of one of the parents, we found a strong correlation between the origins of nuclear, chloroplast and mitochondrial DNA. The hybrids contained chloroplast DNA (cpDNA) and most mtDNA fragments of the parent predominating in the nucleus.

Apparently, a viable somatic hybrid between distantly related species requires that the chloroplasts, mtDNA frag-

Communicated by P. Maliga

A. M. A. Wolters · H. C. H. Schoenmakers · M. Koornneef (✉)
Department of Genetics, Wageningen Agricultural University, Dreijenlaan 2, NL-6703 HA Wageningen, The Netherlands

ments and nuclear DNA be mainly or solely derived from one parental species. This raised the question whether there is also a preference for mtDNA and cpDNA of the same parental species in somatic hybrids between relatively related species, e.g. between *L. esculentum* and *S. tuberosum*. The presence of nucleo-cytoplasmic incongruity between these two species is indicated by the impossibility of obtaining true cybrids combining a *L. esculentum* nucleus with *S. tuberosum* chloroplasts (Wolters et al. 1991).

In the present article we report the analysis of organellar DNA composition of (near) triploid and (near) tetraploid somatic hybrid plants between diploid tomato and monoploid potato. Hybrids were selected in two ways. After fusion of potato with a cytoplasmic albino tomato genotype, green calli were selected, i.e. calli containing potato chloroplasts (A7-hybrids). The same potato genotype was also fused with a nitrate reductase-deficient mutant of tomato. In this latter case calli which proliferated on medium containing nitrate as the sole nitrogen source were selected, and thus selection was based on a nuclear encoded trait from potato (C7-hybrids, Schoenmakers et al. 1992).

We investigated (1) if tomato (+) potato hybrids selected for nuclear encoded traits show a random distribution of chloroplast types, (2) if selection for the presence of potato chloroplasts influences the mtDNA composition of the resulting tomato (+) potato hybrids, and (3) if there is a correlation between nuclear and organellar DNA composition in these hybrids. Furthermore,(4) recombination between tomato and potato mtDNAs was analyzed by (co)segregation analysis of mtDNA restriction fragments.

Materials and methods

Plant materials

The triploid somatic hybrids between *Lycopersicon esculentum* ALRCxM8-7 (a cytoplasmic albino mutant) and monoploid *Solanum tuberosum* 7322 (designated as A7-hybrids) and between *L. esculentum* C31-244 (a nitrate reductase-deficient mutant) and monoploid *S. tuberosum* 7322 (designated as C7-hybrids) that were used in this study have been described by Schoenmakers et al. (1993). The tetraploid hybrids were obtained from the same fusion combinations. Individual A7- or C7-hybrid calli were numbered consecutively. Independent shoots regenerated from a single hybrid callus were distinguished by different letters: A, B, C or D. The hybrid nature of the somatic hybrid calli was confirmed by isozyme analyses (Schoenmakers et al. 1993). For organelle DNA analyses we used *L. esculentum* ALRC, the female parent of ALRCxM8-7, as the parental species for the A7-hybrids. We used *L. esculentum* cv 'GT', the wild type of C31-244 (Schoenmakers et al. 1993), as the parental species for the C7-hybrids. No differences in cpDNA or mtDNA restriction fragment length polymorphism (RFLP) pattern were detected between these two tomato genotypes. Chromosome numbers of the triploid and tetraploid somatic hybrids used in this study were analyzed as described in Schoenmakers et al. (1993).

DNA isolation, restriction and Southern transfer

Leaves of the hybrids and of parental species *L. esculentum* ALRC grown in vitro, and of *L. esculentum* 'GT' and *S. tuberosum* 7322 grown in the greenhouse, were used for isolation of total DNA according to Rogers and Bendich (1988). DNA concentrations were measured with a mini-fluorometer (Hoefer) according to the supplier's instructions. Total DNA (4 µg) was digested with *Eco*RI or *Hin*dIII. The DNA fragments were separated on 0.8% agarose gels and subsequently alkaline blotted onto Gene Screen Plus (DuPont) membranes according to Chomczynski and Qasba (1984).

Organelle DNA probes and hybridization

For the characterization of cpDNA the 2.4-kb *Bam*HI fragment of *Petunia* cpDNA probe pPCY64 (de Haas et al. 1986) and the 1.8-kb *Eco*RI/*Hin*dIII fragment of *Petunia* cpDNA probe pPCY20-1 (Overbeeke et al. 1984) were used. We characterized the mtDNA composition using the 0.45-kb *Eco*RI-*Sal*I fragment of the *Pcf* gene from *Petunia* (Young and Hanson 1987), the 6.0-kb *Bam*HI fragment of *Zea diploperennis* mtDNA containing the 18S+5S rRNA genes (Gwynn et al. 1987), the 1.2-kb *Bam*HI/*Hin*dIII fragment of the ATPase α-subunit (*atpA*) gene from *Oenothera* mtDNA (Schuster and Brennicke 1986) and the 2.7-kb *Hin*dIII fragment of *Zea mays* mtDNA containing the gene encoding ATPase subunit 6 (*atp6*, Dewey et al. 1985). In order to isolate inserts from the plasmids the correct restriction fragment was cut out of an agarose gel, and the DNA was recovered with the Prep-A-Gene DNA Purification kit from Bio-Rad. Probes were radioactively labeled with the Boehringer Mannheim Random Primed DNA Labeling kit. We performed hybridizations in glass bottles using a HYBAID hybridization oven, at 65°C for 16 h. The blots were rinsed to a stringency of 0.2×SSC, 1% SDS at 65°C. After autoradiography the probe was removed from the blots according to the supplier's instructions, and the membranes were re-used.

Results

Table 1 shows the chromosome numbers of the 17 A7-hybrids and 18 C7-hybrids used in this study. Thirteen A7-hybrids and 9 C7-hybrids were triploid (2n=2x+x=36) or near-triploid, with 1 or 2 chromosomes missing or with 1 chromosome extra. One A7-hybrid (A7-74B) formed roots with cells containing 36 chromosomes in addition to roots with cells containing 37 chromosomes. Four A7-hybrids were tetraploid (2n=2x+2x=48) and 9 C7-hybrids were tetraploid or hypotetraploid, with 1 or 2 chromosomes missing.

Nuclear hybridity of all 35 plants was confirmed by the presence of tomato and potato satellite chromosomes. The satellite of tomato chromosome 2 of the genotypes used in this study is significantly larger than the satellite of potato chromosome 2 (cf. Schoenmakers et al. 1993). All triploid A7- and C7-hybrids, except C7-149A and C7-167A, possessed 1 potato and 2 tomato satellite chromosomes. Hybrids C7-149A and C7-167A, which both had 35 chromosomes, contained 1 tomato and 1 potato chromosome 2. All of the tetraploid A7- and C7-hybrids, except C7-49A, possessed 2 tomato and 2 potato satellite chromosomes, indicating that they resulted from the fusion of 1 diploid tomato protoplast with 1 diploid or 2 haploid potato protoplasts. Hybrid C7-49A contained 2 tomato and 1 potato chromosome 2.

Total DNA from the A7-hybrids, the C7-hybrids and their respective parents was digested with *Eco*RI, electrophoresed and subsequently blotted. The blots were hybridized with the inserts of plasmids pPCY64 and pPCY20-1

Table 1 Chromosome complement, chloroplast DNA (cpDNA) type and mitochondrial DNA (mtDNA) composition of parental genotypes and somatic hybrids between *L. esculentum* ALRCxM8-7 and *S. tuberosum* 7322 (A7-hybrids) and between *L. esculentum* C31-244 and *S. tuberosum* 7322 (C7-hybrids)

Genotype	Number of chromosomes	cpDNA	Number of mtDNA fragments[a]					MtDNA group[b]
			C	L	S	R	total	
ALRCxM8-7/C31-244	24	L	8	10	–	–	18	L
7322	12	S	8	–	9	–	17	S
A7-hybrids								
58A	36	S	8	7	6	–	21	LS
73C	36	S	8	6	3	–	17	L
74B	36/37	S	8	9	5	1	23	L
82A	36	S	8	10	–	–	18	L
105B	37	S	8	9	2	–	19	L
105C	37	S	8	9	4	1	22	L
120D	35	S	8	7	2	2	19	L
146D	34	S	8	2	9	–	19	S
169A	35	S	8	10	–	–	18	L
170B	34	S	8	9	1	–	18	L
194A	36	S	8	10	2	–	20	L
196B	35	S	8	10	–	–	18	L
208A	35	S	8	9	2	1	20	L
3D	48	S	8	8	1	–	17	L
76B	48	S	8	8	1	–	17	L
97B	48	S	8	9	3	1	21	L
98C	48	S	8	7	2	–	17	L
C7-hybrids								
22A	35	L	8	4	9	–	21	S
72B	34	L+S	8	8	3	–	19	L
76A	36	S	8	9	1	–	18	L
112B	36	S	8	3	9	–	20	S
113A	36	S	8	5	7	–	20	S
116A	36	S	7	9	3	–	19	L
133A	36	S	8	3	8	–	19	S
149A	35	L	7	8	3	–	18	L
167A	35	L	7	7	3	–	17	L
15C	48	L	8	7	2	–	17	L
23A	48	S	8	2	9	–	19	S
42B	48	L	7	9	2	–	18	L
49A	47	S	8	3	8	–	19	S
74A	47	L	8	4	7	–	19	S
107B	47	S	7	4	8	–	19	S
109C	46	S	8	2	9	–	19	S
142A	48	L	8	2	9	2	21	S
147A	48	S	8	1	8	–	17	S

[a] C, common; L, *L. esculentum*-specific; S, *S. tuberosum*-specific; R, new fragment
[b] L group: number of L mtDNA fragments/number of S mtDNA fragments (L/S)>1.4; LS group: 0.8<L/S<1.4; S group: L/S<0.8

(Fig. 1). The results obtained with the first probe were confirmed by the second probe. As expected, all A7-hybrids possessed the cpDNA fragments specific for *S. tuberosum*. The C7-hybrids showed a random distribution of chloroplast types (Fig. 1, Table 1): 10 hybrids contained chloroplasts from potato, 7 had tomato chloroplasts and 1 hybrid possessed chloroplasts from both parents (C7-72B).

Blots containing *Eco*RI- or *Hin*dIII-digested total DNA of the hybrids and their parents were hybridized with four mtDNA-specific probes: the *Pcf* gene of *Petunia* mtDNA (consisting of part of the *cox*II gene and part of the *atp9-1* gene), *atp6*, *atpA* and the 18S+5S rRNA genes (Fig. 2). Figure 2A shows an autoradiogram of total DNA from *L. esculentum* (lane 1), *S. tuberosum* (lane 2) and the A7-hybrids (lanes 3–19) digested with *Eco*RI and hybridized with mtDNA probe *atpA*. The tomato parent shows a single band of 5.4 kb (L8, see Table 2) and the potato parent one band of 4.9 kb (S7). The hybrids contain only the tomato-specific band (lanes 4, 6–9, 11, 12, 14, 16–18), only the potato-specific band (lanes 10,19), or both mtDNA bands (lanes 3, 5, 13, 15). In this enzyme/probe combination we found no new, non-parental fragments.

Figure 2B shows an autoradiogram of the same blot as in Fig. 2A, but hybridized with the 18S+5S rRNA genes. The strongly hybridizing band of 3.1 kb is the cpDNA fragment carrying the chloroplast 16S rRNA gene (this fragment has the same size as the tobacco cpDNA fragment containing this gene, data not shown). Two tomato-specific mtDNA fragments of 5.0 kb (L5) and 2.0 kb (L6) and 2 potato-specific mtDNA fragments of 4.3 kb (S5) and 2.9

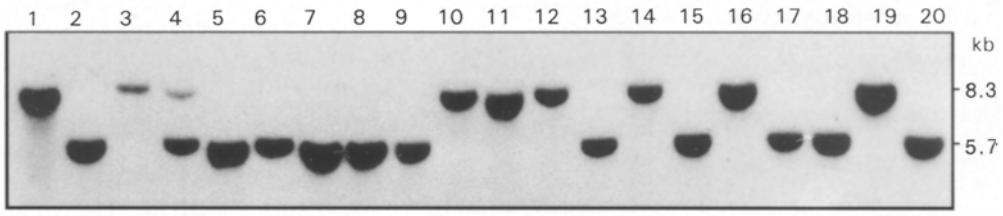

Fig. 1 Autoradiogram of a blot containing *Eco*RI-digested total DNA of *L. esculentum* GT (*lane 1*), *S. tuberosum* 7322 (*lane 2*) and C7-hybrids 22A, 72B, 76A, 112 B, 113A, 116A, 133A, 149A, 167A, 15C, 23A, 42B, 49A, 74A, 107B, 109C, 142A and 147A (*lanes 3–20*), hybridized with chloroplast probe pPCY20-1. Sizes of tomato- and potato-specific fragments are indicated

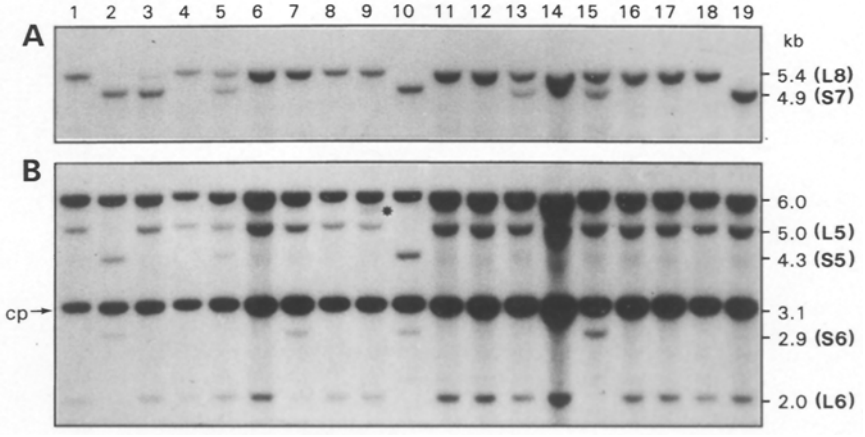

Fig. 2A, B Autoradiograms of a blot containing *Eco*RI-digested total DNA of *L. esculentum* ALRC (*lane 1*), *S. tuberosum* 7322 (*lane 2*) and A7-hybrids 58A, 73C, 74B, 82A, 105B, 105C, 120D, 146D, 169A, 170B, 194A, 196B, 208A, 3D, 76B, 97B, and 98C (*lanes 3–19*)hybridized with mtDNA probes *atpA* (**A**) or 18S+5S rRNA (**B**). Sizes of fragments are indicated. In **B** the cpDNA fragment carrying the 16S rRNA gene is indicated with an *arrow*. A novel, non-parental fragment in hybrid A7-120D (*lane 9*) is indicated with an *asterisk*

kb (S6) can be seen. Thirteen hybrids contain both the L5 and L6 bands and 1 hybrid (A7-146D, lane 10) contains both the S5 and S6 bands. Hybrid A7-74B (lane 5) contains the S5 band besides the L5 and L6 bands, and hybrid A7-105B (lane 7) contains the S6 band besides the L5 and L6 bands. Finally, 1 hybrid (A7-208A, lane 15) contains 1 tomato-specific band (L5) and 1 potato-specific band (S6). Hybrid A7-120D (lane 9) shows a new, non-parental band of about 5.7 kb.

For each enzyme/probe combination the number of common bands (bands of a particular size displayed by both parents), *L. esculentum*-specific bands, *S. tuberosum*-specific bands and novel (non-parental) bands were counted per hybrid. The sum of these numbers per hybrid are shown in Table 1. Details of the tomato-specific and the potato-specific mtDNA and cpDNA fragments are given in Table 2.

For each hybrid the number of *L. esculentum*-specific mtDNA fragments was plotted against the number of *S. tuberosum*-specific mtDNA fragments, and the cpDNA type was indicated (Fig. 3A). No correlation between chloroplast type and mtDNA composition in the hybrids was observed. Some hybrids possessing tomato chloroplasts contained predominantly tomato mtDNA fragments and others contained predominantly potato mtDNA fragments. When only the A7-hybrids, which all possessed potato chloroplasts, were considered it is remarkable that almost all of them contained predominantly *L. esculentum*-specific mtDNA fragments (see Table 1). A similar plot as in Fig. 3A was made in which, instead of the chloroplast type, the ploidy level of the hybrids was indicated (Fig. 3B). Again, no correlation was apparent between nuclear DNA content, i.e. the presence of one or two potato genomes, and mtDNA composition.

The total number of mtDNA fragments per hybrid observed in our hybridizations varied between 17 and 23 (Table 1), which is equal to or slightly higher than the total numbers of the parents. This is a clear indication that in these somatic hybrids the heteroplasmic state of two complete mtDNA genomes was unstable and segregation took place. Hybrids containing many tomato mtDNA fragments had few potato mtDNA fragments, and vice versa. The regression lines in Fig. 3A and B have a correlation coefficient (r) of –0.91.

Random segregation per mtDNA fragment was tested in relation to the mean segregation ratio of all *Lycopersicon*-specific (L) or all *S. tuberosum*-specific (S) mtDNA fragments analyzed (Table 3) to find out if any of the mtDNA fragments showed a biased transmission. In Table 3 the number of hybrids containing a specific mtDNA fragment (L1–L10 or S1–S9, see Table 2) and the number of hybrids in which this fragment was absent are indicated. A χ^2 test was performed in which these numbers were compared with the mean ratio for L or for S mtDNA fragments.

Table 2 Details of species-specific cpDNA and mtDNA fragments

Fragment	Size (kb)	Digest	Probe	Remarks
CpDNA				
tomato-specific				
cpL1	0.86	*Eco*RI	pPCY64	
cpL2	8.3	*Eco*RI	pPCY20-1	
potato-specific				
cpS1	0.73	*Eco*RI	pPCY64	
cpS2	5.7	*Eco*RI	pPCY20-1	
MtDNA				
tomato-specific				
L1	3.4	*Eco*RI	*Pcf*[a]	*cox*II-containing fragment, cf. Guri et al. (1988)[b]
L2	1.6	*Eco*RI	*Pcf*	*cox*II-containing fragment, cf. Guri et al. (1988)
L3	7.7	*Hin*dIII	*Pcf*	
L4	3.0	*Hin*dIII	*Pcf*	*cox*II-containing fragment, cf. Guri et al. (1988)
L5	5.0	*Eco*RI	18S+5S rRNA	
L6	2.0	*Eco*RI	18S+5S rRNA	
L7	10.5	*Hin*dIII	18S+5S rRNA	cf. Bonnema and O'Connell (1992)
L8	5.4	*Eco*RI	*atpA*	
L9	3.2	*Eco*RI	*atp6*	
L10	1.4	*Hin*dIII	*atp6*	
potato-specific				
S1	0.76	*Eco*RI	*Pcf*	*atp9*-containing fragment, cf. Dell'Orto et al. (1993)
S2	8.8	*Hin*dIII	*Pcf*	
S3	2.3	*Hin*dIII	*Pcf*	*cox*II-containing fragment, cf. Guri et al. (1988)
S4	1.6	*Hin*dIII	*Pcf*	*cox*II-containing fragment, cf. Guri et al. (1988)
S5	4.3	*Eco*RI	18S+5S rRNA	
S6	2.9	*Eco*RI	18S+5S rRNA	
S7	4.9	*Eco*RI	*atpA*	
S8	1.5	*Eco*RI	*atp6*	
S9	6.3	*Hin*dIII	*atp6*	

[a] The 0.45-kb *Eco*RI-*Sal*I fragment of the *Pcf* gene used for hybridization consists of part of the *cox*II gene and part of the *atp9-1* gene of *Petunia* mtDNA (Young and Hanson 1987)
[b] Guri et al. (1988) mention the size of fragments obtained after hybridization of *Eco*RI- and *Hin*dIII-digested mitochondrial DNA of *L. esculentum* and *S. nigrum* with mtDNA probe *cox*II

Fragments L1, L7, L9+10, S4 and S5 showed a significant deviation of the mean ratio. These deviations were also found when A7- and C7-hybrids were tested separately (data not shown).

To evaluate a possible cosegregation of specific mtDNA fragments, the presence or absence of each mtDNA fragment in the hybrids was compared with the presence or absence of every other mtDNA fragment and of the cpDNA fragments. Independent assortment was tested with a χ^2 assay. Because of the large number of pairwise comparisons an error rate of $P=0.001$ was used for each combination in order to obtain an approximate overall error rate $P=0.05$. The results are shown in Table 4. A strong cosegregation ($P<10^{-4}$) is apparent between L2 and L4 (both *cox*II-containing tomato mtDNA fragments), L2 and L5, L2 and L6, L4 and L5 (tomato *cox*II and tomato 18S+5S rRNA), L5 and L6 (both 18S+5S rRNA-containing tomato mtDNA fragments) and S3 and S5 (potato *cox*II and potato 18S+5S rRNA). Strong repulsion exists between L2 and S3, L4 and S3 (tomato *cox*II vs. potato *cox*II), L5 and S3, L7 and S4 (tomato 18S+5S rRNA vs. potato *cox*II), L2 and S5, L4 and S5 (tomato *cox*II vs. potato 18S+5S rRNA) and L5 and S5, L6 and S5, L6 and S6 (tomato 18S+5S rRNA vs. potato 18S+5S rRNA). L8, the tomato mtDNA fragment containing *atpA*, cosegregates, although less frequently, with L2, L5 and L6, and is in repulsion with S3, S5 and S6, whereas S8 and S9 (potato *atp6*-carrying fragments) are in repulsion with L2, L4 (tomato *cox*II) and L5 (tomato 18S+5S rRNA) and cosegregate with S2 and S5 (potato 18S+5S rRNA). Furthermore, a strong cosegregation is apparent between L9 and L10 (the *Eco*RI respectively *Hin*dIII tomato mtDNA fragment carrying the *atp6* gene), between S8 and S9 (the *Eco*RI respectively *Hin*dIII potato mtDNA fragment containing the *atp6* gene) and between S1 (potato *atp9*) and S3 (potato *cox*II). A strong repulsion exists between L3 and S2 (probably tomato *atp9* vs. potato *atp9*), L8 and S7 (tomato *atpA* vs. potato *atpA*) and between cpL and cpS.

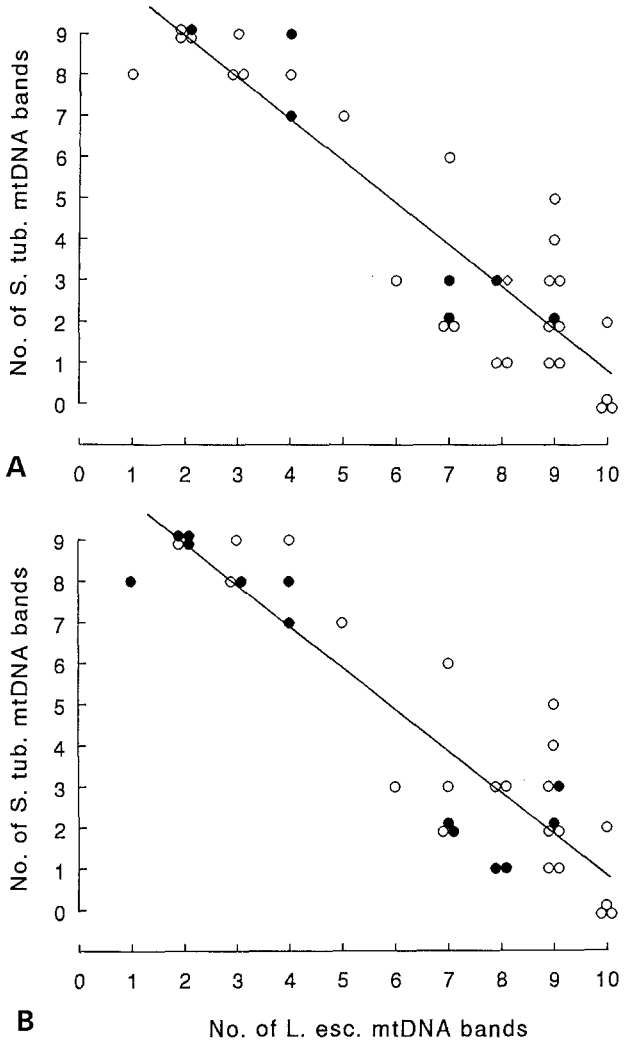

Fig. 3A, B Scatter diagrams showing the number of tomato-specific and potato-specific mtDNA fragments per hybrid with respect to chloroplast type (**A**) or ploidy level of the hybrids (**B**). **A** ○ hybrid containing potato chloroplasts, ● hybrid containing tomato chloroplasts, ◇ hybrid containing both tomato and potato chloroplasts. **B** ○ triploid hybrid, ● tetraploid hybrid

Table 3 Segregation of mtDNA fragments and χ^2 assay to test for a biased transmission of specific mtDNA fragments from *L. esulentum* (L1–L10) or from *S. tuberosum* (S1–S9), described in Table 2

Fragment	Number of hybrids		χ^2_1
	Present	Absent	
L1	14	21	7.13[a]
L2	23	12	0.21
L3	22	13	0.01
L4	24	11	0.66
L5	24	11	0.66
L6	24	11	0.66
L7	5	30	33.67[a]
L8	25	10	1.34
L9+10	34	1	18.42[a]
Mean	21.7	13.3	
S1	20	15	1.03
S2	18	17	0.11
S3	13	22	1.83
S4	30	5	19.33[a]
S5	11	24	4.12[a]
S6	13	22	1.83
S7	16	19	0.11
S8+9	15	20	0.46
Mean	17	18	

[a] Significant deviation ($P<0.05$) from the mean segregation ratio

Discussion

The analysis of the chloroplast DNA type of 18 tomato (+) potato somatic hybrids selected for nuclear traits from both parents (the C7-hybrids) revealed that the chloroplast segregation ratio in these hybrids is approximately 1:1. This is in accordance with the results of Schiller et al. (1982) who reported that the frequency distribution of cpDNAs of either parent among 12 tomato (+) potato somatic hybrids was compatible with random selection. Both the triploid and the tetraploid hybrids we analyzed showed this 1:1 segregation. The tetraploid hybrids resulted from fusion of one diploid tomato protoplast with two monoploid potato protoplasts or with one somatically doubled and thus dihaploid potato protoplast. Since the number of chloroplasts in a leaf mesophyll cell is related to the number of genomes (Butterfass 1973), it seems likely that the original heterokaryons which yielded triploid hybrids contained on average fewer potato chloroplasts than the heterokaryons that gave rise to tetraploid hybrids. Therefore, it is remarkable that the triploid C7-hybrids showed the same segregation ratio with respect to cpDNA as the tetraploid C7-hybrids. Hung et al. (1993) observed the same phenomenon. They produced diploid, triploid and tetraploid somatic hybrids between haploid *Nicotiana plumbaginifolia* and haploid *N. sylvestris*, and observed a 1:1 segregation of chloroplast types, irrespective of the ratio of parental nuclear genomes.

One somatic hybrid contained chloroplasts from both parents, even though the total DNA for organelle analysis was isolated from hybrids that had been maintained as shoot cultures in vitro for 2½ years. Although chloroplast segregation is usually completed before the onset of shoot regeneration, hybrid plants containing chloroplasts from both parents are occasionally found up to 3 years after fusion (Glimelius et al. 1981; Derks et al. 1991; Wachocki et al. 1991).

The mtDNA composition of the hybrids seemed not to be influenced by nuclear DNA composition, i.e. one or two potato genomes. Similarly, Landgren and Glimelius (1990) could find no correlation between nuclear constitution and mtDNA type in *Brassica napus* (+) *Eruca sativa* somatic hybrids that showed preferential elimination of *E. sativa* chromosomes (Fahleson et al. 1988). However, Bonnema et al. (1992) reported that highly asymmetric somatic hybrids between *L. esculentum* and *L. pennellii* had more tomato-specific mitochondrial sequences than symmetric somatic hybrids. It is likely that specific nuclear genes of one

Table 4 Pairwise comparison of the presence or absence of specific mtDNA fragments from *L. esculentum* (L1–L10) or from *S. tuberosum* (S1–S9) and of cpDNA fragments (cpL, cpS), described in Table 2. Results of a χ^2 test for independent assortment are indicated. 0, Independent assortment; +, – dependent assortment ($P<10^{-3}$); + +, – – dependent assortment ($P<10^{-4}$); +, + + cosegregation; –, – – repulsion

Fragment	L1	L3	L2	L4	L5	L6	L7	L8	L9	L10	
L3	+										
L2	0	0									
L4	0	0	+ +								
L5	0	0	+ +	+ +							
L6	0	0	+ +	+	+ +						
L7	0	0	0	0	0	0					
L8	0	0	+	0	+	+	0				
L9	0	0	0	0	0	0	0	0			
L10	0	0	0	0	0	0	0	0	+ +		
cpL	0	0	0	0	0	0	0	0	0	0	
Fragment	S1	S4	S3	S2	S5	S6	S7	S8	S9		
S4	0										
S3	+ +	0									
S2	0	0	0								
S5	+	0	+ +	+							
S6	0	0	0	0	+						
S7	0	0	0	0	0	+					
S8	0	0	0	+	+	0	0				
S9	0	0	0	+	+	0	0	+ +			
cpS	0	0	0	0	0	0	0	0	0		
Fragment	L1	L3	L2	L4	L5	L6	L7	L8	L9	L10	cpL
S1	0	0	–	–	–	0	0	0	0	0	0
S4	0	0	0	0	0	0	– –	0	0	0	0
S3	0	0	– –	– –	– –	–	0	–	0	0	0
S2	–	– –	–	0	–	0	0	0	0	0	0
S5	0	0	– –	– –	– –	– –	0	–	0	0	0
S6	0	0	–	0	–	– –	0	–	0	0	0
S7	0	0	0	0	0	–	0	– –	0	0	0
S8	0	0	–	–	–	0	0	0	0	0	0
S9	0	0	–	–	–	0	0	0	0	0	0
cpS	0	0	0	0	0	0	0	0	0	0	– –

parental species are required for the mitochondrial genes of that species to be functional.

The mtDNA composition of our hybrids seemed not to be affected by the chloroplast type. Independent segregation of mitochondria and chloroplasts has also been reported by Walters and Earle (1993) for somatic hybrids of *Brassica oleracea* genotypes containing either a *B. oleracea* cytoplasm or a *Raphanus sativus* cytoplasm. The A7-hybrids, which were selected for the presence of potato chloroplasts, did not contain more potato-specific mtDNA fragments than the C7-hybrids; conversely, they showed mostly tomato-specific mtDNA fragments. Both the A7- and C7-hybrids were obtained from fusions between leaf mesophyll protoplasts of both parents, thus eliminating the influence of different sources of protoplasts on mitochondrial segregation, as proposed by Landgren and Glimelius (1990). Possibly, leaf cells of the albino tomato parent of the A7-hybrids, which contain only proplastids and grow heterotrophically, possess more mitochondria or mitochondria with a higher replication rate than leaf cells of normal green tomato or potato genotypes.

Some specific mtDNA fragments show a biased segregation (Table 3). L9 and L10, the tomato mtDNA fragments carrying the *atp6* gene, are present in 34 of the 35 hybrids analyzed. In contrast, the corresponding potato *atp6* gene (fragments S8 and S9) is present in 15 of the 35 hybrids. The only hybrid that lacks the tomato *atp6* gene possesses the potato *atp6* gene. Although tomato-specific fragments are on average retained in 60% of the hybrids, the L7 fragment is only present in 5 A7-hybrids that lack S4. The S4 fragment is significantly more frequently retained in the hybrids than the other potato-specific mtDNA fragments. Temple et al. (1992) reported that some regions in the mtDNA genomes of *Brassica campestris* (+) *B. oleracea* (Ogura cms) somatic hybrids are always derived from one fusion partner. Possibly, some specific mtDNA regions of one or the other parent are needed for hybrid callus growth, for the regeneration thereof or for some other essential process. This suggests that hybrids with particular mtDNA compositions are more viable than others carrying a different mtDNA.

Between individual *Lycopersicon* (+) *Solanum* hybrids large differences in greenhouse performance and leaf and flower morphology were observed (Schoenmakers et al. 1993). Possibly, the mtDNA composition determines some of these traits. For example, in tobacco it has been shown that mutations in the mtDNA can cause changes in floral morphology (Bonnett et al. 1991; Kofer et al. 1991,1992) or leaf variegation and abnormal vegetative growth (Bonnett et al. 1993). However, it cannot be excluded that dif-

ferences in chromosome complements also affect these traits. Hybrid A7-82A, a euploid triploid hybrid containing potato chloroplasts and only tomato-specific mtDNA fragments (Table 1), showed a good viability in the greenhouse and regularly shaped leaves and flowers, whereas A7-146D, an aneuploid triploid hybrid with 34 chromosomes, potato chloroplasts and mtDNA fragments mainly from potato, was less viable and showed abnormal leaves and flowers (Schoenmakers et al. 1993).

The present analysis does not indicate that in tomato (+) potato somatic hybrids there is a requirement for organelles to be predominantly derived from one or the other parental species. Many possible nucleus-chloroplast-mitochondrion combinations seem to be functional in these fusion products. This could be a factor explaining the higher frequency of *Lycopersicon* (+) *Solanum* somatic hybrids as compared with *Lycopersicon* (+) *Nicotiana* somatic hybrids (Wolters et al. 1993b). The latter hybrids apparently require a more specific fine-tuning of nucleus and organelles, and thus the fraction of viable fusion products in the total population of fusion products is lower.

When, by asymmetric somatic hybridization or by backcrossing, many nuclear genes from one of the parents are lost, the situation may be different for tomato (+) potato somatic hybrids. In that case nucleo-cytoplasmic interactions may be more critical for chloroplasts than for mtDNA genomes, because mtDNAs recombine easily whereas cpDNAs usually do not recombine. In previous experiments we could not obtain true cybrids combining a tomato nucleus with potato chloroplasts, not even when the potato protoplasts had been heavily irradiated (Wolters et al. 1991). Probably, potato chloroplasts can only be functional when a certain amount of potato nuclear DNA is present. In contrast, cybrids combining a tomato nucleus with mtDNA fragments from both tomato and potato have been reported by Melchers et al. (1992). Thus, the proteins encoded by genes located on the potato mtDNA fragments in these cybrids can either be functional when only tomato nuclear DNA is present, or at least they do not interfere with the interaction between tomato mtDNA-encoded and nuclear DNA-encoded proteins.

It is remarkable that only 6 of the 35 analyzed tomato (+) potato hybrids showed 1 or 2 new, non-parental mtDNA fragments. The majority of the intertribal hybrids between *Lycopersicon* spp. and *Nicotiana* spp. (Wolters et al. 1993b), which were analyzed with three mtDNA probes, but also of the intrageneric hybrids between tomato and *L. pennellii*, analyzed with seven cosmid clones containing tomato mtDNA (Bonnema et al. 1992), showed several novel mtDNA fragments. Possibly, more A7- and C7-hybrids could be shown to contain new mtDNA fragments using additional mtDNA probes, since novel fragments mainly seem to occur in distinct regions of the mitochondrial genome (Temple et al. 1992).

Not only novel fragments are an indication of recombination between the parental mtDNAs, but also new combinations of specific mtDNA fragments from both parents. Thus, recombination can also be observed when analyzing cosegregation of mtDNA restriction fragments. Closely linked genes will often be retained together in a somatic hybrid, whereas genes between which recombination occurs frequently, e.g. because they are located on different subgenomic molecules, are more often separated. The results of the cosegregation analysis suggest that on both tomato and potato mitochondrial DNA, genes encoding *cox*II and 18S+5S rRNA are closely linked (on the same subgenomic molecule). Segregation of the original mixture of parental mtDNAs therefore resulted in most cases in hybrid cells containing either tomato *cox*II and 18S+5S rRNA genes, or potato *cox*II and 18S+5S rRNA genes. Probably, two tomato 18S+5S rRNA genes and two potato *cox*II genes are involved, since L7 (tomato 18S+5S) is not cosegregating with L5 and L6, and S4 (potato *cox*II) segregates independently from S3. The L8 fragment seems to be linked to the tomato *cox*II and 18S+5S rRNA genes, but at a larger genetic distance than the distance between *cox*II and 18S+5S rRNA. Similarly, S8 and S9, the *Eco*RI and *Hin*dIII potato mtDNA fragments, respectively, carrying the *atp6* gene, seem to be linked to the potato 18S+5S rRNA gene.

Cosegregation of mtDNA fragments may not only result from physical linkage, but may also occur when for the proper functioning of the somatic hybrid cell mitochondrial genes encoding different proteins have to originate from the same parental species. To distinguish between linkage and somatic incongruity as an explanation for cosegregation, a comparison of cosegregation data with physical maps of *Lycopersicon* and *Solanum* mitochondrial genomes, which are not yet available, will be necessary.

Acknowledgements The authors thank J. W. van Ooijen for statistical advice and C. Heyting for critically reading the manuscript. This work was supported by the Dutch Programme Committee on Agricultural Biotechnology (PcLB).

References

Akada S, Hirai A (1986) Studies on the mode of separation of chloroplast genomes in parasexual hybrid calli. III. Random separation of two types of chloroplast genomes in a hybrid callus. Jpn J Genet 61:437–445

Belliard G, Pelletier G, Vedel F, Quétier F (1978) Morphological characteristics and chloroplast DNA distribution in different cytoplasmic parasexual hybrids of *Nicotiana tabacum*. Mol Gen Genet 165:231–237

Bonnema AB, O'Connell MA (1992) Molecular analysis of the nuclear organellar genotype of somatic hybrid plants between tomato (*Lycopersicon esculentum*) and *Lycopersicon chilense*. Plant Cell Rep 10:629–632

Bonnema AB, Melzer JM, Murray LW, O'Connell MA (1992) Nonrandom inheritance of organellar genomes in symmetric and asymmetric somatic hybrids between *Lycopersicon esculentum* and *L. pennellii*. Theor Appl Genet 84:435–442

Bonnett HT, Kofer W, Håkansson G, Glimelius K (1991) Mitochondrial involvement in petal and stamen development studied by sexual and somatic hybridization of *Nicotiana* species. Plant Sci 80:119–130

Bonnett HT, Djurberg I, Fajardo M, Glimelius K (1993) A mutation causing variegation and abnormal development in tobacco is associated with an altered mitochondrial DNA. Plant J 3:519–525

Butterfass T (1973) Control of plastid division by means of nuclear DNA amount. Protoplasma 76:167–195

Chomczynski P, Qasba PK (1984) Alkaline transfer of DNA to plastic membrane. Biochem Biophys Res Commun 122:340–344

Daunay MC, Chaput MH, Sihachakr D, Allot M, Vedel F, Ducreux G (1993) Production and characterization of fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L. Theor Appl Genet 85:841–850

Dell'Orto P, Moenne A, Graves PV, Jordana X (1993) The potato mitochondrial ATP synthase subunit 9: gene structure, RNA editing and partial protein sequence. Plant Sci 88:45–53

Derks FHM, Wijbrandi J, Koornneef M, Colijn-Hooymans CM (1991) Organelle analysis of symmetric and asymmetric hybrids between *Lycopersicon peruvianum* and *Lycopersicon esculentum*. Theor Appl Genet 81:199–204

Dewey RE, Levings III CS, Timothy DH (1985) Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79:914–919

Fahleson J, Råhlén L, Glimelius K (1988) Analysis of plants regenerated from protoplast fusions between *Brassica napus* and *Eruca sativa*. Theor Appl Genet 76:507–512

Gilissen LJW, van Staveren MJ, Verhoeven HA, Sree Ramulu K (1992) Somatic hybridization between potato and *Nicotiana plumbaginifolia*. 1. Spontaneous biparental chromosome elimination and production of asymmetric hybrids. Theor Appl Genet 84:73–80

Glimelius K, Chen K, Bonnett HT (1981) Somatic hybridization in *Nicotiana*: segregation of organellar traits among hybrid and cybrid plants. Planta 153:504–510

Guri A, Levi A, Sink KC (1988) Morphological and molecular characterization of somatic hybrid plants between *Lycopersicon esculentum* and *Solanum nigrum*. Mol Gen Genet 212:191–198

Gwynn B, Dewey RE, Sederoff RR, Timothy DH, Levings III CS (1987) Sequence of the 18S-5S ribosomal gene region and the cytochrome oxidase II gene from mtDNA of *Zea diploperennis*. Theor Appl Genet 74:781–788

Haas JM de, Boot KJM, Haring MA, Kool AJ, Nijkamp HJJ (1986) A *Petunia hybrida* chloroplast DNA region, close to one of the inverted repeats, shows sequence homology with the *Euglena gracilis* chloroplast DNA region that carries the putative replication origin. Mol Gen Genet 202:48–54

Hung CY, Lai YK, Feng TY, Chen CC (1993) Chloroplast segregation in somatic hybrids of *Nicotiana plumbaginifolia* and *N. sylvestris* having different ratios of parental nuclear genomes. Plant Cell Rep 13:83–86

Kemble RJ, Barsby TL, Wong RSC, Shepard JF (1986) Mitochondrial DNA rearrangements in somatic hybrids of *Solanum tuberosum* and *Solanum brevidens*. Theor Appl Genet 72:787–793

Kofer W, Glimelius K, Bonnett HT (1991) Modifications of mitochondrial DNA cause changes in floral development in homeotic-like mutants of tobacco. Plant Cell 3:759–769

Kofer W, Glimelius K, Bonnett HT (1992) Fusion of male-sterile tobacco causes modifications of mtDNA leading to changes in floral morphology and restoration of fertility in cybrid plants. Physiol Plant 85:334–338

Landgren M, Glimelius K (1990) Analysis of chloroplast and mitochondrial segregation in three different combinations of somatic hybrids produced within *Brassicaceae*. Theor Appl Genet 80:776–784

Melchers G, Mohri Y, Watanabe K, Wakabayashi S, Harada K (1992) One-step generation of cytoplasmic male sterility by fusion of mitochondrial-inactivated tomato protoplasts with nuclear-inactivated *Solanum* protoplasts. Proc Natl Acad Sci USA 89: 6832–6836

Overbeeke N, Haring MA, Nijkamp HJJ, Kool AJ (1984) Cloning of *Petunia hybrida* chloroplast DNA sequences capable of autonomous replication in yeast. Plant Mol Biol 3:235–241

Pehu E, Karp A, Moore K, Steele S, Dunckley R, Jones MGK (1989) Molecular, cytogenetic and morphological characterization of somatic hybrids of dihaploid *Solanum tuberosum* and diploid *S. brevidens*. Theor Appl Genet 78:696–704

Rogers SO, Bendich AJ (1988) Extraction of DNA from plant tissues. Plant molecular biology manual A6. Kluwer Academic Publ, Dordrecht

Rothenberg M, Hanson MR (1987) Recombination between parental mitochondrial DNA following protoplast fusion can occur in a region which normally does not undergo intragenomic recombination in parental plants. Curr Genet 12:235–240

Rothenberg M, Boeshore ML, Hanson MR, Izhar S (1985) Intergenomic recombination of mitochondrial genomes in a somatic hybrid plant. Curr Genet 9:615–618

San LH, Vedel F, Sihachakr D, Rémy R (1990) Morphological and molecular characterization of fertile tetraploid somatic hybrids produced by protoplast electrofusion and PEG-induced fusion between *Lycopersicon esculentum* Mill. and *Lycopersicon peruvianum* Mill. Mol Gen Genet 221:17–26

Schiller B, Herrmann RG, Melchers G (1982) Restriction endonuclease analysis of plastid DNA from tomato, potato and some of their somatic hybrids. Mol Gen Genet 186:453–459

Schoenmakers HCH, Nobel EM, Koornneef M (1992) Use of leaky nitrate reductase-deficient mutants of tomato (*Lycopersicon esculentum* Mill.) for selection of somatic hybrid cell lines with wild type potato (*Solanum tuberosum* L.). Plant Cell Tissue Org Cult 31:151–154

Schoenmakers HCH, Wolters AMA, Nobel EM, de Klein CMJ, Koornneef M (1993) Allotriploid somatic hybrids of diploid tomato (*Lycopersicon esculentum* Mill.) and monoploid potato (*Solanum tuberosum* L.). Theor Appl Genet 87:328–336

Schuster W, Brennicke A (1986) Pseudocopies of the ATPase α-subunit gene in *Oenothera* mitochondria are present on different circular molecules. Mol Gen Genet 204:29–35

Taguchi T, Sakamoto K, Terada M (1993) Fertile somatic hybrids between *Petunia hybrida* and a wild species, *Petunia variabilis*. Theor Appl Genet 87:75–80

Temple M, Makaroff CA, Mutschler MA, Earle ED (1992) Novel mitochondrial genomes in *Brassica napus* somatic hybrids. Curr Genet 22:243–249

Wachocki SE, Bonnema AB, O'Connell MA (1991) Comparison of the organization of the mitochondrial genome in tomato somatic hybrids and cybrids. Theor Appl Genet 81:420–427

Walters TW, Earle ED (1993) Organellar segregation, rearrangement and recombination in protoplast fusion-derived *Brassica oleracea* calli. Theor Appl Genet 85:761–769

Wijbrandi J, van Capelle W, Hanhart CJ, van Loenen Martinet-Schuringa EP, Koornneef M (1990) Selection and characterization of somatic hybrids between *Lycopersicon esculentum* and *Lycopersicon peruvianum*. Plant Sci 70:197–208

Wolters AMA, Schoenmakers HCH, van der Meulen-Muisers JJM, van der Knaap E, Derks FHM, Koornneef M, Zelcer A (1991) Limited DNA elimination from the irradiated potato parent in fusion products of albino *Lycopersicon esculentum* and *Solanum tuberosum*. Theor Appl Genet 83:225–232

Wolters AMA, Koornneef M, Gilissen LJW (1993a) The chloroplast and mitochondrial DNA type are correlated with the nuclear composition of somatic hybrid calli of *Solanum tuberosum* and *Nicotiana plumbaginifolia*. Curr Genet 24:260–267

Wolters AMA, Vergunst AC, van der Werff F, Koornneef M (1993b) Analysis of nuclear and organellar DNA of somatic hybrid calli and plants between *Lycopersicon* spp. and *Nicotiana* spp. Mol Gen Genet 241:707–718

Young EG, Hanson MR (1987) A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. Cell 50:41–49