

A. Lössl · N. Adler · R. Horn · U. Frei · G. Wenzel

## Chondriome-type characterization of potato: mt $\alpha$ , $\beta$ , $\gamma$ , $\delta$ , $\varepsilon$ and novel plastid-mitochondrial configurations in somatic hybrids

Received: 7 November 1998 / Accepted: 30 November 1998

**Abstract** One hundred and eighty dihaploid clones used for protoplast fusions, and 144 tetraploid German potato cultivars were analysed for their cytoplasm using 11 homologous mt DNA-probes, and were classified as mitochondrial (mt) types  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , and  $\varepsilon$  according to their RFLP patterns. From the 4x cultivars, 79 had the typical mt-type  $\beta$  of *Solanum tuberosum* being different from the 46 cvs which had the mt- $\alpha$  type and 19 others with mt- $\gamma$ . A dendrogram shows their relationships to other *Solanum* species. The distantly related mt- $\varepsilon$  was only found in di-haploids, and particularly in clones deriving from *Solanum phureja* and *Solanum andigena*. Accessory mt types will be actualized on website (<http://www.edv.agrar.tu-muenchen.de/pbpz/mm/mt/all.htm>). In order to evaluate the genetic potential of novel plastid-mitochondrial configurations we have analyzed four representative populations, which derive from different fusion-combination classes: [ $\alpha$  (+)  $\beta$ ], [ $\alpha$  (+)  $\gamma$ ], [ $\alpha$  (+)  $\delta$ ] and [ $\alpha$  (+)  $\varepsilon$ ]. On the mitochondrial expression level, hybrids from an [ $\alpha$  (+)  $\varepsilon$ ] fusion could be distinguished by *in-organello* translation from [ $\alpha$  (+)  $\beta$ ] hybrids, and other di-haploids, by an additional translation product of 15 kDa. In fusion

parents with mt- $\alpha$  and - $\gamma$  an additional *atp6* reading frame is detectable in sub-stoichiometric amounts by the use of specific PCR primers. The gene differs from the original 211 bp 3' from the stop codon. Novel RFLP-patterns in 10% of the somatic hybrids were due to a high-rate replication of this pre-existing parental genome region. A second characteristic for somatic hybrids was the partial addition of parental mt sub-genomes. The major part of them revealed a new organization in their mt genomes at the mt-type characteristic loci *rpl5*, *rps14*, *cob*, *rps10*, *coxI* and *rpl2*, which contain recombination-specific repeats homologous to *Petunia* spp. and *Nicotiana*. A schematic model for the formation of novel mitochondrial genomes in potato somatic hybrids is provided.

**Key words** Potato · Somatic hybrids · Mt types · Rearrangements · Substoichiometric mtDNA

### Introduction

The production of symmetric hybrids by protoplast fusion is a useful tool in breeding vegetatively propagated crops. This technique is widely accepted by the breeding industry and particularly in potato (Hofferbert 1996). Somatic hybridization has been used to combine different valuable traits whilst maintaining a high level of heterozygosity (Möllers and Wenzel 1992; Thach et al. 1993), and also to overcome fertility barriers (Austin et al. 1986; Deimling et al. 1988; Waara et al. 1989; Chaput et al. 1990), based on the analytical synthetic breeding scheme proposed by Wenzel et al. (1979). In contrast to sexual combinations, in somatic fusion the genetic information of the cytoplasm is biparentally inherited. Therefore, it is relevant on the one hand to investigate the common parental plasmas and on the other hand to analyze the new genetic configurations of the hybrids plasmones.

Communicated by K. Glimelius

Andreas Lössl<sup>1</sup> (✉) · N. Adler · U. Frei · G. Wenzel  
Lehrstuhl für Pflanzenbau und Pflanzenzüchtung,  
Technical University München-Weihenstephan,  
D-85354 Freising, Germany  
Fax: + 49 8161 71 5173  
E-mail: loessl@mm.pbz.agrar.tu-muenchen.de

R. Horn  
Institute for Agronomy and Plant Breeding, JLU Giessen,  
Ludwigstrasse 23, D-35392 Giessen, Germany

Former address:

<sup>1</sup>Federal Centre for Breeding Research on Cultivated Plants,  
Institute for Resistance Genetics, D-85461 Grünbach,  
Germany

The influence of different cytoplasm on fusion-combining ability was described by Frei et al. (1998). First reports about field experiments with somatic hybrids and their agronomical value (Möllers et al. 1994; Schwarzfischer 1994) are currently available. In all cases variability between hybrid clones from the same parents is described. This variability can be attributed to both nuclear deviations and cytoplasmic rearrangements. The latter was analysed in detail in the genus *Brassica* by Morgan and Maliga (1987) and Walters and Earle (1993) who investigated the fate of organelles after cell fusion and found that they segregated independently from each other. In interspecific somatic fusion hybrids of the *Brassicaceae* completely new restriction fragments have also been detected (Sakai and Imamura 1992).

Within regenerants of potato, plastids were found to segregate completely into one of the parental types, whereas mitochondrial genomes showed various amounts of recombination (Lössl et al. 1994). Landgren and Glimelius (1990) suggested that most of these recombinations were due to the heteroplasmic status produced after cell fusion.

A thorough differentiation of the affected mitochondrial regions is expected to allow predictions about the effects of distinct cytoplasmic rearrangements. For this purpose it is necessary to obtain an overview about the different plasmids in cultivated potato and their behaviour when combined by fusion.

## Materials and methods

### Plant material

The 144 potato varieties were provided by German plant breeders. The fusion hybrids and the di-haploid material were obtained from the Institute for Resistance Genetics in D-85461 Grünbach. Fusion

population I [FAL2 (+) H86.601/1] consisted of 35 hybrids, population II [H77.421/2 (+) H88.1512/28] of 27 hybrids, population III [H89.2006/10 (+) H80.576/16] of 12 hybrids, and population IV [BP32 (+) H77.417/9] of 46 hybrids. The fusion hybrids have been checked for deviations in their nuclear genomes by using of a set of probes which, as shown by Lössl et al. (1994), covered the 12 chromosomes. The wild *Solanum* species *S. acaule*, *S. ajanhuiri*, *S. berthaultii*, *S. brevidens*, *S. bulbocastanum*, *S. capsibaccatum*, *S. chacoense*, *S. chaucha*, *S. circaefolium*, *S. curtilobum*, *S. gourlayi*, *S. kurtzianum*, *S. maglia*, *S. megistacrolobum*, *S. microdonum*, *S. multidissectum*, *S. phureja*, *S. polytrichon*, *S. spegazinii*, *S. stenotomum*, *S. stoloniferum*, *S. tarijense*, *S. tuberosum andigena*, *S. vernei*, and *S. verrucosum* were provided by the Institute for Plant Genetics and Cultivated Plant Research Gatersleben, D-18190 Groß Lüsewitz (The GLKS accession numbers can be supplied on request).

### Molecular techniques

In all steps of organellar DNA isolation and hybridization the procedures published by Lössl et al. (1994), based on the protocols of Saghai-Marooof et al. (1984), Kemble (1987) and Hosaka and Hannemann (1987), were followed. The methods of the latter have also been used for chloroplast-type differentiation. Sequencing was performed according to Sanger et al. (1977). The potato mtDNA probes can be supplied on request. Accession numbers for the mitochondrial sequences and probes employed are as listed in Table 1.

The cytoplasmic configurations were checked with specific PCR primers. The pair of primers used to detect the mt-type specific *atp6* copy were designed as follows: AL\_Mt1: [5'CAC AAA TCC ATC TTT GTT TAT GC 3'] or AL\_Mt2: [5'CGG TCT GGA ATT AGG TGT AGC 3'] were combined with AL\_Mt3: [5'GCG TTG GCT TAC AGC GAA ACT AG 3']. For mt-type differentiation at the *rps10* upstream region AL\_Mt4 and AL\_Mt5 were employed: AL\_Mt4: [5'AAT AAT CTT CCA AGC GGA GAG 3'] and AL\_Mt5: [5'AAG ACT CGT GAT TCA GGC AAT 3'] yielded a 2.4-kb mt type- $\alpha$  specific product differing from most other mt types (1624 bp). Optimal annealing temperature for mt primers was 57°C. To differ between cp-type T and cp-type S or W primers were combined as follows: AL\_Cp1 [5'TAG AAT CAG GAG GTC TT 3'] with AL\_Cp3 [5'TTA CTC ACG GCA ATC 3']. The optimal annealing temperature was 44°C.

**Table 1** Mitochondrial sequences and probes employed in the present analysis. GenBank entries are found under the listed accession numbers

Sequence	Length	Loci	GenBank accession no.
"atp6_1"	2444 bp	<i>nd1</i> (exon e), <i>atp6</i> (complete)	AF095276
"atp_2"	2195 bp	<i>nd1</i> (exon e), <i>atp6</i> (complete) with deviating 3' region	AF095277
"m100"	2787 bp	<i>rpl5</i> (3'portion), <i>rps14</i> (complete), <i>cob</i> (5'portion)	AF095274
"cob"	1519 bp	<i>cob</i> (5'portion)	AF095281
"rps14"	983 bp	<i>rps14</i> (3'portion) contained in "m100"	AF095274
"orf206"	672 bp	<i>orf206</i> (3'portion) and <i>atp9</i> homology	AF095280
"coxI"	2195 bp	<i>rps10</i> , part of <i>coxI</i> and repeat	AF095275
"nd3-rps12"	1649 bp	<i>nd3</i> (complete), <i>rps12</i> (2 exons, complete)	AF095279
"rpl2"	1594 bp	<i>rpl2</i> (complete) and repeats	AF095278
"rp_coxII"	2373 bp	<i>rps3</i> (3'portion), <i>rpl16</i> (complete), <i>coxII</i> (exon)	AF096321
"orf577"	740 bp	<i>orf577</i> (partial)	AF097743
"atpA"	2195 bp	<i>atpA</i> (heterologous)	EMBL:X53537
"ccb256"	2.0 kb	<i>ccb256</i> (5'-portion)	In preparation

For *in-organello* translation, mitochondria from potato tubers of different mt types were isolated by differential centrifugation and Percoll density gradient centrifugation. The mitochondrially encoded proteins were labelled by *in-organello* translation with  $^{35}\text{S}$ -methionine as described by Horn et al. (1991). The energy source was creatin phosphate. Protein synthesis was stopped after 90 min and aliquots were taken to estimate the incorporation of  $^{35}\text{S}$ -methionine. The mitochondria were washed and pelleted. For SDS-PAGE (15%) the mitochondria were solubilized in Laemmli sample buffer. Approximately equal amounts of TCA-precipitable radioactivity were loaded onto each lane. The gels were submitted to fluorography.

## Results

### Differentiation of different cytoplasmic types

The mt types of potato genotypes were classified as  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  by RFLP techniques using homologous organellar probes. These contained the loci of *cob*, *rps14*, *rpl5*, *rps10*, *coxI*, *rps3*, *rpl16*, *coxII*, *rpl2*, *ccb256*, *nd3*, *rps12*, *atpA*, *orf206* and *atp6*. The probes detected several coding regions and pseudogenes of the mt genome and revealed different organisations at these loci which characterize mainly the types mt- $\alpha$ , - $\beta$  and - $\gamma$ . Table 2 summarizes the data of the representative RFLP patterns with the standard set of probes which characterize the different mt types. The most characteristic patterns are shown in Fig. 1. From 144 tetraploid varieties 46 had mt-type  $\alpha$  (e.g. 'Adretta', 'Karlana', 'Ponto'), 79 mt- $\beta$  (e.g. 'Cilena', 'Desiree', 'Sieglinde'), and 19 mt- $\gamma$  (e.g. the cms cultivars 'Heidrun', 'Assia', 'Helios').

Mt- $\beta$  was linked to cp-type T and differed by at least seven probes from mt- $\alpha$  and mt- $\gamma$ . Mt- $\alpha$ , mt- $\gamma$  and mt- $\delta$  were found in combination with cp-type W, and mt- $\epsilon$  was connected to cp-type S. There was only one exception from this classification (cv 'Mira'). Mt- $\delta$  (e.g. 'H80.576/16') and mt- $\epsilon$  (e.g. 'H77.417/9') were only found in di-haploid clones.

In order to characterize these main mt types, their RFLP patterns have been compared to the related *Solanum* wild species. It was necessary to gain an overview about the different phylogenetic branches for the subsequent selection of fusion combinations. Populations should derive preferably from combinations with far-related cytoplasmic types. For this purpose the fragment patterns which were generated by the probes were used to perform a cluster analysis and to calculate the relationships of  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  to the *Solanum* wild species. According to the dendrogram the mt- $\alpha$  and mt- $\gamma$  type were closely related to the *S. stoloniferum* and *S. demissum* mt genomes (Fig. 2). Mt- $\beta$  is defined as the *S. tuberosum* mt genome and has some similarity to that of *S. berthaultii*. Mt- $\epsilon$  is related to the *S. andigena*, *goniocalyx*, *phureja*, *curtilobum*, *multidissectum*, *ajanhuiri* and *chauca* mt genomes. Mt- $\beta$  had a genetic distance of 0.3 to mt- $\alpha$  and mt- $\gamma$ , whereas both had a distance of 0.4 to mt- $\epsilon$ .

### Variation of mitochondrial expression in fusion parents and hybrids

The mt types were not only compared at the DNA level but also at the protein level. Putative differences in the physiology of distantly related mt types could be relevant for inter-organellar compatibility in somatic hybrids. In order to evaluate the effect of the different cytoplasmic types on their expression level, *in organello* translations were performed with di-haploid potato genotypes with mt genomes  $\alpha$ ,  $\beta$  and  $\epsilon$ . No differences could be found between  $\alpha$  and  $\beta$  mitochondrial expression. But in the distantly related  $\epsilon$  mitochondria an additional mitochondrial translation product of approximately 15 kDa was synthesised. It also appeared in somatic hybrids from an [ $\alpha$ (+) $\epsilon$ ] fusion containing different plastid-mitochondrial configurations (cp-type S and cp-type W). Fusion hybrids between  $\alpha$  and  $\beta$  did not express this protein (Fig. 3).

### Analysis of hybrid cytoplasmic genomes

Four selected populations of somatic hybrids were analysed: population I was a fusion between parents containing  $\alpha$  and  $\beta$  mt genomes, population II of parents with  $\alpha$  and  $\gamma$  mt genomes, population III of parents with  $\alpha$  and  $\delta$ , and population IV with  $\alpha$  and  $\epsilon$ . According to a previous analysis with nuclear probes, within each population the hybrids possessed nearly identical nuclear genomes but were characterised by different cytoplasmic types.

### Plastid-type segregation

In the analyzed varieties and the di-haploids the mt type was tightly coupled with the cp type whereas in somatic hybrids the strict assortment of cp type and mt type was lacking. The cp types combined independently with the novel mt types of somatic hybrids. Plastid segregation could only be observed in hybrid populations I and IV, differing for the parental cp type. In somatic hybrid population I the plastids segregated completely in all hybrids into either parental-type W or T in the ratio of 1:6. In population IV they segregated into the cp-types W and S in the ratio of 1:1. Mixtures of, or recombinations in, their cp genomes have not been observed. With the above given PCR primers, cp-type segregation was found to be complete (Fig. 4).

### Chondriome-type segregation

Hybrid plants of populations I–IV were compared with probes differentiating between their parental mt types (Table 2); for example mt- $\alpha$  and - $\epsilon$ . The initial comparison of restriction fragment patterns revealed a division

**Table 2** Characteristic fragment patterns of mt-type  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\varepsilon$  following hybridization with homologous mtDNA probes. The restriction enzymes used for Southern blots are given. Probes can be provided on request

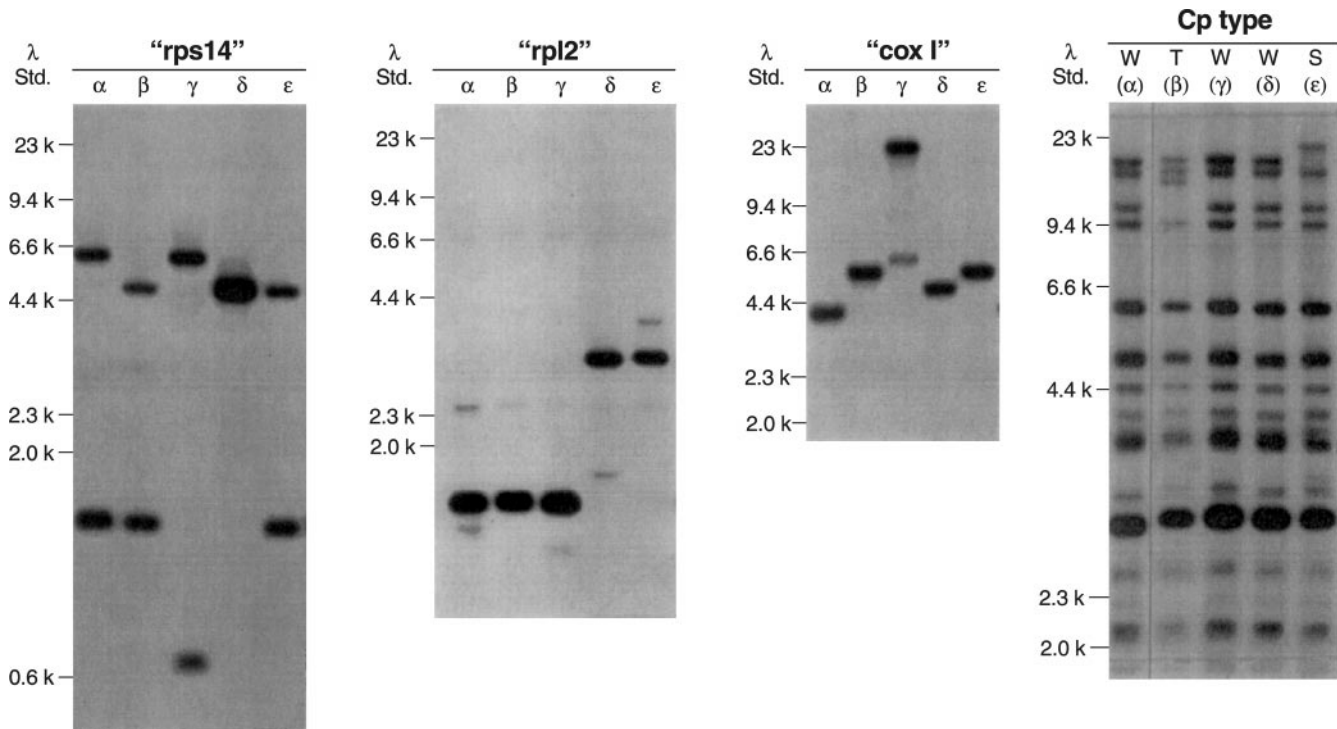
Probed mt locus	DNA digest	Length of fragments (kb)				
		Mt type				
		$\alpha$	$\beta$	$\gamma$	$\delta$	$\varepsilon$
"rps14"	<i>EcoRI</i>	-	-	0.7	-	-
		1.2	1.2	-	-	1.2
		-	4.5	-	4.5	4.5
		-	-	-	4.7	-
		5.5	-	5.5	-	-
"rpl2"	<i>HindIII</i>	-	-	1.2	-	-
		1.3	-	-	-	-
		1.5	1.5	1.5	-	-
		-	-	-	1.8	-
		-	-	-	3.3	3.3
"rpl2"	<i>EcoRI</i>	-	-	-	-	3.8
		0.7	0.7	0.7	-	-
		-	-	-	-	1.1
		-	-	-	1.3	1.3
		-	-	-	-	3.6
"coxI"	<i>EcoRI</i>	3.8	3.8	3.8	3.8	-
		4.2	-	-	-	-
		-	5.0	-	-	5.0
		4.4	-	-	-	-
		-	-	-	4.8	-
"cob"	<i>EcoRI</i>	-	5.2	-	-	5.2
		-	-	6.0	-	-
		-	-	25	-	-
		1.4	1.4	1.4	1.4	1.4
		1.8	-	-	-	-
"ccb256"	<i>BamHI</i>	-	5.0	-	-	5.0
		3.8	3.8	3.8	-	3.8
		-	-	-	5.0	-
"orf206"	<i>EcoRI</i>	6.0	-	6.0	-	6.0
		-	15	-	-	-
		-	0.8	-	-	-
"rp_coxII"	<i>HindIII</i>	1.8	-	1.8	-	1.8
		-	-	-	-	-
		7.5	7.5	7.5	7.5	7.5
"nd3-rps12"	<i>HindIII</i>	2.4	2.4	2.4	2.4	2.4
		3.1	-	-	-	-
"atpA" heterolog.	<i>EcoRI</i>	-	-	-	-	1.4
		3.0	3.0	3.0	3.0	3.0
		-	-	-	-	5.4
		6.6	6.6	6.6	6.6	6.6
"atpA" heterolog.	<i>EcoRI</i>	-	4.7	-	4.7	-
		5.5	-	5.5	-	5.5
		-	-	-	-	-

into hybrids of mt-type  $\alpha$  or  $\varepsilon$ , similar to plastid segregation. However, mixed patterns of mt- $\alpha$  and - $\varepsilon$  appeared as well (data not shown). These mixtures were restricted to single areas within the mt genomes, whereas the observation of other mt-genome sites of the hybrid plant concerned demonstrated clear assortment (even with the highly sensitive PCR primers AL\_Mt4, and AL\_Mt5). Hybridisations with different probes often revealed different segregation patterns within the same hybrid

chondriome. Two phenomena have been observed frequently: amplification of sub-stoichiometric mtDNA and partial addition of parental mtDNA fragments.

#### Amplification of sub-stoichiometric mtDNA

In contrast to the plastome, the hybrid chondriomes could also show patterns which deviated from the



**Fig. 1** The different chondriome types as probed with “rps14”, “coxI” and “rpl2” in comparison to their cp type. The autoradiographs show that potato mt genomes can be categorized into at least five types: mt-Types  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ ; (Std: standard Lambda DNA, cut with *Hind*III). Hybridizations were performed with blots of *Eco*RI-cut DNA of different mt types, ‘rpl2’ was hybridised with a *Hind*III-blot. Plastid types are labelled ‘W’, ‘T’ and ‘S’ according to Hosaka and Hannemann (1988). They were determined by probing *Bam*HI-cut DNA with total cpDNA

parental types by a very high amplification of distinct mt genome regions. This was the case for an additional *atp6*-homologous sequence. The duplication differed from the original *atp6* locus (“*atp6\_1*”) in the termination region 211 bp downstream from the stop codon (“*atp6\_2*”).

PCR-tagging with the primers AL\_Mt2 and AL\_Mt3 revealed that the duplicated reading frame pre-exists in the mt- $\alpha$  and mt- $\gamma$  genomes in sub-stoichiometric amounts. This locus is thus characteristic for mt-types  $\alpha$  and  $\gamma$ . The typical *Tuberosum* cytoplasm of mt- $\beta$  normally lacks this fragment, as shown in Fig. 5.

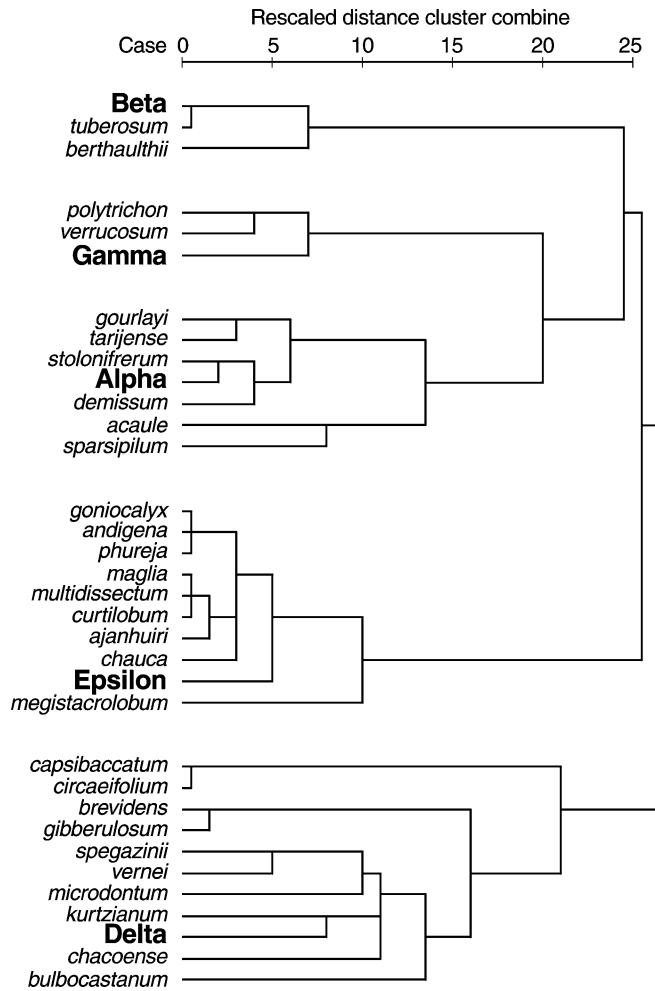
By hybridization with the homologous probe containing *atp6*, the prominent additional fragment becomes visible in 10% of the somatic hybrids but not in their parents. Hybrids affected from this unregulated replication were found in population II [ $\alpha$  (+)  $\gamma$ ] and population IV [ $\alpha$  (+)  $\epsilon$ ]. An example of this occurrence is given in Fig. 6. The same organisation was also found in the far-related wild species *S. polytrichon* and *S. verrucosum*.

Novel fragment patterns have also been detected in hybrids of population II [ $\alpha$  (+)  $\gamma$ ] and population III [ $\alpha$  (+)  $\delta$ ] by hybridization with “coxI” and “rpl2”, two probes which were useful for the detection of mtDNA rearrangements in somatic hybrids. These novel genome compositions only appeared in somatic fusion hybrids which derived from parents with different mt types.

#### Partial addition of parental mtDNA fragments

Usage of the single probe “m100” for differentiation in somatic hybrids detected homogeneous hybrid mt genomes with a restriction pattern similar to their parents, and also several inhomogeneous hybrid mt genomes. Figure 7 illustrates the mutual exchange between the fragment patterns of the  $\alpha$  and  $\beta$  mt genomes. This kind of partial addition of parental fragment patterns was found in hybrids of populations I, II and IV. Hybrids which exhibited these novel mt-genome patterns were classified into the groups “R1” and “R2”. So far no cleavage sites have been detected for these exchanges of mt-genome fragments.

The well-analysed *Bam*HI cloned fragment “m100” showing this occurrence in population I contains the gene cluster *rpl5-rps14* and *cob*. Probe “m100” detected two different RFLPs between the parental mt genomes. The first RFLP was located in the *rps14* upstream flanking region and the second was due to a pseudocopy of *cob*, flanking the *rps10-coxI* gene. This

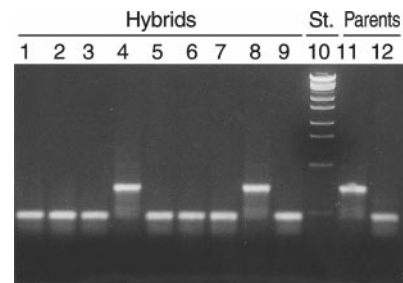
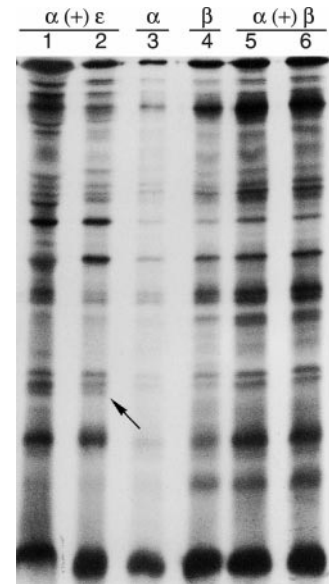


**Fig. 2** Dendrogram of the genetical distances between different *Solanum* mtDNAs. The genetical distances were calculated with a model of Euclidian distance. Distances between clusters were calculated by the method 'Average Linkage between Groups'. RFLP data derive from hybridisation with mt probes containing *cob*, *rps14*, *rpl5*, *coxI*, *rpl2*, *ccb256*, *nd3*, *rps12*, *atpA*, *atp6* and *orf206*

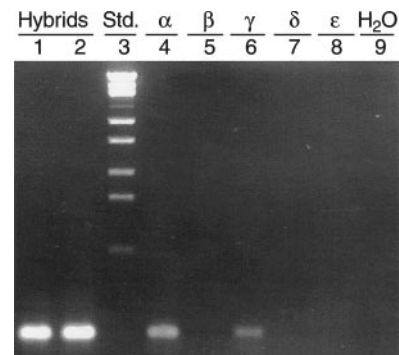
can be proven by hybridization with a subfragment of "m100", involving "rps14" and the probe "cob", which characterized the fusion-parents chondriomes at these loci. A confirmation of this mt-genome organization is possible by hybridization with "coxI", which revealed the same segregation pattern as "cob".

Sequence analysis of the "coxI" probe revealed a sequence which is also present in the polymorphic probe "rpl2". Comparison of "rpl2" with the database for *Nicotiana sylvestris* (Vitart et al. 1992) brought to light a homology of 404-bp of recombinant mtDNA beginning 100 bp upstream of *rpl2*. Both probes "rpl2" and "coxI" share a homology of 91 bp with a recombination repeat region of *Petunia* (Conklin and Hanson 1993).

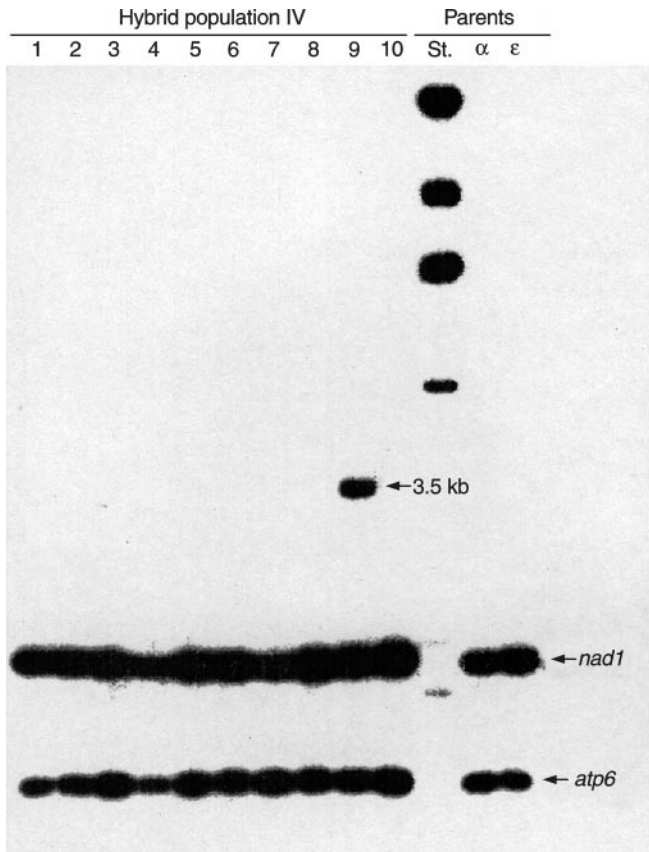
**Fig. 3** Different mitochondrially encoded translation products. At the mitochondrial expression level hybrids from an  $[\alpha (+) \epsilon]$  fusion (lanes 1, 2) could be distinguished by *in-organello* translation from  $[\alpha (+) \beta]$  hybrids (lanes 5, 6) and di-haploids (lanes 3, 4). The characteristic additional translation product of 15 kDa deriving from mt-type  $\epsilon$  is marked by an arrowhead. The  $[\alpha (+) \epsilon]$  hybrids differed in their plastid genomes (lane 1: cp-type S, lane 2: cp-type W)



**Fig. 4** Complete cp-type segregation shown by PCR. The reaction with AL\_Cp1 and AL\_Cp3 yields a 622-bp PCR product. Cp-type T differs from cp-type W by a fragment which is 241-bp shorter (lanes 11: cp W and 12: cp T). Plastid-type segregated completely within hybrids of fusion population I (lanes 1-9). (St., lane 10: standard Lambda DNA, cut with *Eco130I*). The PCR products were separated on a 1.5% agarose gel stained with ethidium bromide



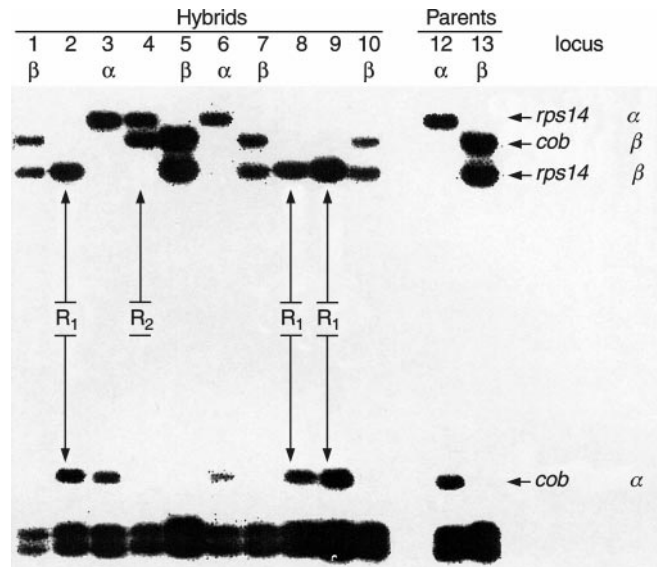
**Fig. 5** A PCR-tagged additional *atp6* copy. By using a PCR product of 623 bp the primer combination AL\_Mt2 and AL\_Mt3 made visible the additional *atp6* copy which is present in a large amount in some somatic hybrids of population IV (lanes 1 and 2). In the parental genomes mt- $\alpha$  (lane 4) and mt- $\gamma$  (lane 6) this organization of *atp6* pre-exists in sub-stoichiometric amounts. (St., lane 3: standard Lambda DNA, cut with *Eco130I*). The PCR products were separated on a 1.5% agarose gel stained with ethidium bromide



**Fig. 6** An additional fragment containing *atp6*. Hybrids of fusion population IV (lanes 1–10), (St: standard Lambda DNA, cut with *Hind*III) and parents (lanes 12, 13) cut with *Eco*RI, hybridised with probe “atp6\_1”. In the deviating hybrid (lane 9) the blot makes visible a 3.5-kbp fragment, containing an additional *atp6* gene with sequence deviation beginning 211-bp 3' from the stop codon. The signals of the lower bands represent the normal *atp6/nad1* fragments

## Discussion

This rather comprehensive study of the various potato mt genomes serves as a basis for the characterization of newly generated cytoplasmic following somatic hybridization. Cytoplasmic analysis of the parents used for protoplast fusion allowed us to recognize differences within a series of potato clones in the organisation of their organellar genomes. Using mt-DNA probes the potato cultivars and di-haploid clones could be grouped according to their mt types  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ . Accessory mt types, which can be detected by the use of additional mt probes, are to be updated on website (<http://www.edv.agrar.tu-muenchen.de/pbpz/mm/mt/all.htm>). Potato plastome-type W was mostly found in combination with mt types  $\alpha$  as well as  $\gamma$  and  $\delta$ . Mt-type  $\beta$  was the only chondriome in linkage with cp-type T, reflecting a co-evolution of both organelles and a common uniparental inheritance. A similar relationship



**Fig. 7** Partial addition of parental mtDNA leading to novel mt-types R1 and R2. The autoradiography shows signals of probe “m100” with total DNA from hybrids cut with *Eco*RI (lanes 1–10). The probe contains the *rpl5*, *rps14* and *cob* genes. Lanes 12 and 13 represent the fusion parents mt-types  $\alpha$  and  $\beta$ . The different banding patterns detect the origin,  $\alpha$  or  $\beta$ , of the corresponding genome regions in the hybrids. R1 and R2 identify the generated recombination types

between plastome and chondriome was observed for the *Chenopodiaceae* by Samitou-Laprade et al. (1991).

According to their percentages, mt-types  $\alpha$  and  $\gamma$  are the most common ‘wild’-type cytoplasmic in cultivated potato in Germany. The almost extinct ancient *Andigena* mt type is positioned within the  $\epsilon$  cluster, showing a high genetic distance to common potato mt types. Differences between the *Andigena* and *Tuberosum* chloroplast-encoded RuBisCo large subunits were reported by Gatenby and Cocking (1978). The di-haploid fusion parent ‘H77.417/9’ containing the *Andigena*-related mt-type  $\epsilon$  differed from mt- $\alpha$  and - $\beta$  in its mitochondrial translation products. The additional mt- $\epsilon$ -specific 15 kDa protein was detected in [ $\alpha$  (+)  $\epsilon$ ]-fusion hybrids containing cp-type S and cp-type W. *In-organello*-translation demonstrated that novel mitochondrial configurations in fusion hybrids can have an effect on the expression level independent of the cp type.

Variant expression of organellar genomes could be a reason for the incompatibilities between cellular compartments. The additional 15-kDa protein is an indication of the physiological differences between the cytoplasmic used in potato breeding. The presence of additional mt proteins is also a possible source of phenotypic variation in somatic hybrids.

Analysis of cytoplasmic organelle components within somatic hybrid populations revealed large differences between the hybrids with respect to their cp and

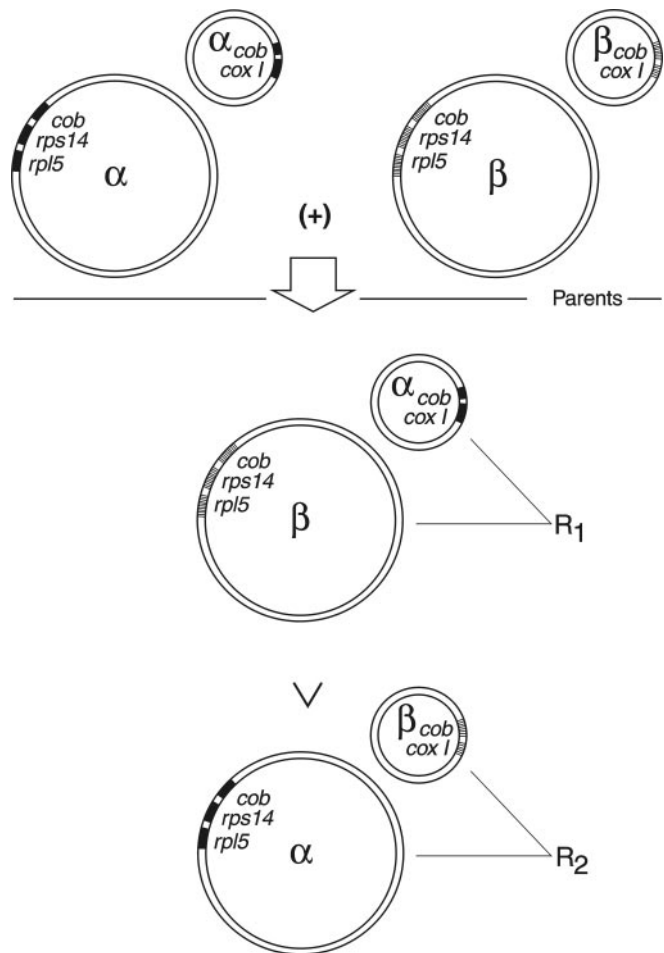
mt genomes, whereas at the nuclear level they were nearly identical. The skewed segregation ratio after the regeneration phase of 1:6 in cp-types W and T was a first indication of cytoplasmic effects in fusion population I. This deviation from random plastid segregation could be due to different frequencies of replication and organelle division (Glimelius et al. 1981; Donaldson et al. 1994) and is indicative of a higher performance for cp-type T during the *in-vitro* regeneration phase (Schweis 1992).

In the analysed populations I, II, III, IV the segregated plastid genomes did not show any recombination and were highly conserved, whereas mitochondrial genomes of the hybrids revealed novel DNA organisations. In hybrids between *Nicotiana tabacum* and *S. tuberosum* Thanh and Medgyesy (1989) demonstrated that, under stringent conditions, even the plastid genome recombined, overcoming nuclear-cytoplasmic incompatibility. Horvath et al. (1992) suggested that the recombinations involved are processes of adaptation, which improve the nuclear-cytoplasmic interaction.

In different fusion populations a series of identical mitochondrial rearrangements have been found which might be due to a particular selection pressure. In this context Kemble et al. (1986) discussed “hot spots”, sequences which tend to a high recombination activity. The accumulation of an additional *atp6* gene in some hybrids probably derives from such a “hot spot”. This could be explained by so-called ‘sublimons’ according to Leaver et al. (1988): these circular DNA molecules, found in sub-stoichiometric amounts in sorghum, maize, sunflower, artichoke and *Nicotiana*, can integrate into a master circle and become visible by a selective amplification. We have found a large sequence homology of the polymorphic probes “rpl2” and “coxI” to the sub-stoichiometric recombinant mtDNA of *N. sylvestris* (Vitart et al. 1992) and to a *Petunia hybrida* repeat region (Conklin and Hanson 1993). These repeats are believed to serve as branching points for re-organization of the mt genome. Another example is found in the potato-specific small repeat: “GAGAG-GGAGCCACTTGACTGTAAGGAGAG” within the *coxII* intron region which, with a slight variation, is inserted in at least at two other sites, the *coxI* 3' region and *orf577*.

Rearrangements after the fusion process could be triggered by inverted repeats dispersed within the genome. In evolution, and probably in somatic hybrids as well, the process of excision and insertion elsewhere results in novel conformations which are rarely found in conventionally bred clones.

In the present study the rearrangements generated novel configurations between plastomes and chondriomes, such as mt-types R1 and R2, which were not observed in the mt genomes of 200 different clones representing distinct evolutionary branches. Moreover, in view of the multiple copies of ‘normal’ mt chromo-



**Fig. 8** Model of parental mt genomes  $\alpha$  and  $\beta$  and recombinant hybrid mt genomes R1 and R2. Probe “m100” shows new rearrangements, which simultaneously affect the gene *rps14* and copies of the *cob* (next to *rps10* and *coxI*) gene. After protoplast fusion parts of mitochondrial genome type  $\alpha$  are re-placed by sub-genomes from chondriome type  $\beta$  and vice versa. Fusion hybrids with deviating mitochondrial types show a recombinant pattern. R1 and R2 have no cleavage sites for the recombination event. The different parental mt sub-genomes are not necessarily integrated in the main genome. They can be exchanged independently with the other genome parts

somes in a single cell, it remains unknown precisely how a distinct recombinant mt type asserts itself during the regeneration phase.

The results show that both parents can contribute to the new mt genome of the hybrid regenerant. Partial addition of parental mt genome regions indicates that subunits of the mitochondrial chromosome coexist with the main chromosome of these organelles. At least during the reorganisation of the mt genome after the fusion process these subgenomes seem to be excluded from the master circle, so that they can be exchanged independently from the mt main chromosome. The appearance of sub-circles, which are often generated by recombination, is the result of homologous recombination between sequence repeats (Newton 1988). If these



repeats are not located in an inverted direction they can initiate the replication of two sub-genomes (Schuster and Brennicke 1994). Repeated sequences and pseudocopies of mitochondrial reading frames are due to these events. Pseudogenes of *rps14* and *cob* in potato have been reported by Quiñones et al. (1996), and the homology of the probes “*rpl2*” and “*coxI*” to the recombination repeat of *Petunia* (Conklin and Hanson 1993) is possibly involved in the reorganization of hybrid mt genomes. In view of the various chondriome types observed within a potato fusion hybrid population the parental and progeny mt genomes are supposed to be organized in a manner similar to the schematic model given in Fig. 8. The mixtures and partial additions provide an indication of the exchanges of mt-genome units which apparently exist in the structure of sub-genomes. Somatic hybridization combines the  $\alpha$  and  $\beta$  mt sub-genomes into a new composition. This form of organisation could explain the new constitution of hybrid types R1 and R2 and could also explain the mixtures between different loci which can be found in some hybrids. But a pre-condition for this preliminary model is a multipartite structure of the potato mt genome, as postulated for example in maize by Fauron et al. (1989).

Summarizing the results of our hybrid analysis, we have found that in somatic hybrids the replication of the mt genome was affected by the irregular amplification of distinct mtDNA regions. Partial addition of parental mt-genome regions took place in the neighbourhood of repeated sequences. Other than in parental cytoplasm the strict assortment of cp type and mt type was discontinued and cp types combined independently with the novel mt types. In this study we did not focus on any phenotypic consequences, e.g. sterility, but, in general, hybrids with homogeneous chondriomes exhibited a higher vigour than hybrids affected by extreme recombinations. In this respect various cp-mt configurations in fusion populations of types I-IV need to be further evaluated in field experiments.

**Acknowledgements** The authors are grateful to Mr. A. Barth, Mrs. E. Gerick and Mrs. M. Ammerseder for technical assistance and to Dr. K. Schüler for providing material from the gene bank. This work was supported by grants of the BMBF under the number 0310768.

## References

- Austin S, Ehlenfeld MK, Baer MA, Helgeson JP (1986) Somatic hybrids produced by protoplast fusion between *S. tuberosum* and *S. brevidens*: phenotypic variation under field conditions. *Theor Appl Genet* 71: 682–690
- Chaput MH, Sihachakr D, Ducreux G, Marie D, Barghi N (1990) Somatic hybrid plants produced by electrofusion between dihaploid potatoes: BF 15 (H1), Aminca (H6), Cardinal (H3). *Plant Cell Rep* 9: 411–414
- Conklin PL, Hanson MR (1993) A truncated recombination repeat in the mitochondrial genome of a *Petunia* CMS line *Curr Genet* 23: 477–482
- Deimling S, Zitzelsperger J, Wenzel G (1988) Somatic fusion for breeding tetraploid potatoes. *Plant Breed* 101: 181–189
- Donaldson PA, Bevis E, Pandaya RS, Gleddie SC (1994) Random chloroplast segregation and frequent mtDNA rearrangements in fertile somatic hybrids between *Nicotiana tabacum* L. and *N. glutinosa* L. *Theor Appl Genet* 87: 900–908
- Fauron CM-R, Havlik M, Rettel RIS (1989) The mitochondrial genome organization of a maize fertile cms T revertant line is generated through recombination between two sets of repeats. *Genetics* 124: 423–428
- Frei U, Stattmann M, Lössl A, Wenzel G (1998) Aspects of fusion combining ability of di-haploid *S. tuberosum* L.: influence of the cytoplasm. *Potato Res* 41: 155–162
- Gatenby AA, Cocking EC (1978) Fraction-I protein and the origin of the european potato. *Plant Sci Lett* 12: 177–181
- Glimelius K, Chen K, Bonett T (1981) Somatic hybridization in *Nicotiana*: segregation of organellar traits among hybrid and cybrid plants. *Planta* 153: 504–510
- Hofferbert, RH (1996) Erstellung und Selektion dihaploider Genotypen zur gezielten Kombinationszüchtung bei Kartoffeln. PhD thesis, Technische Universität München
- Horn R, Köhler R, Zetsche K (1991) A 16-kDa protein is associated with cytoplasmic male-sterility in sunflower. *Plant Mol Biol* 17: 29–36
- Horvath G, Thanh N, Rusu A, Raschova L, Medgyesy P (1992) Nucleus-mitochondrion incompatibility drives the DNA recombination-mediated adaption of the mitochondrial genome. HFSP-Workshop, Interaction of three genomes, S. 115, Berlin, 1992
- Hosaka K, Hannemann RE (1987) A rapid and simple method for determination of chloroplast DNA type. *Am Potato J* 64: 345–352
- Hosaka K, Hannemann RE (1988) The origin of the cultivated tetraploid potato based on chloroplast DNA. *Theor Appl Genet* 76: 172–176
- Kemble RJ (1987) A rapid, single-leaf, nucleic assay for determining the cytoplasmic organelle complement of *Brassica* species. *Theor Appl Genet* 73: 364–370
- 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
- Landgren M, Glimelius K (1990) Analysis of chloroplast and mitochondrial segregation in three different combinations of somatic hybrids produced within the *Brassicaceae*. *Theor Appl Genet* 80: 776–784
- Leaver CJ, Isaac PG, Small ID, Bailey-Serres J, Liddell AD, Hawkesford MJ (1988) Mitochondrial genome diversity and cytoplasmic male sterility in higher plants. *Phil Trans R Soc Lond* 319: 165–176
- Lössl A, Frei U, Wenzel G (1994) Interaction between cytoplasmic composition and yield parameters in somatic hybrids of *S. tuberosum* L. *Theor Appl Genet* 89: 873–878
- Möllers C, Wenzel G (1992) Somatic hybridization of diploid potato protoplasts as a tool for potato breeding. *Bot Acta* 105: 133–139
- Möllers C, Frei U, Wenzel G (1994) Field evaluation of tetraploid somatic potato hybrids. *Theor Appl Genet* 88: 147–152
- Morgan A, Maliga P (1987) Rapid chloroplast segregation and recombination of mitochondrial DNA in *Brassica* cybrids. *Mol Gen Genet* 209: 240–246
- Newton KJ (1988) Plant mitochondrial genomes: organization, expression and variation. *Annu Rev Plant Physiol Plant Mol Biol* 39: 503–532
- Quiñones V, Zanlungo S, Moenne A, Gomez I, Holuigue L, Litvak S, Jordana X (1996) The *rpl5-rps14-cob* gene arrangement in *Solanum tuberosum*: *rps14* is a transcribed and unedited pseudogene. *Plant Mol Biol* 31: 937–943
- Saghai-Marooof MA, Soliman KM, Jörgensen RA, Allard RW (1984) Ribosomal spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc Natl Acad Sci USA* 81: 15–20

- Sakai T, Imamura J (1992) Alteration of mitochondrial genomes containing *atpA* genes in the sexual progeny of cybrids between *Raphanus sativus* and *Brassica napus* cv Westar. *Theor Appl Genet* 84:923–929
- Sanger F, Nicklen S, Coulson (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463
- Samitou-Laprade P, Pannenbecker G, Boutin-Stadler V, Michaelis G, Vernet P (1991) Plastid DNA diversity in natural populations of *Beta maritima* showing additional variation in sexual phenotype and mitochondrial DNA. *Theor Appl Genet* 81:533–536
- Schuster W, Brennicke A (1994) The plant mitochondrial genome: physical structure, information content, RNA editing, and gene migration to the nucleus. *Annu Rev Plant Physiol Plant Mol Biol* 45:61–78
- Schwarzfischer A (1994) Use of somatic hybrids in an applied breeding programme. *Eucarpia report 1994, Potato Res* 37:346–347
- Schweis A (1992) Quantitative Klassifizierungs und Segregationanalysen nach somatischer Hybridisierung und Cybridisierung bei Kartoffeln. PhD Thesis, Eberhard-Karls Universität Tübingen
- Thach NQ, Frei U, Wenzel G (1993) Somatic fusion for combining virus resistances in *Solanum tuberosum* L.. *Theor Appl Genet* 85:863–867
- Thanh ND, Medgyesy P (1989) Limited chloroplast gene transfer via recombination overcomes plastome-genome incompatibility between *Nicotiana tabacum* and *Solanum tuberosum*. *Plant Mol Biol* 12:87–93
- Vitart V, De Paepe R, Mathieu C, Chetrit P, Vedel F (1992) Amplification of substoichiometric recombinant mitochondrial DNA sequences in a nuclear, male-sterile mutant regenerated from protoplast culture in *Nicotiana sylvestris*. *Mol Gen Genet* 233:193–200
- Waara S, Tegelstorm H, Wallin A, Eriksson T (1989) Somatic hybridization between anther-derived dihaploid clones of potato (*Solanum tuberosum* L.) and the identification of hybrid plants by isozyme analysis. *Theor Appl Genet* 77:49–56
- Walters TW, Earle ED (1993) Organellar segregation, rearrangement and recombination in protoplast fusion-derived *Brassica oleracea* calli. *Theor Appl Genet* 85:761–769
- Wenzel G, Schieder O, Prezewzny T, Sopory SK, Melchers G (1979) Comparison of single-cell-culture-derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. *Theor Appl Genet* 55:49–55