Theor Appl Genet (2000) 101:336–343

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Analysis of the plastome and chondriome origin in plants regenerated after asymmetric *Solanum* ssp. protoplast fusions

Received: 7 October 1999 / Accepted: 3 December 1999

Abstract Protoplasts from potato cultivars used as recipient parents were fused with irradiated protoplasts from wild *Solanum* donor species. Regenerated plants were analysed by RAPDs to identify hybrids. Irradiation of donor protoplasts with ionizing irradiation induced a broad range of donor nuclear DNA elimination in the asymmetric hybrids. Usage of chloroplast (cp)- and mitochondrial (mt)-specific PCR markers made it possible to trace the different origins of the cp genome in seven fusion combinations, as well as the mt genomes in two fusion combinations. Regenerated plants with recipient nucleus and plastome markers from the donors were found in six of the seven analysed fusion combinations. Protoplast fusion has generated novel mt genome combinations consisting of different portions of the mt genomes from the fusion partners. Selection of heterofusion products based on fluorescence markers is an efficient method to obtain asymmetric *Solanum* hybrids and cybrids from most fusion combinations. Possible models for cybrid formation are discussed.

Key words Potato · Mitochondria · Chloroplast · Asymmetric hybrids · Cybrids

Introduction

Intraspecific protoplast fusion (Rasmussen et al. 1998) and interspecific protoplast fusion (reviewed by Waara and Glimelius 1995) are supplements to conventional breeding within certain plant families. Fertility and crossing barriers can be bypassed and agronomically important traits can be combined by protoplast fusion (Thach et al. 1993; Rasmussen et al. 1996). Interspecific protoplast fusion between *Solanum tuberosum* and tuber-bearing (Serraf et al. 1991; Mattheij et al. 1992) and non-tuber bearing wild *Solanum* species (Austin et al. 1985) has resulted in somatic hybrids with new traits. In order to get rid of unfavourable wild-species traits in the somatic hybrids a number of backcrossings to the cultivated potato are needed (Mattheij et al. 1992). A method to reduce the number of backcrossings by reducing the amount of genomic DNA from the wild species in the hybrid is asymmetric protoplast fusion. Asymmetric protoplast fusion has been used to introduce fragments of the nuclear genome from one species (donor) into the intact genome of another species (recipient) (Sjödin and Glimelius 1989; Rasmussen et al. 1997; Liu et al. 1999). The fragmentation of the donor genome can be induced by UV irradiation (Forsberg et al. 1998) or ionizing irradiation (Sidorov et al. 1987; Rasmussen et al. 1997) of the donor protoplasts prior to protoplast fusion. Asymmetric hybrids with a pathogen resistance from the donor species has been obtained in a fusion between *Brassica napus* recipients and *Phoma lingam*-resistant *Brassica* accessions (Sjödin and Glimelius 1989).

Many asymmetric protoplast fusion protocols have been developed in order to produce potato cybrids with new nuclear-cytoplasm combinations (Perl et al. 1991; Melchers et al. 1992; Sidorov et al. 1994). Analysis of the plastome background in European potato breeding cultivars revealed that the β-type chondriome only occurred in combination with T-type chloroplasts whereas chloroplast type W was found in combination with the three chondriome types α, γ and δ (Lössl et al. 1999). The cytoplasm-type of the fusion parents is found not to be a suitable marker for predicting the fusion combining ability (FCA) in intraspecific symmetric fusion experiments (Frei et al. 1998). The generation of cybrids between wild *Solanum* species and cultivated potato genotypes may provide breeding material with new nuclear–chondriome–plastome combinations. This is

Communicated by R. Hagemann

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due to the biparental combination of the chloroplasts and mitochondria in somatic hybrids in contrast to the maternal inheritance by sexual hybridization. Analysis of cybrids could be a tool to find valuable sources of cytoplasm among the wild potato species, by using the nuclear background from well-characterized potato cultivars. New plastome–chondriome combinations may be useful when integrating wild *Solanum* species into a breeding programme due to new nucleus-cytoplasm interactions.

The bottleneck in asymmetric protoplast fusion experiments is the selection of the asymmetric hybrids and cybrids. Selection has been based on selectable genes in transgenic donor plants (Fehér et al. 1992), resistance to a fungal toxin (Sjödin and Glimelius 1989), the phenotype of regenerated shoots (Xu et al. 1993), nuclear or cytoplasmic mutants (Sidorov et al. 1987; Gleba et al. 1988), chemical inactivation of cytoplasm (Perl et al. 1991; Melchers et al. 1992), the selection of induced cytoplasmic mutations (Sidorov et al. 1994) and more recently on relative DNA content estimated by flow cytometry (Oberwalder et al. 1998). We have previously described two protocols to isolate and identify asymmetric *S. tuberosum*+wild *Solanum* ssp. nuclear hybrids based on the selection of fluorescent markers either by micromanipulation or by fluorescence-activated cell sorting (FACS) (Rasmussen et al. 1997). These two selection methods provide the ability to produce asymmetric hybrid plants between a broad range of fusion partners. The only constraint to the donor is the ability to isolate protoplasts.

In this paper we describe the production of asymmetric hybrids from two fusion combinations using the protocol developed in order to select the heterofusion products by micromanipulation. The aim of this study is to analyse the plastome–chondriome combination in plants regenerated from asymmetric heterofusion products. Regenerated plants, previously described in Rasmussen et al. (1997), and regenerated plants from the present study were subjected to an analysis of their cytoplasmic origin. This analysis is important in order to fully exploit the asymmetric protoplast fusion products. The perspective of this work is to transfer resistance traits from wild *Solanum* species, along with a limited amount of wild-species DNA, into cultivated potato cultivars.

Materials and methods

Plant material

Regenerated plants (see Table 2) from five recipient – donor protoplast fusion combinations with the recipients *S. tuberosum* cv Matilda (2n=4*x*=48), cv Folva (2n=4*x*=48), cv 161:14 (2n=2*x*=24) and the wild tuber bearing donor species *Solanum spegazzinii* clone V7 (2n=2*x*=24) and *Solanum microdontum*×*Solanum vernei* clone V8 (n=2*x*=24) have been described previously (Rasmussen et al. 1997). Two new asymmetric fusion combinations with the recipients *S. tuberosum* cv Kiva (2n=4*x*=48) and cv Godiva (2n=4*x*=48) and the wild donor species *Solanum multiinterruptum* clone V577 (2n=2*x*=24) are analysed in the present study (see Table 2). All parental plants were kindly donated by the Danish Potato Breeding Foundation, Vandel, Denmark. Donor protoplasts were irradiated by γ-rays from Cs^{137} giving a dose rate of 2.6 Gy/min. Protoplasts were irradiated for 58 min, giving a dose of 150 Gy. Heterofusion products were selected by micromanipulation, cultured in VKM media and regenerated as described in Rasmussen et al. (1997). The analysis of one plant from each regenerating callus are presented in this report. All plants were grown in vitro as described in Rasmussen and Rasmussen (1995).

Isolation of DNA for PCR analysis

DNA was isolated from young leaves of in vitro grown plants following the method of Edwards et. al (1991).

RAPD analysis

Decamer oligonucleotide primers from the series OPAT, OPAQ, OPAC, OPAR, OPF and OPAX (Operon Kit, Alameda, Calif., USA) were tested for the generation of *S. multiinterruptum* clone V577-specific RAPD bands. The PCR-amplification was performed as described earlier (Rasmussen and Rasmussen 1995).

PCR analysis of cytoplasmic genomes

The PCR reactions were performed with Ampli*Taq* DNA Polymerase or Ampli*Taq* Gold DNA Polymerase (Perkin-Elmer corp, Norwalk, Conn., USA) with primer-pairs (synthesized by DNA Technology A/S, Aarhus, Denmark) and annealing temperatures as described by Lössl et al. (1999). Chloroplast (cp) type T could be discriminated from cp type S or type W with the primer-pair AL_Cp1 and AL_Cp3. The primer-pair AL_Mt4 and AL_Mt 5 was used to differentiate the mitochondrial type at the *rps10* upstream region. The mitochondrial-specific *atp6* copy could be detected with the primer-pair AL_Mt1 and AL_Mt3.

Results

All plants examined in the present study were regenerated from individual calli. Analysis of the chloroplast and the mitochondrial genome in plants from the fusion combinations Kiva+V577 (K77) and Godiva+V577 (G77) as well as in plants from five previous described fusion combinations (Rasmussen et al. 1997), were performed in the present study. The plastome-specific primer-pair AL_Cp1+AL_Cp3 (Lössl et al. 1999) revealed that all recipient plants contained the T-type chloroplast, while the donor plants carried the W/S-type chloroplast (Table 1). Analysis of the chondriome with the primer-pair AL_Mt4+AL_Mt5, specific for the mitochondrial *rps10* upstream region, indicated that all recipient plants amplified a 1600-bp fragment. With the same primer-pair the donor plant V577 gave a strong 2200-bp band (Fig. 1). The two donor plants V7 and V8 amplified a 1600-bp fragment. The primer-pair AL_Mt1+AL_Mt3 gave a 1000-bp fragment with V577 donor DNA, while no PCR products were obtained with DNA from the donors V7 and V8 or from the recipient plants. An example of the PCR analysis for the chondriome, plastome and nuclear background in some of the regenerated K77 plants is given in Fig 1.

Table 1 PCR analysis of the recipient and donor plants used for asymmetric protoplast fusions. The primer-pair AL_Mt 4+AL_Mt5 is a mitochondrial (Mt)-specific primer-pair amplifying a region located at the *rps10* upstream region. The primer-pair AL_Mt1+AL_Mt3 results in a mitochondrial specific *atp6* copy. The chloroplast (Cp)-specific primer-pair AL_Cp1+AL_Cp3 discriminates chloroplast type T (380 bp) from chloroplast type W/S (622 bp). The size of PCR fragments is given in bp; nb=no PCR bands

Primer-pair	Organelle	Locus	Recipients					Donors		
			Kiva	Godiva	Matilda	Folva	161:14	V577	V7	V8
AL_Mt4+AL_Mt5	Mt	*rps10*	1600	1600	1600	1600	1600	2200	1600	1600
AL-Mt1+AL_Mt3	Mt	*atp6*	nb	nb	nb	nb	nb	1000	nb	nb
AL_Cp1+AL_Cp3	Cp	5′ to *atpE*	380	380	380	380	380	622	622	622
Plastid type			T	T	T	T	T	W/S	W/S	W/S

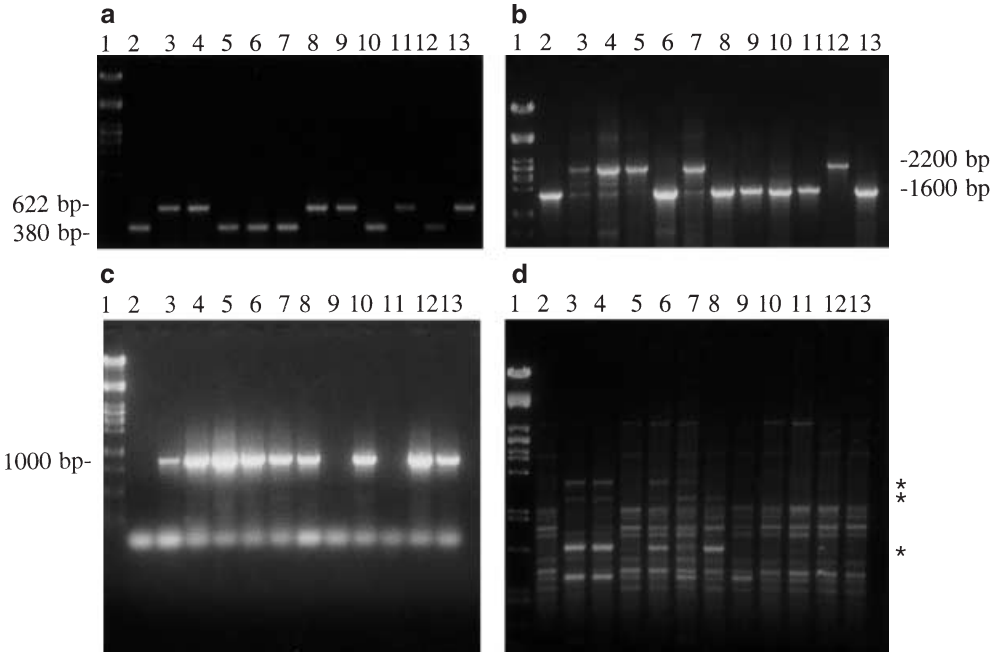

Fig. 1a–d PCR analysis of regenerated K77–0 and K77–150 plants. Plants were analysed with four different primer-pairs to characterize their cytoplasmic origin and to illustrate the presence of donor RAPD bands in some of the plants. The primer-pairs used are: **1a** AL_Cp1+AL_Cp3 to discriminate chloroplast type T (380 bp) from chloroplast type W/S (622 bp); **1b** AL_Mt4+AL_Mt5 to differentiate the mitochondrial type by the *rps10* upstream region (1600 bp or 2200 bp); **1c** AL_Mt1+AL_Mt3 to differentiate the mitochondria specific *atp6* copy (1000 bp). 1d: RAPD analysis with primer AQ07 resulting in three donor-specific bands of 1520 bp, 1250 bp and 820 bp respectively. In **1a–d** the template DNA was isolated from: *lane 2* Kiva, *lane 3* V577, *lane 4* K77–0 symmetric hybrid, *lane 5* K77–0 cybrid, *lanes 6–9* K77–150 asymmetric hybrid clones, *lanes 10–13* K77–150 cybrid clones. *Pst*1-digested λ-DNA was used as a DNA size marker (*lane 1*). * donor-specific RAPD bands

Asymmetric fusions with the *S. multiinterruptum* clone V577 as donor

The two recipient plants *S. tuberosum* cvs Kiva and Godiva and the donor plant *S. multiinterruptum*, clone V577, were analysed for RAPD polymorphism with decamer primers, randomly chosen from six Operon Kits, in order to identify nuclear hybrids. In the symmetric hybrids from the fusion combination K77–0, analysed by 20 donor-specific RAPD markers, a range of 90–100% of the donor-specific RAPD bands was detected (Fig 2). The asymmetric hybrids from the fusion combinations K77–150 and G77–150 were analysed by 34 and 27 donor specific RAPD markers, respectively. A range of 9–70% donor-specific RAPD bands was detected in the K77–150 hybrids while the only asymmetric hybrid from the G77–150 fusion experiment contained 47% donor-specific RAPD bands.

In the symmetric fusion K77–0 we obtained four somatic hybrids from 19 shoot-regenerating calli (Table 2). Analysis of the plastome origin revealed that three hybrids carried the recipient CpT type and one hybrid carried the CpW/S donor type. PCR analysis of the chondriome with the primer-pair AL_Mt4+AL_Mt5 revealed that the *rps10* fragment was of donor origin in the four hybrids. Three of these four hybrids had in addition the donor-specific *atp6* fragment (Table 3). Consequently one hybrid received both the plastome and chondriome markers from the donor parental plant.

Table 2 Analysis of regenerated plants from asymmetric protoplast fusions with seven recipient (Rec)+donor (Don) combinations. One plant was analysed from each regenerating callus. All regenerated plants were analysed for their chloroplast (Cp) type (type T or wild-type W/S). In two fusion combinations the regenerants were analysed for the origin of two mitochondrial (Mt) loci (*rps10* and *atp6*)

Combination Rec+Don	Irradiation dose (Gy)	Fusion code	Total no of analysed plants	Plants with hybrid nucleus							Plants with recipient nucleus						
				No. of plants	Cp from		Mt from			Cp and Mt from Don	No. of plants	Cp from		Mt from			Cp and Mt from Don[b]
					Rec (T)	Don (W/S)	Rec	Don	Rec+ Don[a]			Rec (T)	Don (W/S)	Rec	Don	Rec+ Don[a]	
Kiva+577	0	K77-0	19	4	3	1	0	4	0	1	15	15	0	12	1	2	0
	150	K77-150	52	16	12	4	1	5	10	3	36	31	5	25	2	9	4
Godiva+577	150	G77-150	30	1	0	1	0	0	1	1	29	26	3	27	0	2	2
Folva+V7	0	FV7-0	7	7	7	0	–	–	–	–	0	0	0	–	–	–	–
	150	FV7-150	3(5)[c]	3	3	0	–	–	–	–	–	–	–	–	–	–	–
	300	FV7-300	4	1	1	0	–	–	–	–	3	3	0	–	–	–	–
Folva+V8	300	FV8-300	5	3	0	3	–	–	–	–	2	0	2	–	–	–	–
Matilda+V8	150	MV8-150	4	1	1	0	–	–	–	–	3	1	2	–	–	–	–
161:14+V8	300	16V8-300	9	0	0	0	–	–	–	–	9	7	2	–	–	–	–
161:14+V7	0	16V7-0	3	3	1	2	–	–	–	–	0	0	0	–	–	–	–
	150	16V7-150	3	2	2	0	–	–	–	–	1	0	1	–	–	–	–
	300	16V7-300	4	4	4	0	–	–	–	–	0	0	0	–	–	–	–

[a] One Mt locus from the donor and one Mt locus from the recipient
[b] One or two Mt loci
[c] Only three of the five clones were analysed

Fig. 2 The percentage of donor-specific RAPD bands in hybrids from K77–0 (symbol ◇), K77–150 (symbol ◆) and G77–150 (symbol □) experiments in relation to the total number of donor-specific RAPD bands analysed in the donor plant

Fifteen plants from the fusion combination K77–0 were non-hybrid carrying the recipient nuclear genotype, and all plants also had the recipient plastome CpT genotype (Table 2). Twelve of these plants possessed the recipient *rps10* chondriome fragment (Table 3). One plant had both the *rps10* and the *atp6* fragments from the donor. In addition to the recipient *rps10* fragment one plant also had the donor-specific *atp6* fragment and one plant had both the donor and the recipient *rps10* fragment in combination with the *atp6* donor fragment.

In the asymmetric K77–150 fusion combination we obtained 52 plants and RAPD analysis revealed that 16 of them were asymmetric hybrids. Four of the sixteen asymmetric hybrids had the W/S-type chloroplast from the donor, while 12 had the T-type plastid from the recipient. Analysis of the chondriome origin revealed that five hybrids possessed the donor-specific *rps10* and *atp6* fragments while one hybrid possessed the *rps10* fragment from the recipient. The other ten asymmetric hybrids carried the *rps10* fragment from the recipient and the *atp6* fragment from the donor. Three of the asymmetric hybrids received both plastome and chondriome markers from the donor parent.

In 36 plants from this fusion combination RAPD analysis revealed that all the plants had a recipient nucleus. Plastome analysis of these plants demonstrated that 31 carried the recipient CpT genotype, while five had the donor CpW/S type. Chondriome analysis indicated that 25 regenerants carried the *rps10* fragment from the recipient. Two plants carried both the *rps10* and the *atp6* fragments from the donor. Nine plants had the *rps10* fragment originating from the recipient in combination with the *atp6* fragment from the donor. One of these plants also had the donor *rps10* fragment so this plant appears to contain two different alleles of the *rps10* locus. It was noted that four of the five plants which contain the donor CpW/S chloroplast genome also received a chondriome marker from the donor.

Table 3 The numbers of plants carrying different combinations of mitochondrial markers from donor and recipient in the fusion combinations K77-0, K77-150 and G77-150

Item	Locus	Origin	Plants with hybrid nucleus			Plants with recipient nucleus		
			K77-0	K77-150	G77-150	K77-0	K77-150	G77-150
Total number of plants			4	16	1	15	36	29
Mitochondrial origin	*rps10*	Donor	1	0	0	0	0	0
	rps10 Donor *atp6*	Donor	3	5	0	1	2	0
	rps10 Donor *rps10* Recipient *atp6*	Donor	0	0	0	1	1	0
	rps10	Recipient	0	1	0	12	25	27
	rps10 Recipient *atp6*	Donor	0	10	1	1	8	2

From the fusion combination G77 one asymmetric hybrid was obtained. This hybrid had the W/S-type donor plastome and the recipient *rps10* chondriome fragment in combination with the *atp6* donor fragment. From this fusion combination 29 non-hybrids were obtained. Twenty six plants carried the plastome from the recipient while three plants had the plastome from the donor. Twenty seven plants possessed the *rps10* chondriome fragment from the recipient, and two plants carried the *rps10* recipient fragment in addition to the donor-specific *atp6* fragment.

From all the fusion combinations with the *S. multi-interruptum*-clone V577 as donor, the number of plants with different combinations of the mitochondrial markers from the donor and recipient are summarized in Table 3.

Asymmetric fusions with *S. spegazzinii* clone V7 and *S. microdontum* ×*S. vernei* clone V8 as donors

The RAPD analysis of regenerated plants from the five fusion combinations with the donors V7 and V8 has previously been presented (Rasmussen et al. 1997). PCR-analysis of the plastome type revealed that in the fusion combination FV7 all regenerated plants had the recipient plastome type CpT (Table 2). In the fusion combination FV8–300 all hybrids and non-hybrids carried the donor plastome type CpW/S.

In the fusion combination MV8–150 one asymmetric hybrid with the plastome originating from the recipient was obtained. One of the three non-hybrids from this fusion combination had the plastome from the recipient, while two non-hybrids possessed the donor plastome.

In the fusion combination 16V8–300 none of the shoots from nine regenerating calli were asymmetric hybrids. Plastome analysis of the non-hybrids revealed that two carried the donor plastome, while seven plants carried the recipient plastome.

In the fusion combination 16V7–0 three symmetric hybrids were obtained, of which one had the plastome from the recipient, and two had the plastome from the donor. In the 16V7–150 experiment three plants were regenerated, of which two were asymmetric hybrids with a recipient plastome while one non-hybrid harboured a recipient nucleus and a donor plastome. The four regenerated plants in the 300 Gy experiment gave four asymmetric hybrids, all carrying the recipient plastome type CpT.

Discussion

Regenerated plants from the asymmetric fusion combinations Kiva+V577 (K77–150) and Godiva+V577 (G77–150) were analysed by the RAPD technique for 27–34 donor-specific RAPD bands. Asymmetric hybrids with 9–70% donor-specific RAPD bands were obtained in the two fusion combinations (Fig. 2). These results are at a similar level of donor DNA elimination as was observed in 13 asymmetric hybrids from the fusion combinations FV7, FV8, MV8 and 16V7 (Table 2), where 5–67% donor-specific RAPD bands were obtained (Rasmussen et al. 1997). This donor DNA elimination range is similar to the results from Oberwalder et al. (1998) and Xu and Pehu (1993) who obtained 12–86% and 35–90% wild *Solanum* donor DNA in asymmetric hybrids with *S. tuberosum* recipients respectively. From the interspecific symmetric K77–0 fusion, hybrids with 90–100% donor-specific DNA were obtained. It has previously been reported, for both symmetric intraspecific (Rasmussen et al. 1996) and symmetric interspecific (Mattheij et al. 1992; Rasmussen et al. 1997) protoplast fusions with *Solanum* parentals, that some of the regenerated hybrids are missing chromosomes or chromosome sequences from the fusion parentals. In this analysis we have not analysed the hybrids for missing RAPD bands from the recipient.

Our results demonstrate that irradiation of donor protoplasts with 150 Gy induces a broad range of donor DNA elimination in the asymmetric hybrids. In various asymmetric donor-recipient combinations, such as *Nicotiana plumbaginifolia* and *Atropa belladonna* (Gleba et al. 1988), potato and *Solanum* ssp. (Xu et al. 1993; Rasmussen et al. 1997), increased levels of donor protoplast irradiation did not result in increased levels of

donor DNA elimination. By contrast in fusion experiments with *Lycopersicon esculentum* and *Lycopersicon pennellii* Melzer and O'Connel (1992) observed increased donor chromosome elimination with increased irradiation levels ranging from 50 to 1000 Gy. The donor DNA in asymmetric hybrids has been identified as whole chromosomes (Xu et al. 1993), introgressions (Liu et al. 1999), and as minichromosomes (Feher et al. 1992).

In none of the regenerants from the seven asymmetric fusion combinations and from the three symmetric fusion combinations, listed in Table 2, was a mixture of plastome-specific PCR bands from the donor and the recipient plants found. A mixture of chloroplasts from the two fusion parentals has been found in regenerated somatic *Nicotiana* hybrids (Glimelius et al. 1981), most likely due to a non-completed plastid segregation before shoot regeneration. On two occasions we obtained shoots from the same callus with different plastid types (data not shown). This indicates that the regenerating callus was chimeric, due to plastid segregation in the growing callus before regeneration from the individual cells. In experiments with potato cybrids Perl et al. (1991) also obtained plants with different plastomes, regenerated from the same callus.

The recipient cv Folva was used in two fusion combinations with the V7 and V8 donors. In fusions with the V7 donor all plants from 14 regenerating calli had the T-type plastome from the recipient. In fusions with the V8 donor, all plants from the five regenerating calli had the W/S-type plastome from the donor. This indicates that factors other than the recipient genotype determine chloroplast segregation.

In two asymmetric fusion combinations with the V577 donor, 8 of 65 non-hybrids and 5 of 17 asymmetric hybrids had donor chloroplasts. We did not obtain cybrids with a wild-species plastome and a potato nucleus in the K77–0 experiment as we did in the K77–150 experiment, which indicates that irradiation of donor protoplasts may influence the induction of plastome-nucleus cybrid formation. In the three asymmetric fusion combinations with the V8 donor, 6 of 14 non-hybrids and 3 of 4 asymmetric hybrids had donor chloroplasts, while in two fusion combinations with the V7 donor, 1 of 4 non-hybrids and 0 of 10 asymmetric hybrids had donor chloroplasts. Apparently the two different wild *Solanum* donor species are able to transfer their chloroplasts and form cybrids with different efficiency. In all the asymmetric fusion experiments we obtained 15 (18%) plants with a donor plastome among the 83 plants with a recipient nucleus. In total 23 (20%) of all 114 regenerated plants from the asymmetric experiments analysed had their plastome from the donor. Although we only analysed one plant from each callus we find our result with 15 (13%) cybrids of 114 analysed plants comparable to that of Perl et al. (1991) who analysed 401 putative cybrids isolated from 119 calli in 12 *S. tuberosum* cv Desiree+*Solanum* ssp. fusion combinations. In ten combinations they obtained 78 (20%) cybrids with a donor plastome and in two combinations none of the regenerants had donor plastomes.

Various factors may be involved in chloroplast segregation in the heterofusion products. In the analysis of *Brassica napus+Eruca sativa* hybrids Fahleson et al. (1988) observed a non-random chloroplast segregation, and suggest that different rates of replication or genome-plastome incompatibilities could be involved. Another explanation for a non-random chloroplast segregation could be an unequal input of organelles due to different ploidy levels between the fusion partners, as suggested by Sundberg et al. (1991). Irradiation of donor protoplasts may also influence the competition between chloroplasts, but can not explain the difference observed in this study between donors. A slower growth rate of the asymmetric heterofusion products, and a reduced ability to develop into calli, with an increased irradiation dose of the donor protoplasts has been observed (Xu et al. 1993; Rasmussen et al. 1997). Despite these inhibitory effects, in some heterofusion products the plastomes from the irradiated donor can outdo the non-irradiated recipient plastome. This has also been shown in other asymmetric *S. tuberosum+Solanum* ssp. fusion experiments (Sidorov et al. 1987; Perl et al. 1991). Derks et al. (1992) analysed chloroplast DNA from *Lycopersicon peruvianum* protoplasts irradiated with increasing doses from 50 to 1000 Gy. Doses of 300 Gy did not increase the amount of fragmentated chloroplast DNA, although an increased amount of linear chloroplast DNA molecules was observed with an increasing irradiation dose. These authors suggest that the nuclear DNA is more damaged than the chloroplast DNA at lower irradiation doses, since the radiosensitivity of DNA is relative to target size. The number of nucleotide pairs in the *L. peruvianum* nucleus is more than 10^4-times higher than in the chloroplast genome. Derks et al. (1992) suggested that the high copy number of the chloroplast genome in a cell, and a sorting out of mutated chloroplast genomes before or during shoot regeneration, might explain why chloroplasts are apparently less sensitive to irradiation damage than the nuclear genome. A rare occurrence of chloroplast DNA rearrangements in asymmetric hybrids has been reported (Sidorov et al. 1987) but in most analyses such rearrangements have not been observed (Sidorov et al. 1994; Waara and Glimelius, 1995).

The donors and recipients were analysed by PCR for polymorphisms at the mt *rps10* upstream region (Lössl et al. 1999). A polymorphism for the fusion combinations with the V577 donor and the Kiva and Godiva recipients was found (Fig. 1). Analysis of regenerated K77 and G77 plants revealed a clear assortment of the chondriome type in all but two regenerants. In two regenerants, one K77–0 non-hybrid and one K77–150 non-hybrid, both the *rps10* donor and recipient fragments were found (Table 3). We were unable to determine whether the two chondriome types were located in the same cell, or if different cells have different chondriomes. Chloroplast PCR analysis revealed that these two plants had the Cp type T from Kiva. These regenerants

could have arisen from a non-completed chondriome segregation before regeneration, but the formation of new sublimones (Leaver et al. 1988) could also be a possibility.

The PCR primer-pair AL_Mt1 + AL_Mt3 results in a mitochondrial specific *atp6* fragment in the donor V577 whereas no PCR bands were obtained in the other donor and recipient genotypes. Analysis of the regenerated plants from K77–150 and G77–150 revealed that 21 of 74 plants with the *rps10* recipient PCR fragment also had the mitochondrial-specific *atp6* donor fragment. Isolation of new DNA from regenerants subcultivated two or three times confirmed the reproducibility of the results, eliminating the possibility of contamination.

The presence of mitochondrial-specific PCR fragments located at different positions on the chondriome in the donor and the recipient could be explained by recombination events between the chondriomes from the two fusion parents, as reported for many somatic hybrids (Melchers et al. 1992; Lössl et al. 1994). If recombination had taken place between the *atp6* and the *rps10* loci we would expect to obtain plants with two new donor-recipient combinations between these loci. But we mainly found one combination product with the recipient *rps10* PCR fragment and the donor *atp6* fragment (Table 3). Only in one plant did we find the other combination with a donor *rps10* PCR fragment not in combination with the *atp6* fragment from the recipient. Our results can be interpreted in terms of the model with mitochondrial subgenomes suggested by Lössl et al. (1999). Their results indicate that subunits of the mitochondrial chromosome in sub-stoichiometric amounts coexist with the main mt chromosome of these organelles. According to that model these subunits could be exchanged independently from the main mt chromosome. The mitochondrial-specific *atp6* band found in V577 might be located on both the main chromosome and on a subunit of the mitochondrial chromosome. This explains the strong coupling found between the *rps10* donor band and the *atp6* donor band. In 13 of the 14 plants found with the *rps10* PCR donor band the *atp6* PCR donor band was also present (Table 3). In the single regenerated clone where this coupling was not found, a recombination between the two loci could have occurred. The 22 plants with the *atp6* donor band in combination with the recipient *rps10* band (Table 3) might contain a main mitochondrial chromosome from the recipient and a subunit of the mitochondrial chromosome with an *atp6* copy from the donor. We did not observe any new PCR bands with the primer-pairs.

In some of the selected heterofusion products the recipient nucleus and donor nucleus have fused, which has been followed by a partial donor DNA elimination resulting in an asymmetric hybrid. We have previously observed a slight variation in the RAPD banding pattern in asymmetric hybrids from the same callus (Rasmussen et al. 1997), and asymmetric K77–150 hybrid calli also regenerated shoots with only a slight variation in RAPD-donor banding patterns (data not shown). Thus the donor DNA elimination seems to take place in the early stages of callus development. Preliminary morphological analysis indicates that plants isolated from the same callus, and having a high similarity in RAPD banding pattern, can have a different morphology (data not shown). In six of seven analysed fusion combinations we obtained cybrids with a recipient nucleus and a donor plastome. In the two asymmetric fusion combinations with the V577 donor, 15 of the 65 regenerated plants with a recipient nucleus had either plastome or/and chondriome PCR markers from the donor. These cybrids must have originated either from heterofusion products in which all nuclear donor DNA had been eliminated from the heterokaryon or in which no heterokaryon had been formed. We cannot exclude the possibility that some of the plants categorized as cybrids have a low level of donor nuclear DNA that is not detectable by this RAPD analysis. If donor-chromosome elimination results in cybrid formation, we would expect more asymmetric hybrids with a low content of donor-specific RAPD bands than found in this (Table 1) and our previous (Rasmussen et al. 1997) analysis. Probably the irradiation of the donor protoplasts results in an inactivation of the nuclei, which will then be eliminated during cell division if no heterokaryons have been formed. Cybrids with a potato nucleus and wild-species plastids have been obtained after irradiation at 100 Gy (Perl et al. 1991) and also after irradiation at 1000 Gy (Sidorov et al. 1994), which indicates that nuclear inactivation is important in cybrid formation. In the symmetric fusion experiment K77–0 we obtained two plants with nuclei from the recipient, of which one had both *rps10* and *atp6* chondriome fragments from the donor and the other had the *atp6* copy from the donor. These results support the model of no heterokaryon formation.

This report demonstrates that characterization of the plastome and chondriome origin by specific PCR primers is very useful in order to characterize large populations of regenerated putative hybrids. In the fusion combinations G77 and K77 analysed for both the chondriome and plastome markers all the symmetric and asymmetric hybrids either had chondriome and/or plastome PCR bands from the donor. This indicates that PCR analysis of cytoplasmic DNA could be used to select regenerants that should be analysed further by RAPDs in order to identify asymmetric hybrids.

Based on the results presented in this paper we conclude that chloroplasts and mitochondria segregate independently of each other. New combinations between the fusion parental cp type and mt type have also been obtained in this study.

Until now the published protocols for asymmetric protoplast fusion have been developed in order to obtain cybrids (Perl et al. 1991; Melchers et al. 1992; Sidorov et al. 1994) or asymmetric hybrids (Sidorov et al. 1987; Fehér et al. 1992; Sjödin and Glimelius 1989; Xu et al. 1993; Gleba et al. 1988; Oberwalder et al. 1998) whereas both cybrids and asymmetric hybrids have not been obtained in the same experiments. This paper demonstrates

that selection of heterofusion products based on fluorescent markers is an efficient method to produce asymmetric hybrids and cybrids from most fusion combinations. The aim of this work was to transfer potato late blight resistance from the V577 donor to cultivated potato genotypes by use of asymmetric protoplast fusion. Asymmetric hybrids and cybrids from the K77 experiments will be subjected to future greenhouse and field trials for late blight resistance analysis.

Acknowledgements The authors thank Henriette B. Rasmussen for skilful technical assistance with the PCR analysis and in vitro culture. We are indebted to Dr. K.Tolstrup, Dr. H.G.Kirk and Dr. J.P.Nepper, Danish Potato Breeding Foundation, Vandel, Denmark, for providing the *Solanum* genotypes. The project is supported by The Danish Agricultural Ministry, J.no. 93S-2466-A95–00706.

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