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Organelle analysis of symmetric and asymmetric hybrids between *Lycopersicon peruvianum* and *Lycopersicon esculentum*

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Summary. The organelles of somatic hybrids obtained from symmetric and asymmetric fusions between the *Lycopersicon* species *L. peruvianum* and *L. esculentum* were analyzed by DNA hybridization methods. In the asymmetric fusions the *L. peruvianum* protoplasts were gamma-irradiated at a dose of 50, 300 and 1,000 Gy. The organelles were characterized using the Petunia chloroplast probe pPCY64 and the mitochondrial EcoRI-SalI fragment of the *Pcf* gene. In all symmetric and asymmetric hybrid plants, a total of 73 being analyzed, only one of the parental chloroplast genomes was present, except for one hybrid plant which harbored both parental chloroplast genomes. No recombination and/or rearrangement in the chloroplast genome could be identified with the pPCY64 probe. Irradiation of the *L. peruvianum* protoplasts did not significantly reduce the fraction of asymmetric hybrids with *L. peruvianum* chloroplasts. A novel mitochondrial restriction pattern was present in 5 out of 24 hybrids tested. In 9 hybrids novel combinations of chloroplasts and mitochondria were found, indicating that both organelle types sorted out independently.

Key words: Chloroplast – Mitochondrion – Somatic hybrids – Irradiation – Restriction fragment length polymorphism (RFLP)

Introduction

The transfer of cytoplasm to another nuclear background in both interspecific and intergeneric somatic hybridization experiments has been described predominantly in the genera *Nicotiana, Brassica* and *Solanum* (Zelcer et al. 1978; Gleba and Sytnik 1984; Barsby et al. 1986; Galun and Aviv 1988). The chloroplasts in the fusion products sort out bi- or unidirectionally to homogeneity for one or the other parent, depending on the phylogenetic distance of the species, the presence of selection pressure for one organelle type and the relative number of plastids in each parental protoplast (Menczel et al. 1982; Kumar and Cocking 1987; O'Connell and Hanson 1987; Gleba et al. 1988; Levi et al. 1988). Until now the recombination of chloroplast DNA (cpDNA) in fusion products has only rarely been observed (Medgyesy et al. 1985a; Maliga et al. 1987).

In contrast to what has been observed with the chloroplast genome, recombination and/or rearrangement of mitochondrial DNA (mtDNA) in fusion products is common, resulting in restriction fragment patterns that are unique in comparison to the parental mitochondrial genomes (Barsby et al. 1986; Galun and Aviv 1988; Levi et al. 1988). In most experiments it has been shown that chloroplasts and mitochondria segregate independently (Aviv and Galun 1980; Kumar and Cocking 1987; O'Connell and Hanson 1987; Levi et al. 1988).

In asymmetric fusion experiments gamma or X-rays are often used to inactivate the donor protoplasts (Zelcer et al. 1978). The effect of irradiation on the transfer of chromosomal DNA was extensively studied in hybridization experiments in which a nuclear encoded trait was used for selection (Imamura et al. 1987; Gleba et al. 1988; Yamashita et al. 1989; Famelaer et al. 1989). Menczel et al. (1982) reported the effect of various irradiation doses on chloroplast transfer. They described that plastids could be rescued from the X-irradiated cells (300 Gy) by fusion with untreated protoplasts, but the effect of a high irradiation dose on mtDNA was not discussed.

Although protoplast fusion between *Lycopersicon* species has been described (O'Connell and Hanson 1985, 1987; Adams and Quiros 1985; Kinsara et al. 1986; Wijbrandi et al. 1988), less is known about organelle transmission. Only O'Connell and Hanson (1985, 1987) have analyzed the organelles in a limited number of hybrids

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obtained after symmetric fusion between *L. pennellii* and *L. esculentum*.

The combination between the cultivated tomato (*L. esculentum*) and its outbreeding wild relative *L. peruvianum* (Stevens and Rick 1986) has been studied extensively (Wijbrandi 1989). Symmetric and asymmetric hybrids were formed, and these were selected for nuclear traits and characterized for their morphology, fertility, chromosome number and nuclear DNA composition by RFLP analysis. In the present article we describe an analysis of the organelle composition of these hybrids, and by this means we were able to study the segregation, recombination and the effect of gamma irradiation on organelle transmission in these hybrids.

Materials and methods

Plant material

Regenerated hybrid plants were obtained from symmetric and asymmetric fusions between protoplasts from *L. peruvianum* (PI128650) and the kanamycin-resistant lines of the *L. esculentum* genotypes 'Bellina', LA291 and LA1182, as described previously (Wijbrandi et al. 1988). Under the tissue culture conditions employed, only the *L. peruvianum* protoplasts were able to regenerate into plants. Consequently symmetric hybrids could be selected on their ability to regenerate into plants and their kanamycin resistance. In the asymmetric fusion experiments *L. peruvianum* protoplasts were exposed to 50, 300 and 1,000 Gy of gamma rays. The asymmetric hybrids were selected on the basis of the plant regeneration capacity derived from *L. peruvianum* protoplasts.

DNA isolation and organelle DNA probes

Total DNA from *L. peruvianum* and *L. esculentum* was isolated from 5–8 g frozen fully expanded leaves according to Dellaporta et al. (1983).

For the characterization of cpDNA the Petunia cpDNA probes pPCY64 (de Haas et al. 1986), pPCY20-1 (Overbeeke et al. 1984), and *PST*I fragments 1 and 4 (de Haas et al. 1986) were used.

The mtDNA was characterized using the 0.45-kb EcoRI-SalI fragment of the *Pcf* gene (Young and Hanson 1987), the 2.2-kb XbaI fragment of the maize ATPase subunit 9 gene (Dewey et al. 1985), the 6.0-kb BamHI fragment of the 18S-5S rRNA gene (Chao et al. 1984), the 14-kb BamHI fragment of the maize 26S rRNA gene (Dale et al. 1984), pKL-D (Hensgens et al. 1983) and p*Spom* 1 (de Heij et al. 1985).

DNA to be used as a probe was labelled with α-^{32}P-dATP (Amersham) for 1.5 h at 15 °C with a nick translation kit (Amersham) as described by the supplier. Unincorporated nucleotides were removed by centrifugation on a Sephadex G50-M column (Pharmacia). The probe was boiled for 10 min before being added to the hybridization mixture.

DNA restriction, Southern transfer and hybridization

Total DNA (4 µg) was digested with restriction endonucleases BamHI, BstEII, EcoRI, HindIII and HinfI (10 units/µg DNA) as described by the manufacturer (Boehringer, Mannheim). DNA fragments were separated on a 0.8% (w/v) agarose gel (40 m*M* TRIS, 1 m*M* EDTA, 20 m*M* acetic acid, pH 8.0) for 18 h at 1.25 V/cm. Digests with HinfI were electrophoresed on 1.2% (w/v) agarose gel.

The gels were first incubated in 0.25 *N* HCl for 15 min (Wahl et al. 1979) and subsequently for 30 min in transfer buffer (Chomczynski and Qasba 1984). The DNA was transferred to Gene Screen Plus (Dupont, NEN products, Boston, USA) as described by Chomczynski and Qasba (1984).

The blots were prehybridized for 2 h at 65 °C in 10 × Denhardt's 40 m*M* TRIS pH 8.0, 1 m*M* EDTA, 5 × SSC, 1% (w/v) SDS and 100 µg/ml salmon sperm DNA. The ^{32}P-labelled DNA probe was then added to the hybridization solution and incubated for 18 at 65 °C. After hybridization the blots were washed according to the supplier (Dupont, Boston, USA), sealed in plastic and exposed to X-ray film (Kodak XAR-5) at −70 °C using an intensifying screen.

Results

Chloroplast DNA analysis of L. esculentum and L. peruvianum

To detect a restriction fragment length polymorphism (RFLP) between cpDNA of *L. peruvianum* and *L. esculentum*, total DNA digestion patterns were screened in 20 combinations using four cpDNA specific probes and five restriction endonucleases (BamHI, BstEII, EcoRI, HindIII and HinfI). All combinations resulted in the same hybridization patterns for both parents, except for the HinfI digests when probed with pPCY64 (Fig. 1). This probe hybridized to a 1.4-kb fragment in *L. esculentum* cpDNA and to a 1.55-kb fragment in *L. peruvianum* cpDNA. The same hybridization patterns were detected when cpDNA instead of total DNA was isolated and probed with pPCY64 (results not shown).

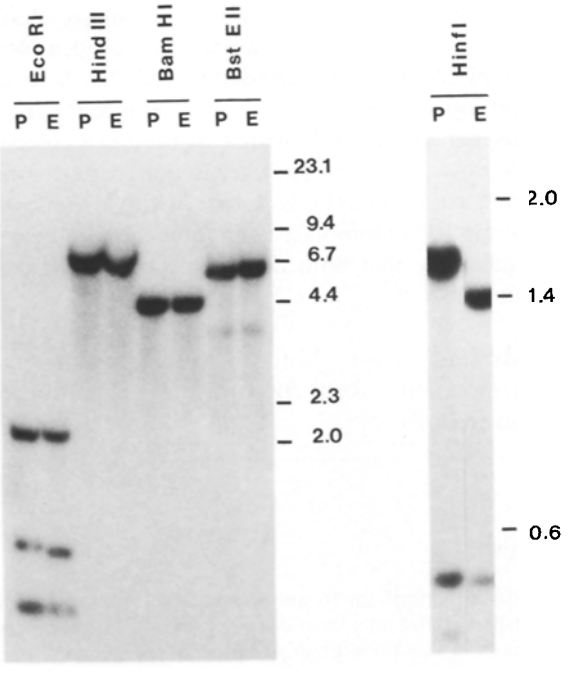

Fig. 1. Analysis of cpDNA. Autoradiographs showing the hybridization patterns of EcoRI, HindIII, BamHI, BstEII and HinfI of *L. esculentum* (*E*) and *L. peruvianum* (*P*) with pPCY64

Chloroplast DNA analysis of symmetric somatic hybrids

These specific marker fragments made it possible to identify the cpDNA present in the somatic hybrid plants (Fig. 2). Hybrid plants regenerated on 34 independent calli obtained after symmetric somatic fusion were examined for their cpDNA type. Table 1 shows that 12 shoots had the cpDNA type of *L. esculentum* and 21 shoots had the *L. peruvianum* chloroplasts. One of the symmetric hybrid plants showed restriction fragments specific to both parental species, in an estimated ratio of about 94% *L. peruvianum* and 6% *L. esculentum*, as determined by densitometric measurements (Fig. 2 H4). This means that sorting out might not yet be completed at the plant level. From one specific hybrid callus that was not included in Table 1, two shoots were formed and analyzed for their cpDNA type. They were found to differ in chloroplast type. In five other cases where two shoots were analyzed no differences in chloroplast types between these shoots were found.

Chloroplast DNA analysis of asymmetric somatic hybrids

In the asymmetric fusions, in which the donor protoplasts were exposed to various doses of gamma rays, there were also chloroplasts of only one of the parents present (Table 1). A comparison of the total number of hybrids from symmetric and asymmetric fusions shows that irradiation did not significantly affect the transmission of chloroplasts, as tested with a contingency Chi-square test ($\chi_1^2 = 1.12$ ($P > 0.05$)). Furthermore, the results do not indicate deviations in the random sorting of the chloroplasts.

Mitochondrial DNA analysis of L. esculentum and L. peruvianum

Similar to what was observed for cpDNA it was difficult to detect differences in mtDNA restriction patterns. For this characterization six mtDNA probes in combination with the five restriction endonucleases were used. No differences were found in the mtDNA pattern of both parents except when EcoRI and HindIII digests were probed with the EcoRI-SalI fragment from the *Pcf* gene of Petunia (Fig. 3). In the mtDNA of *L. peruvianum* two additional bands (0.97 and 0.62 kb) were detected in the EcoRI digest (Fig. 3 A) and one additional band (3.75 kb) in the HindIII digest (Fig. 3 B).

Mitochondrial DNA analysis of the symmetric and asymmetric somatic hybrids

With the *Pcf* probe and the enzymes EcoRI and HindIII the mitochondrial genomes of 12 symmetric and 12 asymmetric hybrids were characterized (Table 2). In most cases either the *L. peruvianum* mtDNA pattern or the *L. esculentum* mtDNA pattern was obtained (Fig. 3). A comparison of symmetric and asymmetric hybrids showed that irradiation affected the distribution of the mitochondria: the number of hybrids exhibiting the *L. peruvianum* mtDNA pattern was significantly decreased (Fisher's exact probability test for 2×2 tables; $P = 0.036$).

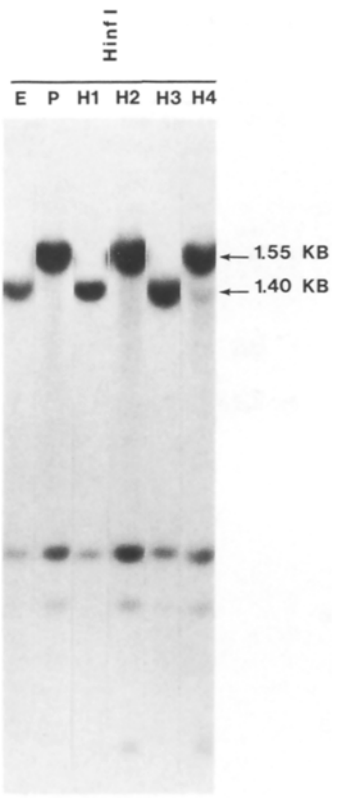

Fig. 2. Analysis of cpDNA.Autoradiograph showing the hybridization pattern of the HinfI digest probed with pPCY64 of *L. esculentum* (*E*), *L. peruvianum* (*P*) and some hybrids (*H1–H4*). *H1* is an asymmetric hybrid and *H2–H4* are symmetric hybrids. The hybrids *H1–H3* correspond to the hybrids in Fig. 3

Table 1. cpDNA analysis in 73 independent hybrid shoots obtained from symmetric and asymmetric fusions between *L. peruvianum* and *L. esculentum*

Chloroplast type[a]	Number of hybrids obtained			
	Without irradiation	With irradiation (dose in Gy)		
		50	300	1,000
P	21	6	13	1
E	12	8	10	1
P+E	1	0	0	0
Total number	34	14	23	2

[a] Chloroplast genome of *L. peruvianum* (P), *L. esculentum* (E) and both cpDNA types (P+E)

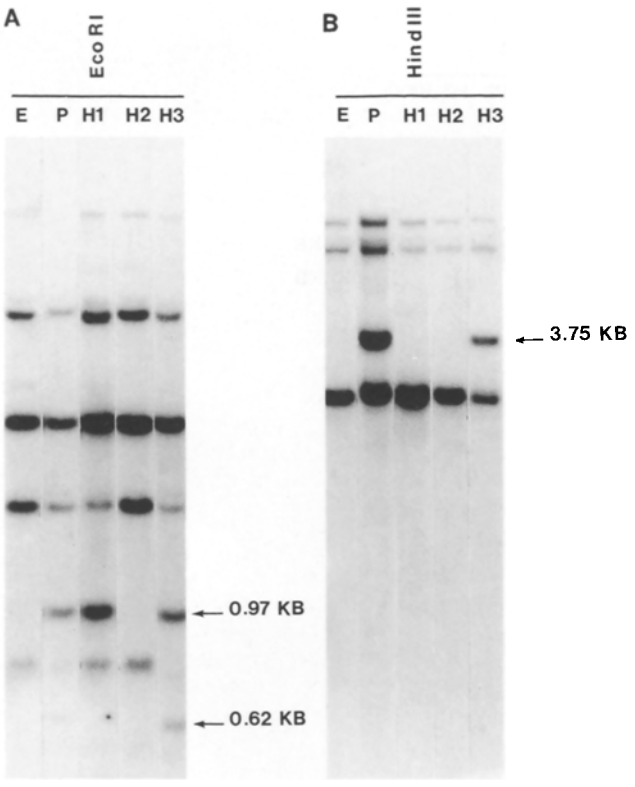

Fig. 3 A, B. Analysis of mtDNA. Autoradiograph showing the hybridization pattern of *L. esculentum* (*E*), *L. peruvianum* (*P*) and some hybrids (*H1–H3*). The EcoRI (*A*) and HindIII (*B*) hybridization pattern probed with the EcoRI-SalI fragment from the *Pcf* gene of Petunia. The hybrids in this figure correspond to the hybrids in Fig. 2

Table 2. Analysis of the mtDNA restriction pattern of 24 independent hybrids obtained from symmetric and asymmetric fusions between *L. peruvianum* and *L. esculentum*

Mitochondrial DNA restriction pattern[a]	Number of hybrids obtained			
	Without irradiation	With irradiation (dose in Gy)		
		50	300	1,000
P	8	1	1	0
E	3	1	2	3
R	1	2	1	1
Total number	12	4	4	4

[a] DNA pattern resembling the mtDNA restriction pattern of *L. peruvianum* (P), *L. esculentum* (E) and a new mitochondrial restriction pattern (R)

A new mtDNA pattern was found in 5 out of 24 tested hybrids. These 5 hybrids showed the *L. esculentum* pattern with an additional 0.97 kb fragment (Table 2, Fig. 3 H1). Surprisingly this pattern was the same for the EcoRI digestion of all 5 hybrids, whereas the HindIII digests showed the normal *L. esculentum* pattern. These results indicate that a recombination of mtDNA had occurred. Although the fraction of recombinants is higher in asymmetric hybrids, more analyses are needed to prove that irradiation enhances recombination.

A comparison was made between the cpDNA and mtDNA composition of the individual hybrids. In the cytoplasm of 9 hybrids, novel combinations of organelles were found (see Figs. 2 and 3 H2 and H3), which means that chloroplasts and mitochondria sorted out independently.

Discussion

In the genus *Lycopersicon* this is the first time that organelles have been analyzed for a large number of somatic hybrids.

RFLPs, which were used to discriminate between the organelles of the parents, were difficult to detect, indicating that the organelle genomes of *L. peruvianum* and *L. esculentum* resemble each other. This result suggests that there is no nucleo-cytoplasmic incongruity and fits well with the unbiased organelle distribution observed in the hybrids.

The irradiation dose had no apparent effect on the segregation of the chloroplasts in the hybrids, which suggests that the activity of the chloroplasts of *L. peruvianum* is not affected by irradiation. This agrees with earlier reports (e.g., Aviv et al. 1980; Sidorov et al. 1981; Aviv and Galun 1986), although in those papers no comparison was made of the various irradiation doses. Famelaer et al. (1989) studied chloroplast distribution in *Nicotiana* hybrids that were selected on the basis of nuclear encoded traits. In contrast to our results only the acceptor chloroplasts were present in their symmetric and asymmetric hybrids. In our study there is a slight indication that high irradiation dose had an effect on the distribution of the mitochondria, which is opposite to what was observed with chloroplasts. Such an effect on mitochondria segregation has never been described and is contrary to results from previous investigations (Zelcer et al. 1978; Aviv and Galun 1980; Galun et al. 1982; Menczel et al. 1983; Medgyesy et al. 1985 b). However, in these earlier reports only irrdiation doses up to 200 Gy were used. Because we characterized only a small population of hybrids (4) for mtDNA, more plants have to be screened to confirm these results.

Using one RFLP marker we were not able to detect recombination in the cpDNA of the hybrids: all hybrids showed either the *L. peruvianum* pattern or the *L. esculentum* pattern. This agrees quite well with other reports on the segregation of chloroplasts (Barsby et al. 1986; Kumar and Cocking 1987). However, intramolecular rearrangements of cpDNA do occur within a chloroplast (Kolodner and Tewari 1979; Palmer and Thompson

1981), and cpDNA recombination has been reported only rarely (Medgyesy et al. 1985a; Maliga et al. 1987; Thanh and Medgyesy 1989).

Sine the same recombinant hybridization patterns was found in all 5 somatic hybrids, which indicates that recombination and/or rearrangements had occured, these results suggest the presence of hot spots or site-specific sequences for recombinations and/or rearrangements. This has been described for *Brassica* (Vedel et al. 1986) and for *Solanum* (Barsby et al. 1986). The ratio of recombinants could probably be considerably increased after a more extensive analysis of the mitochondrial genomes. In our experiment only a single probe which covers only a small proportion of the whole genome was used.

In conclusion this research shows that unbiased chloroplast and mitochondria segregation occurred in symmetric hybrids between *L. peruvianum* and *L. esculentum*. Irradiation had no observable effect on chloroplast distribution, but irradiation at doses 200 times higher than the lethal one appeared to have a negative effect on the multiplication of the mitochondria. In addition, the chloroplasts and mitochondria sorted out independently.

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